CORRESPONDENCE

IL-1RA Antibodies in Myocarditis after SARS-CoV-2 Vaccination

TO THE EDITOR: Myocarditis associated with messenger RNA (mRNA) vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) predominantly affects male adolescents and young male adults (14 to <30 years of age) and typically occurs after receipt of the second vaccine dose.[1,2] In adults with critical coronavirus disease 2019 (Covid-19) and in cases of multisystem inflammatory syndrome in children (MIS-C), we recently discovered neutralizing autoantibodies targeting the endogenous interleukin-1 receptor antagonist (IL-1RA), which inhibits interleukin-1 signaling and inflammation.[3,4]

In this study, we evaluated the prevalence of antibodies neutralizing IL-1RA and progranulin, which inhibits tumor necrosis factor signaling, in 69 patients (14 to 79 years of age) who had clinically suspected myocarditis after SARS-CoV-2 vaccination. A total of 61 patients underwent endomyocardial biopsy.

Myocarditis was confirmed by biopsy in 40 of 61 patients (Fig. 1A). Among patients with histologically confirmed myocarditis, anti–IL-1RA antibodies were found in 9 of 12 patients (75%) younger than 21 years of age, as compared with 3 of 28 patients (11%) 21 years of age or older. Anti–IL-1RA antibodies were not detectable in the 21 patients in whom biopsy ruled out the diagnosis of myocarditis (Fig. 1B and 1C). IL-1RA antibody–positive patients with biopsy-confirmed myocarditis had an early onset of symptoms, which occurred mostly after receipt of the second vaccine dose, and a milder course of myocarditis than patients with biopsy-confirmed myocarditis but without anti–IL-1RA autoantibodies (Tables S1 through S6 and Figs. S1 through S6 in the Supplementary Appendix, available with the full text of this letter at NEJM.org).[1,2]

IL-1RA antibodies were observed in 2 of 214 vaccinated control participants (1%) and in 2 of 125 participants (2%) who had histologically proven myocarditis that had been diagnosed before the Covid-19 pandemic. Previous data that had been obtained from patients with critical Covid-19 did not support the cross-reactivity of purified IL-1RA antibodies with structural proteins of SARS-CoV-2, including the spike protein,[3] which argues against virus- or vaccine-driven molecular mimicry.

Current evidence points toward a transient hyperphosphorylation of IL-1RA preceding a breakdown of peripheral immune tolerance.[3,4] In Western blots of total plasma protein, antibodies to IL-1RA coincided with weaker bands of free IL-1RA, but prominent immune (IgM or IgG)–complexed protein with an atypical IL-1RA isoform occurred exclusively in patients who were seropositive for anti–IL-1RA antibodies (Fig. 1D). This additional IL-1RA isoform was hyperphosphorylated at threonine position 111, which had been observed previously in adult patients with critical Covid-19 and in patients with MIS-C.[3,4] In contrast to our observations in patients with myocarditis after SARS-CoV-2 vaccination, IL-1RA was not hyperphosphorylated in any of the samples that had been obtained from control participants.

At the time of acute myocarditis, the mean (±SD) free IL-1RA plasma level in 15 patients who were seropositive for anti–IL-1RA antibodies was 236±82 pg per milliliter, whereas the level was 1736±312 pg per milliliter in 33 patients without anti–IL-1RA antibodies and 1599±277 pg per milliliter in 21 patients in whom histologic testing ruled out the diagnosis of myocarditis (Fig. 1F). IL-1RA plasma levels correlated with markers of cardiac damage (troponin T, creatine kinase, creatine kinase MB, or pro–B-type natriuretic peptide), cardiac-tissue infiltration of CD3+ T cells and CD68+ macrophages, and systemic inflammation (C-reactive protein). There was a negative correlation between markers of cardiac damage and IL-1RA plasma levels in patients with anti–IL-1RA antibodies (Fig. 1E). Interleukin-1 signaling reporter assay experiments showed direct

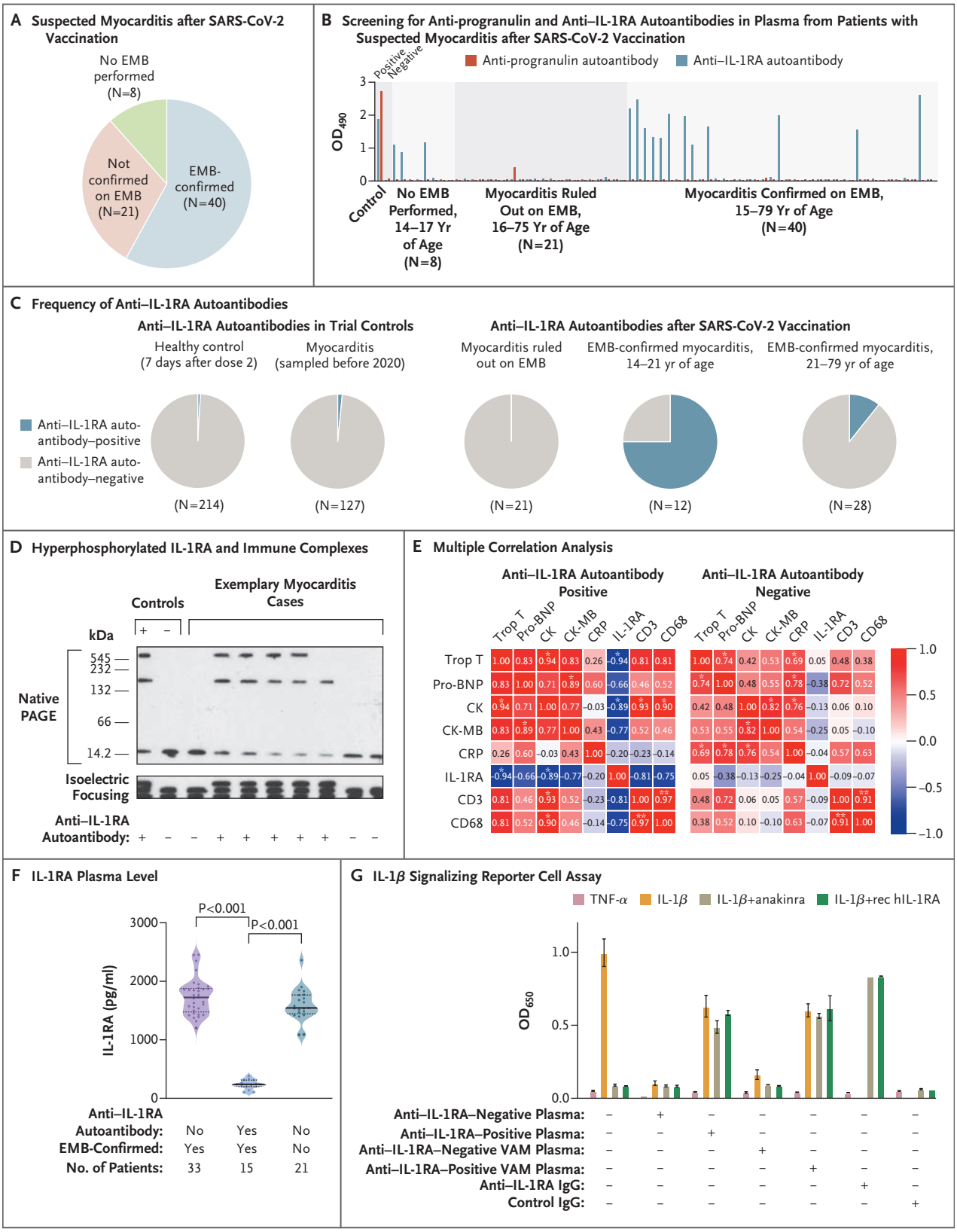
A Suspected Myocarditis after SARS-CoV-2 Vaccination
No EMB performed (N=8)
Not confirmed on EMB (N=21)
EMB-confirmed (N=40)
B Screening for Anti-progranulin and Anti–IL-1RA Autoantibodies in Plasma from Patients with Suspected Myocarditis after SARS-CoV-2 Vaccination
Positive
Negative
Anti-progranulin autoantibody
Anti–IL-1RA autoantibody
OD490
Control
No EMB Performed, 14–17 Yr of Age (N=8)
Myocarditis Ruled Out on EMB, 16–75 Yr of Age (N=21)
Myocarditis Confirmed on EMB, 15–79 Yr of Age (N=40)
C Frequency of Anti–IL-1RA Autoantibodies
Anti–IL-1RA Autoantibodies in Trial Controls
Anti–IL-1RA Autoantibodies after SARS-CoV-2 Vaccination
Healthy control (7 days after dose 2)
Myocarditis (sampled before 2020)
Myocarditis ruled out on EMB
EMB-confirmed myocarditis, 14–21 yr of age
EMB-confirmed myocarditis, 21–79 yr of age
Anti–IL-1RA auto-antibody–positive
Anti–IL-1RA auto-antibody–negative
(N=214)
(N=127)
(N=21)
(N=12)
(N=28)
D Hyperphosphorylated IL-1RA and Immune Complexes
Controls
Exemplary Myocarditis Cases
kDa
545
232
132
66
14.2
Native PAGE
Isoelectric Focusing
Anti–IL-1RA Autoantibody:
E Multiple Correlation Analysis
Anti–IL-1RA Autoantibody Positive
Anti–IL-1RA Autoantibody Negative
Trop T
Pro-BNP
CK
CK-MB
CRP
IL-1RA
CD3
CD68
1.0
0.5
0.0
−0.5
−1.0
F IL-1RA Plasma Level
P<0.001
P<0.001
IL-1RA (pg/ml)
Anti–IL-1RA Autoantibody:
EMB-Confirmed:
No. of Patients:
No Yes No
Yes Yes No
33 15 21
G IL-1β Signaling Reporter Cell Assay
TNF-α
IL-1β
IL-1β+anakinra
IL-1β+rec hIL-1RA
OD650
Anti–IL-1RA–Negative Plasma:
Anti–IL-1RA–Positive Plasma:
Anti–IL-1RA–Negative VAM Plasma:
Anti–IL-1RA–Positive VAM Plasma:
Anti–IL-1RA IgG:
Control IgG:

Figure 1 (facing page). Autoantibodies Targeting IL-1RA in Myocarditis after SARS-CoV-2 Vaccination.

Blood plasma samples were obtained from 69 patients with suspected myocarditis after receipt of vaccine against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). In 61 patients, endomyocardial biopsy (EMB) was performed, and myocarditis was confirmed on EMB in 40 patients (**Panel A**). Plasma samples that were obtained from 8 patients with no confirmatory investigation on EMB, from 21 patients in whom the diagnosis of myocarditis was ruled out, and from 40 patients in whom myocarditis was confirmed on EMB were analyzed for antibodies against endogenous interleukin-1 receptor antagonist (IL-1RA) and progranulin by enzyme-linked immunosorbent assay (ELISA). Data are shown sorted according to the age of the study participants (**Panel B**). OD_{490} denotes optical density as measured at a wavelength of 490 nm. The frequency of anti–IL-1RA antibodies in plasma samples from patients with vaccine-associated myocarditis was confirmed or ruled out on EMB and was sorted according to age. Control participants were 214 healthy adults who had samples obtained 1 week after receipt of the second dose of SARS-CoV-2 vaccine and 127 patients with myocarditis whose samples were obtained before 2020 (**Panel C**).

Western blots of native gradient polyacrylamide gel electrophoresis (PAGE) revealed immune-complexed IL-1RA and weakened bands resembling free IL-1RA (16 kDa) in plasma samples seropositive for anti–IL-1RA. In identical samples, isoelectric focusing of IL-1RA revealed a differentially charged IL-1RA isoform (**Panel D**). Multiple Spearman's correlation analyses were conducted of IL-1RA plasma levels in anti–IL-1RA–positive patients (left graph) and autoantibody-negative patients (right graph) with the use of troponin T (Trop T; in units per milliliter), creatine kinase (CK and CK-MB; in picograms per milliliter), pro–B-type natriuretic peptide (pro-BNP; in units per milliliter), and CD3+ cells (normal value, <7 per square millimeter) and CD68+ cells (per square millimeter) infiltrating the tissue of the right or left ventricle, respectively, as well as C-reactive protein (CRP; in milligrams per deciliter). Numbers indicate the respective Spearman's r (*P<0.05, and **P<0.01) (**Panel E**). IL-1RA plasma levels were determined by ELISA in patients with vaccination-associated myocarditis. Data are shown as violin plots; in each plot, dots indicate individual samples, the solid horizontal line the median, dotted horizontal lines the upper and lower quartiles, and the shaded area the probability density. Data were analyzed by Brown-Forsythe and Welch analysis of variance and Dunnett's T3 multiple comparisons test (**Panel F**). Human embryonic kidney IL-1 reporter cells (releasing secreted embryonic alkaline phosphatase on IL-1β signaling) were incubated with tumor necrosis factor α (TNF-α), IL-1β , and IL-1β with anakinra or recombinant human IL-1RA (rec hIL-1RA). Plasma from adult patients with critical coronavirus disease 2019, without and with IL-1RA antibodies, and from patients with myocarditis after SARS-CoV-2 vaccination, without or with IL-1RA antibodies, were added (all plasma in 1:20 dilution). Recombinant anti–IL-1RA antibody and recombinant anti–stomatin-like protein 2 antibody were used as positive and negative controls, respectively (**Panel G**). I bars indicate the standard deviation of the mean. OD_{650} denotes optical density as measured at a wavelength of 650 nm, and VAM vaccination-associated myocarditis.

impairment of IL-1RA bioactivity after the addition of anti–IL-1RA antibodies from patients' plasma (Fig. 1G).

Our study of SARS-CoV-2 vaccination–associated myocarditis and anti–IL-1RA antibodies should be interpreted within the context that the transiency of hyperphosphorylation (as previously reported in patients with critical Covid-19 or MIS-C[3,4]) and patients' HLA haplotypes were not known. In our study, neutralizing antibodies against IL-1RA and a hyperphosphorylated IL-1RA isoform were observed in young male patients with biopsy-confirmed myocarditis after the receipt of SARS-CoV-2 mRNA vaccine. These antibodies impaired IL-1RA bioactivity in vitro, were associated with low circulating levels of IL-1RA, and were found in patients with biomarker evidence of cardiac damage and inflammation.

Lorenz Thurner, M.D.

Saarland University
Homburg, Germany
lorenz.thurner@uks.eu

Christoph Kessel, Ph.D.

University Children's Hospital Muenster
Muenster, Germany

Natalie Fadle
Evi Regitz

Saarland University
Homburg, Germany

Franziska Seidel, M.D.

German Heart Center Berlin
Berlin, Germany

Ingrid Kindermann, M.D.
Stefan Lohse, Ph.D.
Igor Kos, M.D.

Saarland University
Homburg, Germany

Carsten Tschöpe, M.D.

Charité University Medicine Berlin
Berlin, Germany

Parastoo Kheiroddin, M.Sc.

University Children's Hospital Regensburg
Regensburg, Germany

Daniel Kiblboeck, M.D.
Johannes Kepler University
Linz, Austria

Marie-Christin Hoffmann
Birgit Bette
Gabi Carbon
Onur Cetin, M.D.
Klaus-Dieter Preuss, Ph.D.
Konstantinos Christofyllakis, M.D.
Joerg T. Bittenbring, M.D.
Saarland University
Homburg, Germany

Thomas Pickardt, M.D.
Competence Network for Congenital Heart Defects
Berlin, Germany

Yvan Fischer, Ph.D.
Rheinisch-Westfälische Technische Hochschule Aachen
Aachen, Germany

Holger Thiele, M.D.
Heart Center Leipzig
Leipzig, Germany

Stephan Baldus, M.D.
University Hospital Cologne
Cologne, Germany

Karl Stangl, M.D.
Charité University Medicine Berlin
Berlin, Germany

Stephan Steiner, M.D.
St. Vincenz Hospital
Limburg, Germany

Frank Gietzen, M.D.
Sebastian Kerber, M.D.
Thomas Deneke, M.D.
Cardiovascular Center Bad Neustadt–Saale
Bad Neustadt–Saale, Germany

Stefanie Jellinghaus, M.D.
Axel Linke, M.D.
Technische Universität Dresden
Dresden, Germany

Karim Ibrahim, M.D.
Klinikum Chemnitz
Chemnitz, Germany

Ulrich Grabmaier, M.D.
Steffen Massberg, M.D.
Ludwig-Maximilians-University
Munich, Germany

Christian Thilo, M.D.
RoMed Klinikum
Rosenheim, Germany

Simon Greulich, M.D.
Meinrad Gawaz, M.D.
University of Tübingen
Tübingen, Germany

Ertan Mayatepek, M.D.
University Children's Hospital Duesseldorf
Duesseldorf, Germany

Lars Meyer-Dobkowitz, M.D.
Klinikum Westbrandenburg
Potsdam, Germany

Michael Kindermann, M.D.
CaritasKlinikum St. Theresia
Saarbrücken, Germany

Einat Birk, M.D.
Merav Birk, M.D.
Schneider Children's Medical Center of Israel
Petach Tikva, Israel

Mitja Lainscak, M.D.
University of Ljubljana
Ljubljana, Slovenia

Dirk Foell, M.D.
University Children's Hospital Muenster
Muenster, Germany

Philipp M. Lepper, M.D.
Robert Bals, M.D.
Marcin Krawczyk, M.D.
Saarland University
Homburg, Germany

Dror Mevorach, M.D.
Hadassah Medical Center
Jerusalem, Israel

Tal Hasin, M.D.
Shaare Zedek Medical Center
Jerusalem, Israel

Andre Keren, M.D.
Hadassah Hebrew University Hospital
Jerusalem, Israel

Michael Kabesch, M.D.
University Children's Hospital Regensburg
Regensburg, Germany

Hashim Abdul-Khaliq, M.D.
Sigrun Smola, M.D.
Moritz Bewarder, M.D.
Saarland University
Homburg, Germany

Bernhard Thurner, M.D.
Klinikum Kempten
Kempten, Germany

Michael Böhm, M.D.
Jochen Pfeifer, M.D.
Saarland University
Homburg, Germany

Karin Klingel, M.D.
University Hospital Tübingen
Tübingen, Germany

Drs. L. Thurner and Kessel and Drs. Pfeifer and Klingel contributed equally to this letter.

Supported by a young investigator NanoBioMed fund of the University of Saarland (to Dr. L. Thurner); by Deutsche Herzstiftung and Covid-19 Projektförderung Land Baden-Württemberg (to Dr. Klingel); by the Competence Network for Congenital Heart Defects, Federal Ministry of Education and Research (grant number, 01GI0601); and by the National Register for Congenital Heart Defects, Federal Ministry of Education and Research (grant number, 01KX2140). The SaarCoVac registry was funded by the federal state of Saarland. The CoKiBa trial was supported by the Blue Sisters and by the project Post Covid Kids Bavaria of the Bavarian Ministry of Health. Dr. Smola received funding from the Dr. Rolf M. Schwiete Stiftung and from the Staatskanzlei of the federal state of Saarland. Drs. Kessel and Foell were supported by the Center for Interdisciplinary Clinical Research at University Hospital Muenster (F 2/018/20) and by the European Union Horizon 2020 research and innovation program (grant agreement number, 779295; ImmunAID). Dr. Gawaz was supported by the German Research Foundation (project number, 374031971–TRR 240) and by the Ministry of Science, Research, and the Arts of the federal state of Baden-Württemberg (coronavirus disease 2019 funding).

Disclosure forms provided by the authors are available with the full text of this letter at NEJM.org.

This letter was published on September 21, 2022, at NEJM.org.

1. Mevorach D, Anis E, Cedar N, et al. Myocarditis after BNT162b2 mRNA vaccine against Covid-19 in Israel. N Engl J Med 2021;385:2140-9.

2. Ling RR, Ramanathan K, Tan FL, et al. Myopericarditis following COVID-19 vaccination and non-COVID-19 vaccination: a systematic review and meta-analysis. Lancet Respir Med 2022; 10:679-88.

3. Thurner L, Fadle N, Bewarder M, et al. Autoantibodies against progranulin and IL-1 receptor antagonist in critically ill COVID-19. April 26, 2021 (https://www.biorxiv.org/content/10.1101/2021.04.23.441188v1). preprint.

4. Pfeifer J, Thurner B, Kessel C, et al. Autoantibodies against interleukin-1 receptor antagonist in multisystem inflammatory syndrome in children: a multicentre, retrospective, cohort study. Lancet Rheumatol 2022;4(5):e329-e337.

DOI: 10.1056/NEJMc2205667