Circulation

ORIGINAL RESEARCH ARTICLE

Circulating Spike Protein Detected in Post–COVID-19 mRNA Vaccine Myocarditis

Lael M. Yonker, MD*; Zoe Swank, PhD*; Yannic C. Bartsch, PhD*; Madeleine D. Burns, MS; Abigail Kane, MD; Brittany P. Boribong, PhD; Jameson P. Davis, BS; Maggie Loiselle, BS; Tanya Novak, PhD; Yasmeen Senussi, MBBS; Chi-An Cheng, PhD; Eleanor Burgess, MS; Andrea G. Edlow, MD; Janet Chou, MD; Audrey Dionne, MD; Duraisamy Balaguru, MD; Manuella Lahoud-Rahme, MD; Moshe Arditi, PhD; Boris Julg, MD, PhD; Adrienne G. Randolph, MD; Galit Alter, PhD; Alessio Fasano, MD†; David R. Walt, PhD†

BACKGROUND: Cases of adolescents and young adults developing myocarditis after vaccination with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–targeted mRNA vaccines have been reported globally, but the underlying immunoprofiles of these individuals have not been described in detail.

METHODS: From January 2021 through February 2022, we prospectively collected blood from 16 patients who were hospitalized at Massachusetts General for Children or Boston Children's Hospital for myocarditis, presenting with chest pain with elevated cardiac troponin T after SARS-CoV-2 vaccination. We performed extensive antibody profiling, including tests for SARS-CoV-2–specific humoral responses and assessment for autoantibodies or antibodies against the human-relevant virome, SARS-CoV-2–specific T-cell analysis, and cytokine and SARS-CoV-2 antigen profiling. Results were compared with those from 45 healthy, asymptomatic, age-matched vaccinated control subjects.

RESULTS: Extensive antibody profiling and T-cell responses in the individuals who developed postvaccine myocarditis were essentially indistinguishable from those of vaccinated control subjects, despite a modest increase in cytokine production. A notable finding was that markedly elevated levels of full-length spike protein (33.9±22.4 pg/mL), unbound by antibodies, were detected in the plasma of individuals with postvaccine myocarditis, whereas no free spike was detected in asymptomatic vaccinated control subjects (unpaired *t* test; $P<0.0001$).

CONCLUSIONS: Immunoprofiling of vaccinated adolescents and young adults revealed that the mRNA vaccine–induced immune responses did not differ between individuals who developed myocarditis and individuals who did not. However, free spike antigen was detected in the blood of adolescents and young adults who developed post-mRNA vaccine myocarditis, advancing insight into its potential underlying cause.

Circulation FIRST PROOF ONLY

Key Words: COVID-19 ■ mRNA vaccine ■ myocarditis ■ spike protein, SARS-CoV-2

Editorial, see p XXX

Within 9 months of coronavirus disease 2019 (COVID-19) reaching pandemic proportions, novel, lifesaving severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)–targeted mRNA vaccine technologies received emergency regulatory approvals and were distributed to many developed countries. Following a tiered approach that prioritized high-risk individuals, infants ≥6 months of age are now eligible for vaccination with SARS-CoV-2 mRNA vaccines in select countries. SARS-CoV-2 mRNA vaccines

Correspondence to: Lael M. Yonker, MD, Massachusetts General Hospital, 55 Fruit St, Jackson 14, Boston, MA 02114. Email ; or David R. Walt, PhD, Brigham and Women's Hospital, 60 Fenwood Rd, Boston, MA 02115. Email

*L.M. Yonker, Z. Swank, and Y.C. Bartsch contributed equally.

†A. Fasano and D.R. Walt contributed equally.

Supplemental Material is available at

For Sources of Funding and Disclosures, see page XXX

© 2023 American Heart Association, Inc.

Circulation is available at

Downloaded from by on January 12, 2023

Clinical Perspective

What Is New?

- Adolescents and young adults who developed myocarditis after severe respiratory syndrome coronavirus disease 2 (SARS-CoV-2) mRNA vaccination display persistently elevated circulating levels of full-length Spike protein, unbound by antibodies.
- No evidence of autoantibody production, concomitant viral infections, or excessive antibody responses to the anti–SARS-CoV-2 mRNA vaccines was identified in postvaccine myocarditis cases.

What Are the Clinical Implications?

- Understanding the immunopathological mechanisms associated with postvaccine myocarditis will help improve safety and guide the development of future coronavirus disease 2019 (COVID-19) vaccines.
- These results do not alter the risk-benefit ratio favoring vaccination against COVID-19 to prevent severe clinical outcomes.

Nonstandard Abbreviations and Acronyms

COVID-19	coronavirus disease 2019
CRP	C-reactive protein
Ig	immunoglobulin
IL	interleukin
IRB	institutional review board
MIS-C	multisystem inflammatory syndrome in children
RBD	receptor binding protein
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2

have been shown to dramatically reduce disease severity and mortality of COVID-19 in adults[1,2] and children,[3,4] and vaccinated children may be less likely to develop the severe, life-threatening post–COVID-19 complication multisystem inflammatory syndrome in children (MIS-C).[5,6] Rarely, some individuals develop myocarditis after mRNA vaccination. The immune response driving postvaccine myocarditis has not yet been elucidated. Understanding the immunophenotype associated with mRNA vaccine–induced myocarditis is an essential first step in preventing negative complications resulting from this novel vaccine technology.

Roughly 1 to 2 cases per 100 000 individuals have developed myocarditis or pericarditis after mRNA vaccination.[7–9] Previous authors have hypothesized that vaccination may trigger a robust overactive or aberrant innate and acquired immune response in genetically predisposed individuals,[10,11] or given the predilection for male individuals, hormones, specifically testosterone, may alter immune responses, eliciting a more aggressive T helper 1 cell–type immune response.[12] Alternatively, it has been proposed that in certain individuals, vaccination might generate autoantibodies from polyclonal B-cell expansion or immune complex formation and inflammation,[10,11] or potentially the molecular mimicry between the spike protein and self-antigens may result in cardiac-targeted antibodies.[10] One group performed extensive immunoanalysis on a case of post–mRNA-1273 (Moderna) vaccine myocarditis and compared the findings with those from vaccinated control subjects. Although the individual with myocarditis displayed increased natural killer cell subsets, elevated cytokines, and several autoantibodies, a specific signature could not be identified.[13] Thus, the cause of post–SARS-CoV-2 mRNA vaccine myocarditis remains unclear. Understanding the immunopathological mechanisms driving postvaccine myocarditis could help improve safety and guide the development of future COVID-19 vaccines.

In this study, blood samples were collected for immunophenotyping from 61 adolescents and young adults who either developed myocarditis (n=) had no vaccine-related complications after vaccination with either the Pfizer BNT162b2 or Moderna mRNA-1273 COVID-19 mRNA vaccine. We performed extensive humoral profiling, quantified and profiled SARS-CoV-2–specific T-cell responses, and measured cytokines and SARS-CoV-2 antigens in the collected plasma samples.

Circulation FIRST PROOF ONLY

METHODS

Patient Enrollment and Sample Collection

Adolescents or young adults presenting with myocarditis after SARS-CoV-2 mRNA vaccination, along with healthy, vaccinated control subjects and children with MIS-C, were enrolled in the institutional review board (IRB)–approved Pediatric COVID-19 Biorepository at Massachusetts General Hospital (Mass General Brigham IRB No. 2020P0000955)[14] or the IRB-approved Taking on COVID-19 Together Biorepository at Boston Children's Hospital (BCH IRB No. P00035409). All participants provided written or verbal consent before participation in accordance with the IRB-approved protocols.

The Centers for Disease Control and Prevention definition for postvaccine myocarditis was used; both confirmed and probable cases were included.[15] Healthy vaccinated, control adults (>18 years of age) were enrolled in a specimen collection study conducted at Brigham and Women's Hospital.[16] All studies were IRB approved. Samples were collected and processed as outlined in Figure S1.

Immunophenotyping

In brief, analysis of plasma included anti–SARS-CoV-2 antibody profiling, comprehensive serological profiling for autoantibodies and antibodies against previous infections, SARS-CoV-2 spike

Downloaded from by on January 12, 2023

protein–specific T-cell responses, cytokine analysis and hematological profiling, and testing for circulating SARS-CoV-2 antigens. The data that support the findings of this study are available from the corresponding author on reasonable request. In-depth descriptions of assays used for single and longitudinal time points are detailed in the Supplemental Methods.

Statistical Analysis

For serological, cytokine, and antigen comparisons between the myocarditis and control groups, we used the 2-sided nonparametric Mann-Whitney *U* test. The Benjamini-Hochberg method was used to correct for multiple *P* values in the serological assays. For T-cell and hematological comparison, unpaired *t* tests were used to analyze comparisons between the myocarditis and control groups.

RESULTS

Sixty-one adolescents and young adults between 12 and 21 years of age, including 16 individuals with vaccine-associated myocarditis, provided a blood sample for analysis (Figure 1). Demographics are described in the Table 1. The majority of individuals with postvaccine myocarditis were male (n=13 of 16; 81%), and symptom onset typically occurred within the first week after vaccination (median, 4 days; range, 1–19 days). In the postvaccine myocarditis cohort, most of the individuals (n=12 of 16; 75%) developed myocarditis after the second dose, although 2 experienced symptoms of myocarditis after the first dose and 2 after the third booster dose. All patients presented with chest pain, and all were found to have elevated cardiac troponin T (median, 260 ng/L; interquartile range, 215–1114 ng/L; upper limit of normal, 14 ng/L) and C-reactive protein (CRP) levels (median, 29.75 mg/L; interquartile range, 15.53–50.58 mg/L; upper limit of normal, 8 mg/L; Table S1). Samples were also collected from 45 age-matched asymptomatic vaccinated control subjects up to 3 weeks after the second mRNA vaccination.

Given the desired goal of humoral protection against SARS-CoV-2 infection, serological responses were compared between the individuals who developed postvaccine myocarditis and age-matched, asymptomatic vaccinated control subjects. Because antibody responses are dynamic over time, only samples collected within 11 days after the second vaccine dose were analyzed to allow a more evenly matched comparison of antibody responses (myocarditis cohort, n=10; healthy vaccinated control subjects, n=17). In this subgroup, no differences were seen in anti-spike or anti-RBD (anti-receptor binding protein) immunoglobulin (Ig) M, IgG, or IgA levels (Figure 2A and 2B). To more deeply assess potential qualitative differences in the serological responses associated with postvaccine myocarditis, we evaluated the ability of the antibody to engage Fc receptors and additional Fc effector functions. No overall difference in binding to FcγR2a, FcγR2b, FcγR3a, or FcγR3b was observed (Figure 2C). Consequently, opsonophagocytic and complement-activating potentials were comparable between the groups (Figure 2D). Collectively, these data indicate that individuals who developed myocarditis have a humoral

Circulation

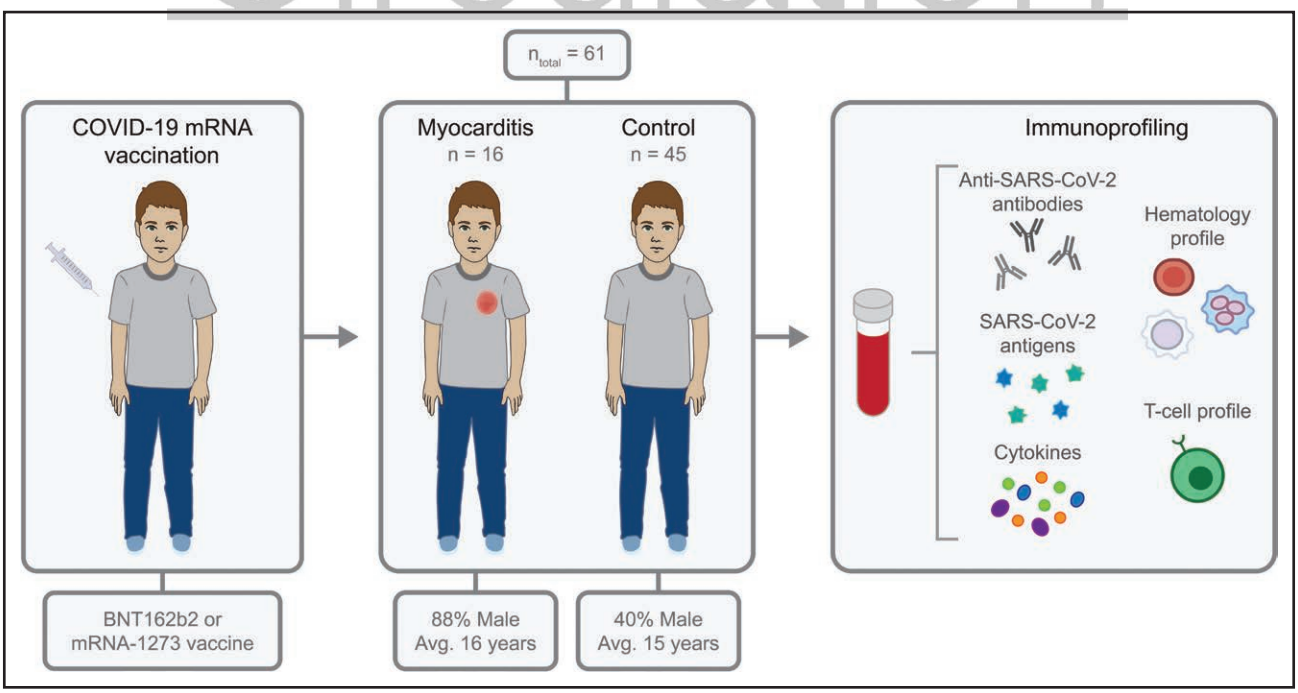

Figure 1. Study overview.
After they received a coronavirus disease 2019 (COVID-19) mRNA vaccination, blood samples were collected from adolescents and young adults who developed myocarditis or who had no vaccine-related complications. The concentrations of anti–severe respiratory syndrome coronavirus disease 2 (SARS-CoV-2) antibodies, SARS-CoV-2 antigens, and cytokines were measured, and hematology and T-cell profiling was performed with the collected blood samples.

Downloaded from by on January 12, 2023

Table 1. Demographics of Adolescents and Young Adults Who Developed Myocarditis After COVID-19 Vaccination and Pediatric Vaccinated Control Subjects

Patient characteristics	Postvaccine myocarditis (n=16)	Vaccinated control subjects (n=45)
Age at enrollment, average (minimum, maximum), y	16 (12, 21)	15 (12, 20)
Male, n (%)	13 (81)	18 (40)
Race, n (%)		
Asian	2 (13)	4 (9)
Black	0 (0)	1 (2)
White	11 (69)	23 (51)
Other	2 (13)	10 (22)
Unknown	1 (6)	7 (16)
Hispanic, n (%)	5 (31)	9 (20)
Time between vaccination and sample collection, median (minimum, maximum), d	4 (1, 19)	14 (4, 21)

COVID-19 indicates coronavirus disease 2019.

immune response comparable to that of asymptomatic adolescents and young adults (Figure 2E). We found no indication that a specific antibody response is associated with myocarditis, but instead, all adolescents and young adults mounted a substantial immune response, conferring protection against SARS-CoV-2 after vaccination.

In addition to vaccine-related responses, we looked more broadly at the antibody response in a subset of patients and vaccinated control subjects by performing phage immunoprecipitation sequencing for self-antibodies (Figure 2F) and a phage immunoprecipitation sequencing–based VirScan, a phage display approach for in-depth characterization of antibodies to the human relevant virome (Figure 2G). No significant levels of self-antibodies were noted in the myocarditis group compared with the healthy vaccinated control group. Strong antibody responses to common pathogens, including other respiratory viruses (respiratory syncytial virus, influenza, and β-coronaviruses), herpes viruses (herpes simplex virus type 1, cytomegalovirus, and Epstein-Barr virus), and vaccine strains (measles, rubella, and mumps), were detected. However, no specific difference tracking with the development of myocarditis stood out in this limited cohort.

We then sought to characterize T-cell responses by multiparameter flow cytometry. We first compared the distribution of the different memory subsets, including all $CD4^+$ and $CD8^+$ T cells, between a subset of individuals who developed postvaccine myocarditis and asymptomatic vaccinated control subjects using age- and sex-matched samples with comparable time from vaccination. Overall, there were no differences in the frequencies of naive ($CD45RA^+CCR7^+$), central memory ($CD45RA^-CCR7^+$), effector memory ($CD45RA^-CCR7^-$), and terminally differentiated effector memory ($CD45RA^+CCR7^-$) T cells between the 2 groups except that individuals with myocarditis had slightly higher frequencies of effector memory cells (*t* test, *P*=0.047; Figure S2A and S2B). We also did not see a difference in the proportions of memory subsets in SARS-CoV-2 spike–specific $CD4^+$ T cells (Figure S2C). Furthermore, the frequencies of interferon-γ secreting and degranulating, as determined by CD107a expression, $CD8^+$ T cells, and $CD4^+$ T cells on stimulation with SARS-CoV-2 spike peptides, were indistinguishable between both groups (Figure S2D–S2G). The only noticeable difference in the T-cell signature was a higher frequency of PD-1–expressing bulk $CD4^+$ T cells in the individuals with postvaccine myocarditis (unpaired *t* test, *P*=0.02; Figure S2H and S2I), likely reflecting variability in expansion after immunization but potentially also suggesting a higher level of exhaustion in this cell subset.

Although minimal differences were seen in the T-cell populations, individuals with myocarditis displayed distinct cytokine profiles reminiscent of the profile seen in MIS-C,[17–19] suggesting likely innate inflammatory activation, with significantly elevated levels of interleukin (IL)-8, IL-6, tumor necrosis factor-α, IL-10, interferon-γ, and IL-1β and lower IL-4 levels compared with healthy vaccinated control subjects (Figure 3A–3G) with no notable differences in IL-5, IL-12p70, or IL-22 (Figure 3H–3K). To further characterize the inflammatory profile, complete blood counts were analyzed. Although mostly within normal ranges in both cohorts, total leukocytes, specifically neutrophils, were significantly increased in individuals with postvaccine myocarditis compared with vaccinated control subjects (unpaired *t* test, *P*=0.007 and *P*=0.01, respectively), whereas platelet counts were decreased compared with vaccinated control subjects (unpaired *t* test, *P*=0.03; Figure S3). These results suggest that postvaccine myocarditis is associated with normal adaptive and T-cell immunity but modest innate activation.

To further characterize immune responses and to assess for potential stimuli of innate inflammation, ultrasensitive single-molecule array antigen assays were used to measure the vaccine-stimulated production of both the full-length SARS-CoV-2 spike protein and its cleaved subunit, S1, in the blood after intramuscular vaccination. Given that S1 antigen levels were seen to drop quickly in a healthy adult cohort, we sought to assess the clearance of S1 in our adolescent cohorts. As seen in adults, in analyses of adolescent plasma samples, which were collected primarily after their second vaccination, freely circulating S1 was not detected in most of the samples (Figure 4A).

We then sought to determine whether S1 was undetectable because the antigen had been effectively cleared or because the antigen was bound and masked by circulating antibodies. To release antibody-bound

Downloaded from by on January 12, 2023

Circulation
American Heart Association.
FOR PERSONAL USE ONLY

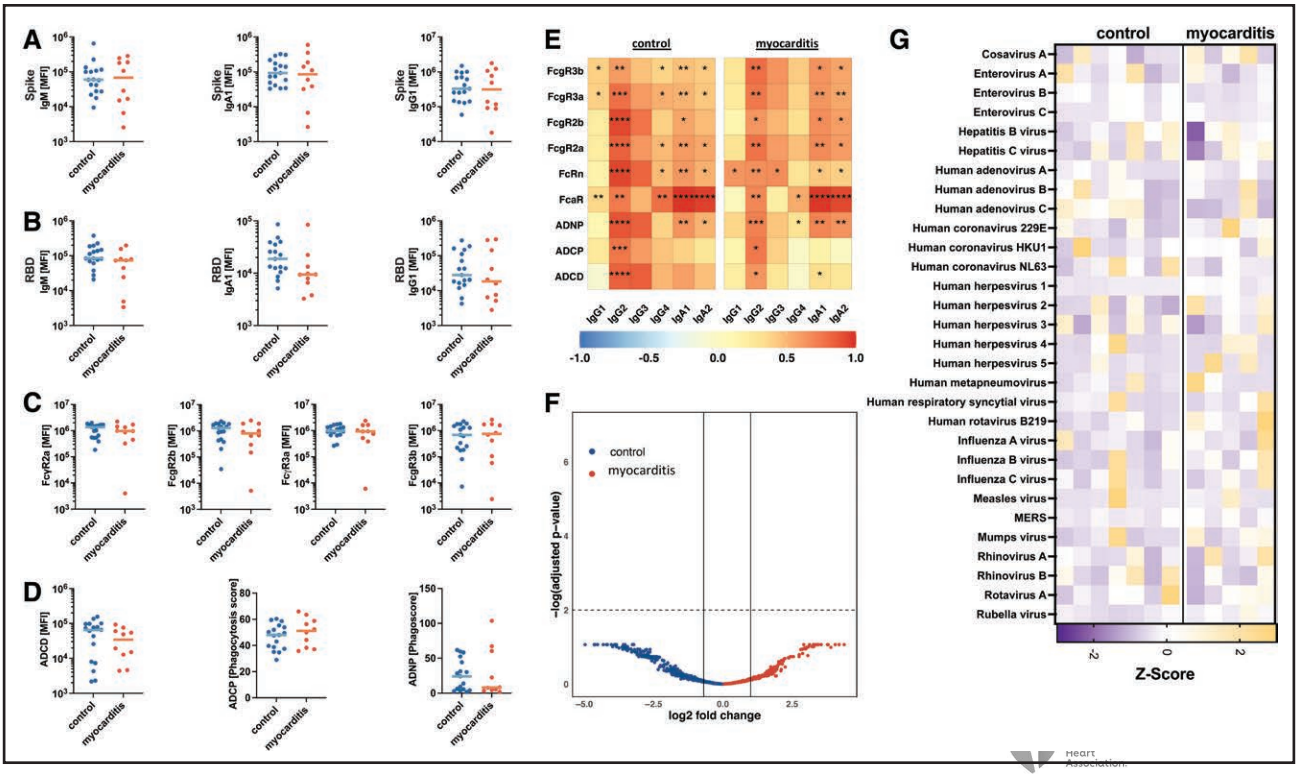

Figure 2. Humoral responses.

Humoral responses of adolescents and young adults with postvaccine myocarditis (n=10) compared with age-matched postvaccine control subjects (n=17). **A** and **B**, Immunoglobulin (Ig) M/G/A to spike and RBD (receptor binding protein); (**C**) Fc receptors to Spike; (**D**) antibody-dependent complement deposition (ADCD), neutrophil phagocytosis (ADNP), and cellular phagocytosis (ADCP); (**E**) correlation heat map; (**F**) phage immunoprecipitation sequencing; and (**G**) heat map of VirScan. **A** through **E** include only samples that were collected within 11 days after the second vaccination dose to allow time-equivalent comparison of severe respiratory syndrome coronavirus disease 2 (SARS-CoV-2)–specific responses. **F** and **G** include 5 individuals with postvaccine myocarditis and 7 healthy vaccinated control subjects. Analysis by Mann-Whitney *U* map. **P*<0.5. ***P*<0.01. ****P*<0.001. *****P*<0.0001.

Circulation

FIRST PUBLISHED ONLY

antigen, we first treated plasma samples with dithiothreitol to denature the antibodies. After incubation with dithiothreitol, S1 was detected in plasma of 34% of the vaccinated control cohort and 29% of the myocarditis cohort. We repeated this analysis for the adult cohort, but S1 antigen remained undetectable in adults after the second vaccine dose (Figure S4). Although the adult cohort is small (n=13), these findings may suggest an age-related difference in the processing of the mRNA vaccine. Regardless, the frequency with which we could detect S1, as well as the similar mean concentrations between the 2 adolescent cohorts, the findings are therefore unlikely associated with the development of postvaccine myocarditis.

In notable contrast, adolescents who developed myocarditis had markedly higher levels of free full-length spike protein in their plasma (33.9±22.4 pg/mL), unbound by antibodies (Figure 4A), whereas asymptomatic vaccinated control subjects had no detectable free spike protein (unpaired *t* test, *P*<0.0001). Although postvaccine myocarditis clinically occurs more commonly in males, elevated spike was seen equally in both affected females and males (Figure S5). We found a minimal increase in spike levels after dithiothreitol treatment, suggesting that most of the antigen is freely circulating and unbound by antibodies in the individuals with postvaccine myocarditis. Antibody-bound spike was detected in only ≈6% of the vaccinated control subjects. Similarly, we were not able to detect any free or antibody-bound spike in the healthy adult samples (Figure S4).

When analyzed according to time since vaccination, free S1, which was detected in only one patient with postvaccine myocarditis and one vaccinated control subject, was detected only within the first week. However, antibody-bound S1, which was detected in roughly one-third of both cohorts, could be detected up to 3 weeks after vaccination (Figure 4B). In contrast, both free and antibody-bound spike, which was detectable only in patients who developed vaccine-induced myocarditis, remained detectable up to 3 weeks after vaccination (Figure 4B). Longitudinal sampling displays a slow decline in both free and antibody-bound spike, suggesting that collecting blood at only a single time point was unlikely to miss circulating spike in healthy vaccinated control subjects. To assess whether this persistence of spike in patients with myocarditis was attributable to inadequate antibody neutralization, we

Downloaded from

by on January 12, 2023

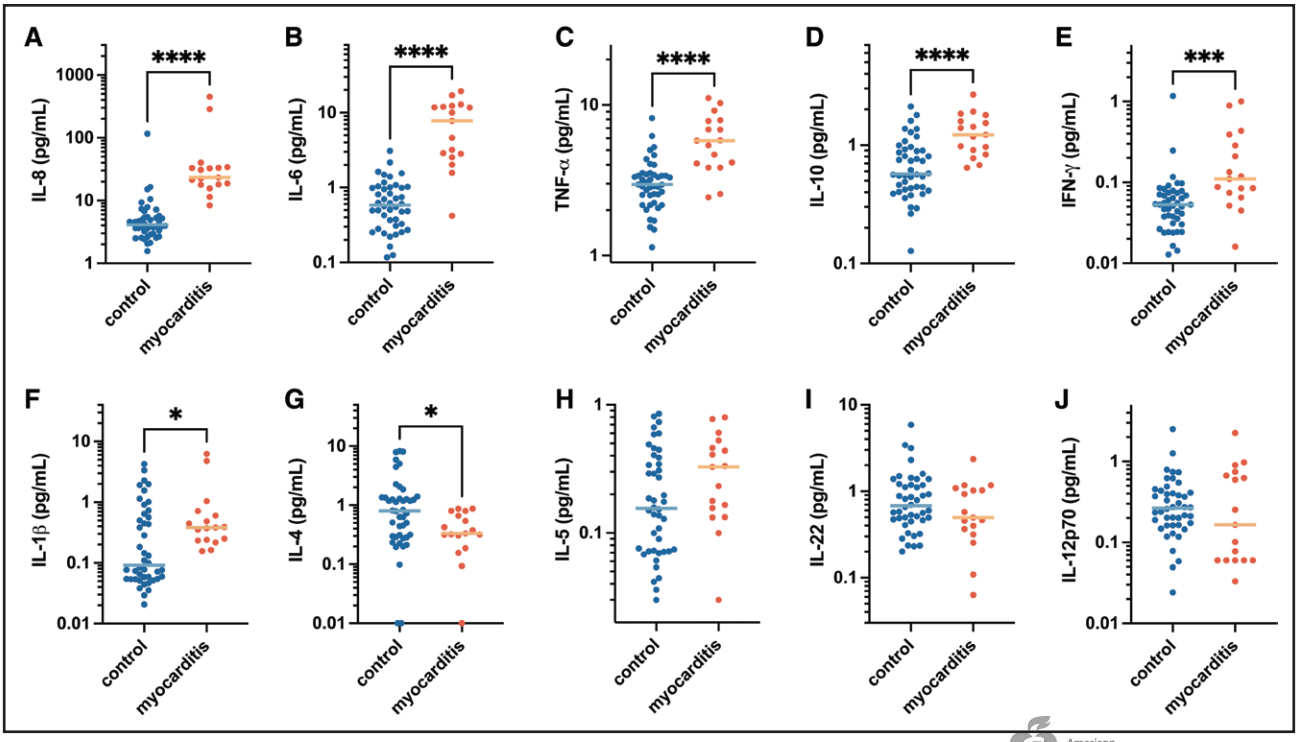

Figure 3. Cytokine profiles.

The concentration of cytokines detected in the plasma of adolescents with postvaccine myocarditis (n=16) compared with age-matched postvaccine control subjects (n=44). Plots are shown in order of decreasing significance between the mean values of each group for the following cytokines: (**A**) interleukin (IL)-6; (**B**) IL-8; (**C**) tumor necrosis factor (TNF)-α; (**D**) IL-10; (**E**) interferon (IFN)-γ; (**F**) IL-1β; (**G**) IL-4; (**H**) IL-5; (**I**) IL-22; and (**J**) IL-12p70. All data represent mean values for duplicate measurements. Significance was calculated with the Mann-Whitney *U* test. **P*<0.05. ***P*<0.01. ****P*<0.001. *****P*< 0.0001.

American Heart Association.

Circulation
FIRST PROOF ONLY

carried out in vitro single-molecule array neutralization assays.[20] We analyzed plasma samples from a subset of patients with myocarditis (n=9) and healthy vaccinated control subjects (n=14) for whom plasma samples were collected within 1 week after the second vaccine dose; however, no significant differences in antibody neutralization capacities were observed (Figure S6).

The persistence of circulating spike in patients with postvaccine myocarditis is similar to the SARS-CoV-2 antigenemia previously reported to be a pathogenic feature of MIS-C.[18,21] For that reason, we compared S1, spike, cardiac troponin T, and CRP levels between individuals who developed myocarditis and those who developed MIS-C with cardiac complications (Figure 5). There were no significant differences in the mean S1 and spike concentrations between the 2 groups. Although both MIS-C and postvaccine myocarditis resulted in elevated cardiac troponin T and CRP compared with healthy vaccinated control subjects, when MIS-C was compared with postvaccine myocarditis, cardiac troponin T levels were significantly elevated for the myocarditis cohort, and CRP levels were significantly elevated for the MIS-C cohort.

For a subset of patients with postvaccine myocarditis (n=4) from whom we obtained repeat blood sampling, we generated longitudinal immunoprofiles, including cardiac troponin T, S1, spike, anti–SARS-CoV-2 antibody, and cytokine levels (Figure S7). Although spike levels can remain elevated for several days to weeks for all patients, any detected S1 was generally cleared. In all 4 patients, anti-N IgG was undetectable, suggesting that natural infection with SARS-CoV-2 was unlikely a contributing factor. In contrast, anti-spike, anti-S1, and anti-RBD IgG, IgA, and IgM levels increased as expected after vaccination. Patients 1 through 3 developed myocarditis after the second vaccine dose; patient 4 developed myocarditis after the first dose. Of the cytokines, IL-8 was most prominent, mirroring cardiac troponin T and antigen levels most closely.

DISCUSSION

Although epidemiological reports describe key clinical features associated with myocarditis after vaccination with BNT162b2 or mRNA-1273,[9,22] here, we provide in-depth immunoprofiling of patients with postvaccine myocarditis. We discovered that individuals who developed postvaccine myocarditis uniquely exhibit elevated levels of free spike protein in circulation, unbound by anti-spike antibodies, which appear to correlate with

Downloaded from by on January 12, 2023

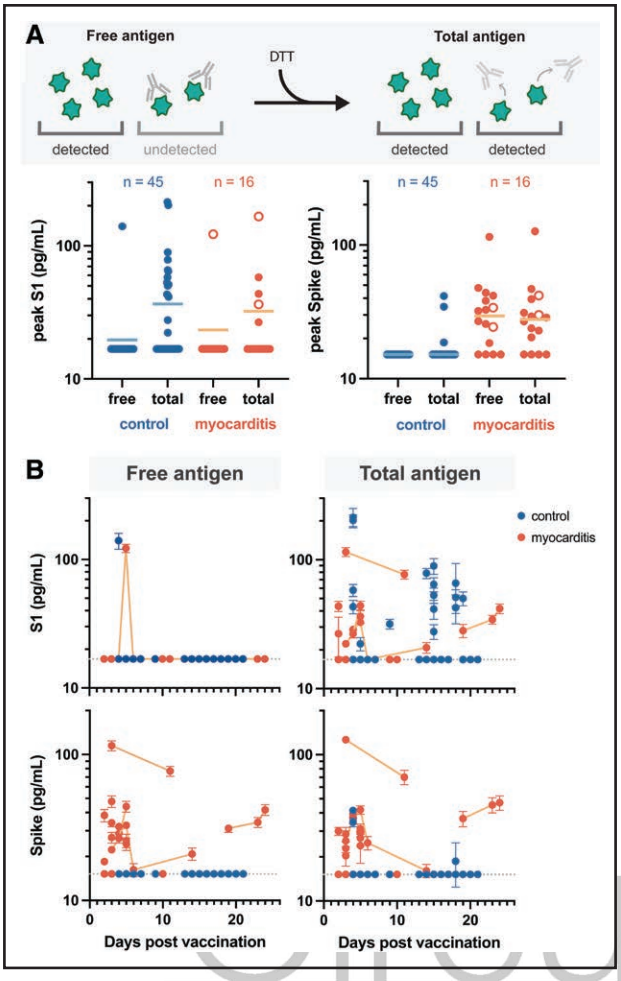

Figure 4. Circulating SARS-CoV-2 antigen.
Free and total S1 and spike levels (**A**) measured in the plasma of the vaccinated control (n=44) and myocarditis (n=16) cohorts. Antigen data corresponding to all individuals who received the BNT162b2 vaccine (●; n=59) and those who received the Moderna vaccine (○; n=2). Free antigen is measured by directly diluting patient plasma, whereas total antigen is measured after treating the plasma with dithiothreitol (DTT) to denature any antigen-bound antibodies. Free and total antigen levels vs days after vaccination (**B**). Longitudinal measurements associated with the same myocarditis patient are connected with lines. All data points represent mean values for duplicate measurements. Dashed gray lines indicate the limit of detection for each assay. SARS-CoV-2 indicates severe acute respiratory syndrome coronavirus 2.

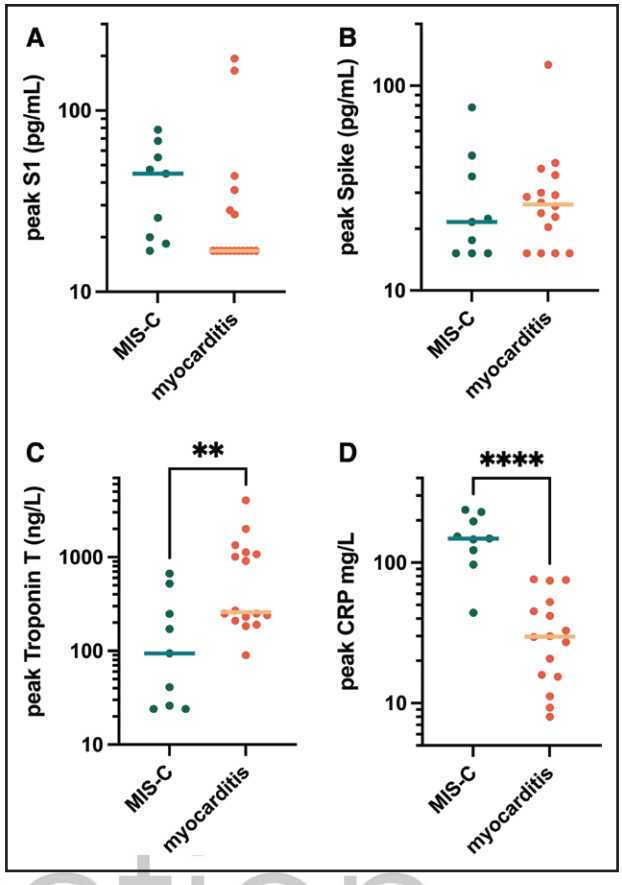

Figure 5. MIS-C vs postvaccine myocarditis.
S1 (**A**), spike (**B**), cardiac troponin T (**C**), and C-reactive protein (CRP; **D**) levels detected in the blood samples of individuals with multisystem inflammatory syndrome in children (MIS-C; n=9) vs those with postvaccine myocarditis (n=17). All data points represent mean values for duplicate measurements. Significance was calculated with the Mann-Whitney *U* test. $^{**}P<0.01$. $^{****}P<0.0001$.

cardiac troponin T levels and innate immune activation with cytokine release. However, adaptive immunity and T-cell responses were essentially indistinguishable from those of asymptomatic age-matched vaccinated control subjects. The postvaccine myocarditis immunoprofile is distinct, however, from acute SARS-CoV-2 infection and the delayed postinflammatory illness MIS-C. Although these findings might provide insight into the immunophenotype of vaccine-related myocarditis, they do not alter the risk-benefit ratio strongly favoring vaccination[10] to protect against severe COVID-19–related complications.

It is notable that spike, which remained intact by evading cleavage and clearance, was associated with myocarditis in this cohort. Whether the circulating spike protein in the setting of mRNA vaccination was pathogenic is unclear. In postvaccine myocarditis, the spike protein appears to evade antibody recognition because the anti-spike antibodies that are generated are produced in adequate quantities with normal functional and neutralization capacity. There is growing in vitro evidence that spike itself can stimulate cardiac pericytes dysfunction[23] or inflame the endothelium, potentially by downregulating angiotensin-converting enzyme 2 expression, by impairing endothelial nitric oxide bioavailability,[24] or by activating integrin-mediated inflammation with hyperpermeability of the endothelial cell layer.[25] Thus, the spike antigen itself, which evades antibody recognition rather than invoking immune hyperactivation, may contribute to myocarditis in these individuals.

T-cell subsets have been implicated in other forms of myocarditis, such as autoimmune myocarditis and dilated cardiomyopathy.[26] Although we did not observe any

Downloaded from by on January 12, 2023

Circulation PROOF ONLY

difference in SARS-CoV-2–specific CD4+ and CD8+ T cells, nor did in vitro stimulation reveal any differences in interferon-γ response between the 2 cohorts, we cannot rule out T-cell involvement on the basis of this analysis alone. However, a slight increase in PD-1–expressing bulk CD4+ T cells in postvaccine myocarditis may indicate some degree of T-cell activation or exhaustion, although it appears distinct from SARS-CoV-2–specific responses. In addition, although we did not detect any autoantibodies as has been seen in other forms of myocarditis, this does not necessarily rule out the potential for T-cell involvement. However, our data suggest that there is a potentially unique mechanism of myocardial injury after SARS-CoV-2 mRNA vaccination associated with a robust innate immune response. Future studies are needed to fully investigate the contribution of T cells in postvaccine myocarditis.

Vaccine-related myocarditis is not unique to the mRNA vaccines; myocarditis can also occur after other vaccines,[27,28] including non–mRNA COVID-19 vaccines, such as influenza and smallpox vaccines. Therefore, it is possible that circulating spike is a biomarker of immune dysregulation leading to myocarditis rather than a causal agent. However, we discovered distinct differences in how adolescents respond to mRNA vaccination compared with adults, which warrant further investigation. It has previously been shown that after the first inoculation of the mRNA-1273 vaccine, the cleaved S1 subunit of spike can be detected in the plasma of healthy adults.[16] However, after the second dose, no antigen was detected,[16] presumably because there are higher levels of circulating anti–SARS-CoV-2 antibodies, which quickly bind any circulating antigen, facilitating its clearance. In contrast, one-third of the adolescents displayed antibody-bound S1 antigenemia after the second vaccination, regardless of the development of myocarditis, a finding not seen in our smaller sample of adults. This suggests that either the immune system of adults responds more quickly to the vaccine-induced production of spike or, because of differences in body mass, the levels of S1 fall below the limit of detection for adults. Alternatively, increased levels of free spike compared with free S1 may be attributable to differences in renal clearance rates; S1 would be expected to clear faster with a molecular weight of 76 kDa, approximately half that of full spike (180 kDa). Because both adults and the adolescents included in our cohort received adult dosing of the mRNA vaccine, this finding suggests an age-related capacity for handling vaccine-introduced antigen. It is important to note that the majority of circulating S1 was bound by specific anti-S1 antibodies, indicating an appropriate immune response for targeting and clearing S1.

Identification of SARS-CoV-2 viral particles in the blood is not unique to postvaccine myocarditis. However, when antigenemia is detected in severe COVID-19[29] or MIS-C,[18,21] a more pronounced hyperinflammatory, superantigen-like response is typically seen, with spike immune complexes triggering hyperactivation of monocyte phagocytosis[30] and neutrophil extracellular trap formation.[31] However, in vaccine-induced myocarditis, spike does not appear to be bound to antibodies, and there is no evidence of excessive immune complex activation of neutrophils, complement, or monocytes. In addition, CRP levels are markedly elevated in MIS-C compared with postvaccine myocarditis.[32] This distinction in hyperinflammation may result from the source of antigenemia. In vaccine-associated myocarditis, antigenemia results after sterile intramuscular inoculation with the liposomally protected mRNA transcript. In contrast, in MIS-C, antigens may leak into the circulation as a result of dysbiosis in the gut, which signals the zonulin-mediated loss of tight junctions, as evidenced by elevated zonulin levels, lipopolysaccharide-binding protein, and soluble CD14.[18] It is also possible that associated endotoxemia exacerbates the inflammatory response against SARS-CoV-2 antigens, accounting for the severe hyperinflammatory response characterizing MIS-C. Furthermore, in MIS-C, the superantigen-like motif[33] of spike/S1 engages with the immune system to produce T-cell receptor skewing[34] and a profound hyperinflammatory response[35]; in postvaccine myocarditis, the circulating free spike antigen appears to evade antibody recognition. Correspondingly, post-mRNA vaccine myocarditis follows a more subdued acute course,[8,36] but long-term outcomes remain to be seen.

Limitations of this study include the relatively small sample size because postvaccine myocarditis is a rare complication, with ≈18 cases occurring for every 1 million vaccine doses administered. Our cohorts were not evenly balanced between the BNT162b2 and mRNA-1273 vaccines: all of our adolescent control cohort and the majority of our myocarditis cohort received the BNT162b2 vaccine (n=15). Furthermore, a more extensive analysis of T-cell subsets, including Th17 cells, which have previously been implicated in non–vaccine-induced myocarditis/dilated cardiomyopathy,[26] or cardiac biopsy tissue analysis would be informative in the future. Nonetheless, our multicenter collaboration allowed meaningful analysis of a considerably sized postvaccine myocarditis cohort. In addition, ultrasensitive antigen detection through single-molecule array[29] does not distinguish whether spike antigenemia is the cause or consequence, and not all patients with myocarditis had detectable antigenemia. Rather, immunophenotyping lays important groundwork to further understand mRNA vaccine–associated complications and provides rationale for future research to aid in vaccine design and dose. These findings also suggest that administration of anti-spike antibodies, if spike antigenemia is detected, could potentially prevent or reverse postvaccine myocarditis.

Downloaded from by on January 12, 2023

Circulation FIRST PROOF ONLY

It is reassuring that neither the BNT162b2 vaccine nor the mRNA-1273 vaccine induces abnormal adaptive immunity or T-cell responses associated with immune activation targeting the myocardium. However, we detected free spike antigen in the blood of adolescents and young adults who developed post-mRNA vaccine myocarditis. Although the implications of this finding must be better understood, these results do not alter the risk-benefit ratio favoring vaccination against COVID-19 to prevent severe clinical outcomes.

ARTICLE INFORMATION

Received May 26, 2022; accepted November23, 2022.

Affiliations

Mucosal Immunology and Biology Research Center (L.M.Y., M.D.B., A.K., B.P.B., J.P.D., M.L., A.F.), Department of Pediatrics (L.M.Y., M.D.B., A.K., B.P.B., J.P.D., M.L., D.B., M.L.-R., A.F.), Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology (A.G.E.), Vincent Center for Reproductive Biology (A.G.E.), and Department of Medicine (B.J.), Division of Infectious Disease, Massachusetts General Hospital, Boston. Harvard Medical School, Boston, MA (L.M.Y., Z.S., Y.C.B., B.P.B., T.N., Y.S., C.-A.C., J.C., A.D., D.B., M.L.-R., B.J., A.G.R., G.A., A.F., D.R.W.). Department of Pathology, Brigham and Women's Hospital, Boston, MA (Z.S., Y.S., C.-A.C., D.R.W.). Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, MA (Z.S., Y.S., C.-A.C., D.R.W.). Ragon Institute of MGH, MIT and Harvard, Cambridge, MA (Y.C.B., E.B., B.J., G.A.). Department of Anesthesiology, Critical Care and Pain Medicine (T.N., A.G.R.), Department of Pediatrics, Division of Immunology (J.C.), and Department of Cardiology (A.D.), Boston Children's Hospital, MA. Department of Pediatrics, Division of Infectious Diseases and Immunology, Infectious and Immunologic Diseases Research Center, and Department of Biomedical Sciences, Cedars-Sinai Medical Center, Los Angeles, CA (M.A.).

Acknowledgments

The authors thank the children and families who participated in this research. They thank Jeni Melo, BS, of Boston Children's Hospital for her assistance in collecting clinical data. Conceptualization and methodology: L.M.Y., Z.S., Y.C.B., G.A., A.F., D.R.W. Investigation: L.M.Y., Z.S., Y.C.B., M.B., A.K., J.P.D., M.L., B.P.B., T.N., Y.S., C.-A.C., B.J., A.G.R., G.A., A.F., D.R.W. Formal analysis and interpretation: L.M.Y., Z.S., Y.C.B., C.-A.C., A.G.E., J.C., A.D., D.B., M.L.-R., M.A., B.J., A.G.R., G.A., A.F., D.R.W. Writing: L.M.Y., Z.S., Y.C.B., G.A., A.F., D.R.W. Writing—review and editing and supervision: L.M.Y., Z.S., Y.C.B., C.-A.C., A.G.E., J.C., A.D., D.B., M.L.-R., M.A., B.J., A.G.R., G.A., A.F., D.R.W.

Sources of Funding

This research was supported by the National Institutes of Health: National Heart, Lung, and Blood Institute (5K08HL143183 to Dr Yonker), the National Institute of Diabetes and Digestive and Kidney Diseases (DK104344 to Dr Fasano), National Institute of Child Health and Human Development (R01HD100022-02S2 to Dr Edlow), and National Institute of Allergy and Infectious Diseases (3R01AI072726-10S1 to Dr Arditi; 3R37AI080289-11S1, R01AI146785 and U19AI42790-01, U19AI135995-02, 1U01CA260476-01, and CIV-IC75N93019C00052 to Dr Alter). They also report funding from the Regione Campania Italy (CUP G58D20000240002-SURF 20004BP000000011 to Dr Fasano), Boston Children's Hospital's Taking on COVID-19 Together Study (to Dr Randolph), and MassGeneral for Children (to Dr Yonker). Funding for the SARS-CoV-2 antigen measurements came from a generous donation from Barbara and Amos Hostetter and the Chleck Foundation. The authors thank Nancy Zimmerman, Mark and Lisa Schwartz, an anonymous donor (financial support), Terry and Susan Ragon, and the Samana Cay Massachusetts General Hospital Research Scholars award for their support. They acknowledge support from the Massachusetts Consortium on Pathogen Readiness, the Musk Foundation, and the Gates Foundation Global Health Vaccine Accelerator Platform funding (OPP1146996 and INV-001650).

Disclosures

Dr Walt has a financial interest in Quanterix Corp, a company that develops an ultrasensitive digital immunoassay platform. He is an inventor of the single-molecule array technology, is a founder of the company, and serves on its board of directors. Dr Walt's interests were reviewed and are managed by Brigham and Women's Hospital and MassGeneral Brigham in accordance with conflict of interest policies. Dr Alter has been employed by Moderna since October 2022; her contributions to this article preceded her employment by Moderna. Dr Alter is also a founder and equity holder of Seromyx Systems, a company developing a platform technology to profile antibody immunity. Drs Julg and Alter are employees and equity holders of Leyden Labs, a company developing pandemic prevention therapeutics. Their interests were reviewed and are managed by Massachusetts General Hospital and MassGeneral Brigham in accordance with their conflict of interest policies. Dr Randolph received funding (to Boston Children's Hospital) from the US Centers for Disease Control and Prevention to study COVID-19 complications in children outside of this work. The other authors report no conflicts.

Supplemental Material

Expanded Methods
Table S1
Figures S1–S11
References 37–42

REFERENCES

1. Polack FP, Thomas SJ, Kitchin N, Absalon J, Gurtman A, Lockhart S, Perez JL, Perez Marc G, Moreira ED, Zerbini C, et al; C4591001 Clinical Trial Group. Safety and efficacy of the BNT162b2 mRNA Covid-19 vaccine. *N Engl J Med*. 2020;383:2603–2615. doi: 10.1056/NEJMoa2034577
2. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, et al. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. *N Engl J Med*. 2021;384:403–416. doi: 10.1056/NEJMoa2035389
3. Olson SM, Newhams MM, Halasa NB, Price AM, Boom JA, Sahni LC, Pannaraj PS, Irby K, Walker TC, Schwartz SP, et al. Effectiveness of BNT162b2 vaccine against critical Covid-19 in adolescents. *N Engl J Med*. 2022;386:713–723. doi: 10.1056/NEJMoa2117995
4. Walter EB, Talaat KR, Sabharwal C, Gurtman A, Lockhart S, Paulsen GC, Barnett ED, Munoz FM, Maldonado Y, Pahud BA, et al; C4591001 Clinical Trial Group. Evaluation of the BNT162b2 Covid-19 vaccine in children 5 to 11 years of age. *N Engl J Med*. 2022;386:35–46. doi: 10.1056/NEJMoa2116298
5. Levy M, Recher M, Hubert H, Javouhey E, Flechelles O, Leteurtre S, Angoulvant F. Multisystem inflammatory syndrome in children by COVID-19 vaccination status of adolescents in France. *JAMA*. 2022;327:281–283. doi: 10.1001/jama.2021.23262
6. Zambrano LD, Newhams MM, Olson SM, Halasa NB, Price AM, Boom JA, Sahni LC, Kamidani S, Tarquinio KM, Maddux AB, et al. Effectiveness of BNT162b2 (Pfizer-BioNTech) mRNA vaccination against multisystem inflammatory syndrome in children among persons aged 12-18 years–United States, July-December 2021. *MMWR Morb Mortal Wkly Rep*. 2022;71:52–58. doi: 10.15585/mmwr.mm7102e1
7. Karlstad O, Hovi P, Husby A, Harkanen T, Selmer RM, Pihlstrom N, Hansen JV, Nohynek H, Gunnes N, Sundstrom A, et al. SARS-CoV-2 vaccination and myocarditis in a Nordic cohort study of 23 million residents. *JAMA Cardiol*. 2022;7:600–612. doi: 10.1001/jamacardio.2022.0583
8. Witberg G, Barda N, Hoss S, Richter I, Wiessman M, Aviv Y, Grinberg T, Auster O, Dagan N, Balicer RD, et al. Myocarditis after Covid-19 vaccination in a large health care organization. *N Engl J Med*. 2021;385:2132–2139. doi: 10.1056/nejmoa2110737
9. Centers for Disease Control and Prevention. Myocarditis and pericarditis after mRNA COVID-19 vaccination. Accessed xxxx. coronavirus/2019-ncov/vaccines/safety/myocarditis.html
10. Bozkurt B, Kamat I, Hotez PJ. Myocarditis with COVID-19 mRNA vaccines. *Circulation*. 2021;144:471–484. doi: 10.1161/circulationaha.121.056135
11. Heymans S, Cooper LT. Myocarditis after COVID-19 mRNA vaccination: clinical observations and potential mechanisms. *Nat Rev Cardiol*. 2022;19:75–77. doi: 10.1038/s41569-021-00662-w
12. Heymans S, Eriksson U, Lehtonen J, Cooper LT Jr. The quest for new approaches in myocarditis and inflammatory cardiomyopathy. *J Am Coll Cardiol*. 2016;68:2348–2364. doi: 10.1016/j.jacc.2016.09.937
13. Muthukumar A, Narasimhan M, Li QZ, Mahimainathan L, Hitto I, Fuda F, Batra K, Jiang X, Zhu C, Schoggins J, et al. In-depth evaluation of a case of presumed myocarditis after the second dose of COVID-19 mRNA vaccine. *Circulation*. 2021;144:487–498. doi: 10.1161/CIRCULATIONAHA.121.056038

Downloaded from by on January 12, 2023

Circulation FIRST PROOF ONLY

14. Lima R, Gootkind EF, De la Flor D, Yockey LJ, Bordt EA, D'Avino P, Ning S, Heath K, Harding K, Zois J, et al. Establishment of a pediatric COVID-19 biorepository: unique considerations and opportunities for studying the impact of the COVID-19 pandemic on children. *BMC Med Res Methodol.* 2020;20:228. doi: 10.1186/s12874-020-01110-y
15. Gargano JW, Wallace M, Hadler SC, Langley G, Su JR, Oster ME, Broder KR, Gee J, Weintraub E, Shimabukuro T, et al. Use of mRNA COVID-19 vaccine after reports of myocarditis among vaccine recipients: update from the Advisory Committee on Immunization Practices–United States, June 2021. *MMWR Morb Mortal Wkly Rep.* 2021;70:977–982. doi: 10.15585/mmwr.mm7027e2
16. Ogata AF, Cheng CA, Desjardins M, Senussi Y, Sherman AC, Powell M, Novack L, Von S, Li X, Baden LR, et al. Circulating severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine antigen detected in the plasma of mRNA-1273 vaccine recipients. *Clin Infect Dis.* 2022;74:715–718. doi: 10.1093/cid/ciab465
17. Diorio C, Henrickson SE, Vella LA, McNerney KO, Chase J, Burudpakdee C, Lee JH, Jasen C, Balamuth F, Barrett DM, et al. Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2. *J Clin Invest.* 2020;130:5967–5975. doi: 10.1172/JCI140970
18. Yonker LM, Gilboa T, Ogata AF, Senussi Y, Lazarovits R, Boribong BP, Bartsch YC, Loiselle M, Rivas MN, Porritt RA, et al. Multisystem inflammatory syndrome in children is driven by zonulin-dependent loss of gut mucosal barrier. *J Clin Invest.* 2021;131:e149633. doi: 10.1172/JCI149633
19. Carter MJ, Fish M, Jennings A, Doores KJ, Wellman P, Seow J, Acors S, Graham C, Timms E, Kenny J, et al. Peripheral immunophenotypes in children with multisystem inflammatory syndrome associated with SARS-CoV-2 infection. *Nat Med.* 2020;26:1701–1707. doi: 10.1038/s41591-020-1054-6
20. Gilboa T, Cohen L, Cheng CA, Lazarovits R, Uwamanzu-Nna A, Han I, Griswold K Jr, Barry N, Thompson DB, Kohman RE, et al. A SARS-CoV-2 neutralization assay using single molecule arrays. *Angew Chem Int Ed Engl.* 2021;60:25966–25972. doi: 10.1002/anie.202110702
21. Sacco K, Castagnoli R, Vakkilainen S, Liu C, Delmonte OM, Oguz C, Kaplan IM, Alehashemi S, Burbelo PD, Bhuyan F, et al. Immunopathological signatures in multisystem inflammatory syndrome in children and pediatric COVID-19. *Nat Med.* 2022;28:1050–1062. doi: 10.1038/s41591-022-01724-3
22. Truong DT, Dionne A, Muniz JC, McHugh KE, Portman MA, Lambert LM, Thacker D, Elias MD, Li JS, Toro-Salazar OH, et al. Clinically suspected myocarditis temporally related to COVID-19 vaccination in adolescents and young adults: suspected myocarditis after COVID-19 vaccination. *Circulation.* 2022;145:345–356. doi: 10.1161/circulationaha.121.056583
23. Avolio E, Carrabba M, Milligan R, Kavanagh Williamson M, Beltrami AP, Gupta K, Elvers KT, Gamez M, Foster RR, Gillespie K, et al. The SARS-CoV-2 Spike protein disrupts human cardiac pericytes function through CD147 receptor-mediated signalling: a potential non-infective mechanism of COVID-19 microvascular disease. *Clin Sci (Lond).* 2021;135:2667–2689. doi: 10.1042/CS20210735
24. Lei Y, Zhang J, Schiavon CR, He M, Chen L, Shen H, Zhang Y, Yin Q, Cho Y, Andrade L, et al. SARS-CoV-2 Spike protein impairs endothelial function via downregulation of ACE 2. *Circ Res.* 2021;128:1323–1326. doi: 10.1161/circresaha.121.318902
25. Robles JP, Zamora M, Adan-Castro E, Siqueiros-Marquez L, Martinez de la Escalera G, Clapp C. The Spike protein of SARS-CoV-2 induces endothelial inflammation through integrin alpha5beta1 and NF-kappaB signaling. *J Biol Chem.* 2022;298:101695. doi: 10.1016/j.jbc.2022.101695
26. Myers JM, Cooper LT, Kem DC, Stavrakis S, Kosanke SD, Shevach EM, Fairweather D, Stoner JA, Cox CJ, Cunningham MW. Cardiac myosin-Th17 responses promote heart failure in human myocarditis. *JCI Insight.* 2016;1:e85851. doi: 10.1172/jci.insight.85851
27. Ling RR, Ramanathan K, Tan FL, Tai BC, Somani J, Fisher D, MacLaren G. Myopericarditis following COVID-19 vaccination and non-COVID-19 vaccination: a systematic review and meta-analysis. *Lancet Respir Med.* 2022;10:679–688. doi: 10.1016/S2213-2600(22)00059-5
28. Neff J, Modlin J, Birkhead GS, Poland G, Robertson RM, Sepkowitz K, Yancy C, Gardner P, Gray GC, Maurer T, et al; Advisory Committee on Immunization Practices and Armed Forces Epidemiological Board. Monitoring the safety of a smallpox vaccination program in the United States: report of the joint Smallpox Vaccine Safety Working Group of the Advisory Committee on Immunization Practices and the Armed Forces Epidemiological Board. *Clin Infect Dis.* 2008;46:S258–S270. doi: 10.1086/524749
29. Ogata AF, Maley AM, Wu C, Gilboa T, Norman M, Lazarovits R, Mao CP, Newton G, Chang M, Nguyen K, et al. Ultra-sensitive serial profiling of SARS-CoV-2 antigens and antibodies in plasma to understand disease progression in COVID-19 patients with severe disease. *Clin Chem.* 2020;66:1562–1572. doi: 10.1093/clinchem/hvaa213
30. Bartsch YC, Wang C, Zohar T, Fischinger S, Atyeo C, Burke JS, Kang J, Edlow AG, Fasano A, Baden LR, et al. Humoral signatures of protective and pathological SARS-CoV-2 infection in children. *Nat Med.* 2021;27:454–462. doi: 10.1038/s41591-021-01263-3
31. Boribong BP, LaSalle TJ, Bartsch YC, Ellett F, Loiselle ME, Davis JP, Gonye ALK, Sykes DB, Hajizadeh S, Kreuzer J, et al. Neutrophil profiles of pediatric COVID-19 and multisystem inflammatory syndrome in children. *Cell Rep Med.* 2022;3:100848. doi: 10.1016/j.xcrm.2022.100848
32. Henderson LA, Canna SW, Friedman KG, Gorelik M, Lapidus SK, Bassiri H, Behrens EM, Ferris A, Kernan KF, Schulert GS, et al. American College of Rheumatology clinical guidance for multisystem inflammatory syndrome in children associated with SARS-CoV-2 and hyperinflammation in pediatric COVID-19: version 2. *Arthritis Rheumatol.* 2021;73:e13–e29. doi: 10.1002/art.41616
33. Cheng MH, Zhang S, Porritt RA, Noval Rivas M, Paschold L, Willscher E, Binder M, Arditi M, Bahar I. Superantigenic character of an insert unique to SARS-CoV-2 Spike supported by skewed TCR repertoire in patients with hyperinflammation. *Proc Natl Acad Sci USA.* 2020;117:25254–25262. doi: 10.1073/pnas.2010722117
34. Porritt RA, Paschold L, Rivas MN, Cheng MH, Yonker LM, Chandnani H, Lopez M, Simnica D, Schultheiss C, Santiskulvong C, et al. HLA class I-associated expansion of TRBV11-2 T cells in multisystem inflammatory syndrome in children. *J Clin Invest.* 2021;131:e146614. doi: 10.1172/JCI146614
35. Porritt RA, Binek A, Paschold L, Rivas MN, McArdle A, Yonker LM, Alter G, Chandnani HK, Lopez M, Fasano A, et al. The autoimmune signature of hyperinflammatory multisystem inflammatory syndrome in children. *J Clin Invest.* 2021;131:e141420. doi: 10.1172/JCI151520
36. Dionne A, Sperotto F, Chamberlain S, Baker AL, Powell AJ, Prakash A, Castellanos DA, Saleeb SF, de Ferranti SD, Newburger JW, et al. Association of myocarditis with BNT162b2 Messenger RNA COVID-19 vaccine in a case series of children. *JAMA Cardiol.* 2021;6:1446–1450. doi: 10.1001/jamacardio.2021.3471
37. Centers for Disease Control and Prevention. Health department-reported cases of multisystem inflammatory syndrome in children (MIS-C) in the United States. Accessed xxxx. tracker/#mis-national-surveillance
38. Norman M, Gilboa T, Ogata AF, Maley AM, Cohen L, Busch EL, Lazarovits R, Mao CP, Cai Y, Zhang J, et al. Ultrasensitive high-resolution profiling of early seroconversion in patients with COVID-19. *Nat Biomed Eng.* 2020;4:1180–1187. doi: 10.1038/s41551-020-00611-x
39. Mina MJ, Kula T, Leng Y, Li M, de Vries RD, Knip M, Siljander H, Rewers M, Choy DF, Wilson MS, et al. Measles virus infection diminishes preexisting antibodies that offer protection from other pathogens. *Science.* 2019;366:599–606. doi: 10.1126/science.aay6485
40. Borducchi EN, Cabral C, Stephenson KE, Liu J, Abbink P, Ng'ang'a D, Nkolola JP, Brinkman AL, Peter L, Lee BC, et al. Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys. *Nature.* 2016;540:284–287. doi: 10.1038/nature20583
41. Keng CT, Zhang A, Shen S, Lip KM, Fielding BC, Tan TH, Chou CF, Loh CB, Wang S, Fu J, et al. Amino acids 1055 to 1192 in the S2 region of severe acute respiratory syndrome coronavirus S protein induce neutralizing antibodies: implications for the development of vaccines and antiviral agents. *J Virol.* 2005;79:3289–3296. doi: 10.1128/JVI.79.6.3289-3296.2005
42. Rivnak AJ, Rissin DM, Kan CW, Song L, Fishburn MW, Piech T, Campbell TG, DuPont DR, Gardel M, Sullivan S, et al. A fully-automated, six-plex single molecule immunoassay for measuring cytokines in blood. *J Immunol Methods.* 2015;424:20–27. doi: 10.1016/j.jim.2015.04.017

Downloaded from by on January 12, 2023

Circulation FIRST PROOF ONLY