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RESEARCH LETTER

Long COVID-19 Cardiac Complications Are Associated With Autoimmunity to Cardiac Self-Antigens Sufficient to Cause Cardiac Dysfunction

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Coronavirus disease 2019 (COVID-19) led to extensive morbidity and mortality. Attention is shifting to long-term effects persisting after acute infection, termed long/post–COVID-19, including cardiac sequelae.[1] Cardiovascular magnetic resonance (CMR) abnormality is present at 6-month follow-up in half of patients recovered from COVID-19 with in-hospital high-sensitivity troponin elevation.[2] Lack of preinfection data renders it hard to exclude causality from pre-existing conditions, rather than infection sequelae. Yet larger studies show increased risk of incident cardiovascular disease in the year after COVID-19,[3,4] suggesting that long/post–COVID-19 cardiac sequelae may become a major public health issue.

Proposed mechanisms of COVID-19 cardiovascular involvement include direct damage by virus, or indirect, mediated by systemic inflammation/autoimmunity.[1] Autoimmunity to cardiac self-antigens could be triggered through "molecular mimicry" of antigens shared between COVID-19 and host cells, and/or through "bystander loss-of-tolerance," where T or B cells, of random self-reactive specificity, are accidentally unleashed after receiving costimulation signals from the infection. The latter hypothesis could explain many features of acute and chronic COVID-19. First, T and B cell antigen specificities vary in every individual, potentially causing pathology in different organs in similarly exposed individuals. Second, autoimmune responses are long-lasting and independent of viral persistence, potentially linking acute and post–COVID-19. Last, bystander loss-of-tolerance requires activated but not yet costimulated self-reactive T and B cells, which would increase with age-related inflammation, potentially explaining the age dependence of long/post–COVID-19.[1] Autoimmunity targeting different tissues has been observed extensively in patients with COVID-19; however, no cardiac antigens have been described so far.[1]

To test whether autoimmunity could be implicated in long/post–COVID-19 cardiac complications, we examined a cohort of 16 patients hospitalized in our hospital due to COVID-19 pneumonia in April and May 2020, without history of previous cardiovascular disease, with in-hospital elevation of high-sensitivity troponin, and who underwent CMR at 6-month follow-up after hospital discharge due to newly observed cardiovascular symptoms (dyspnea, intolerance to physical activity, and palpitations). A CMR abnormality was found in 9 patients (56%), with a nonischemic injury pattern in 7 patients and an ischemic injury pattern, in absence of coronary artery disease history, in 2 patients. To assess their immune state, we examined peripheral blood cells

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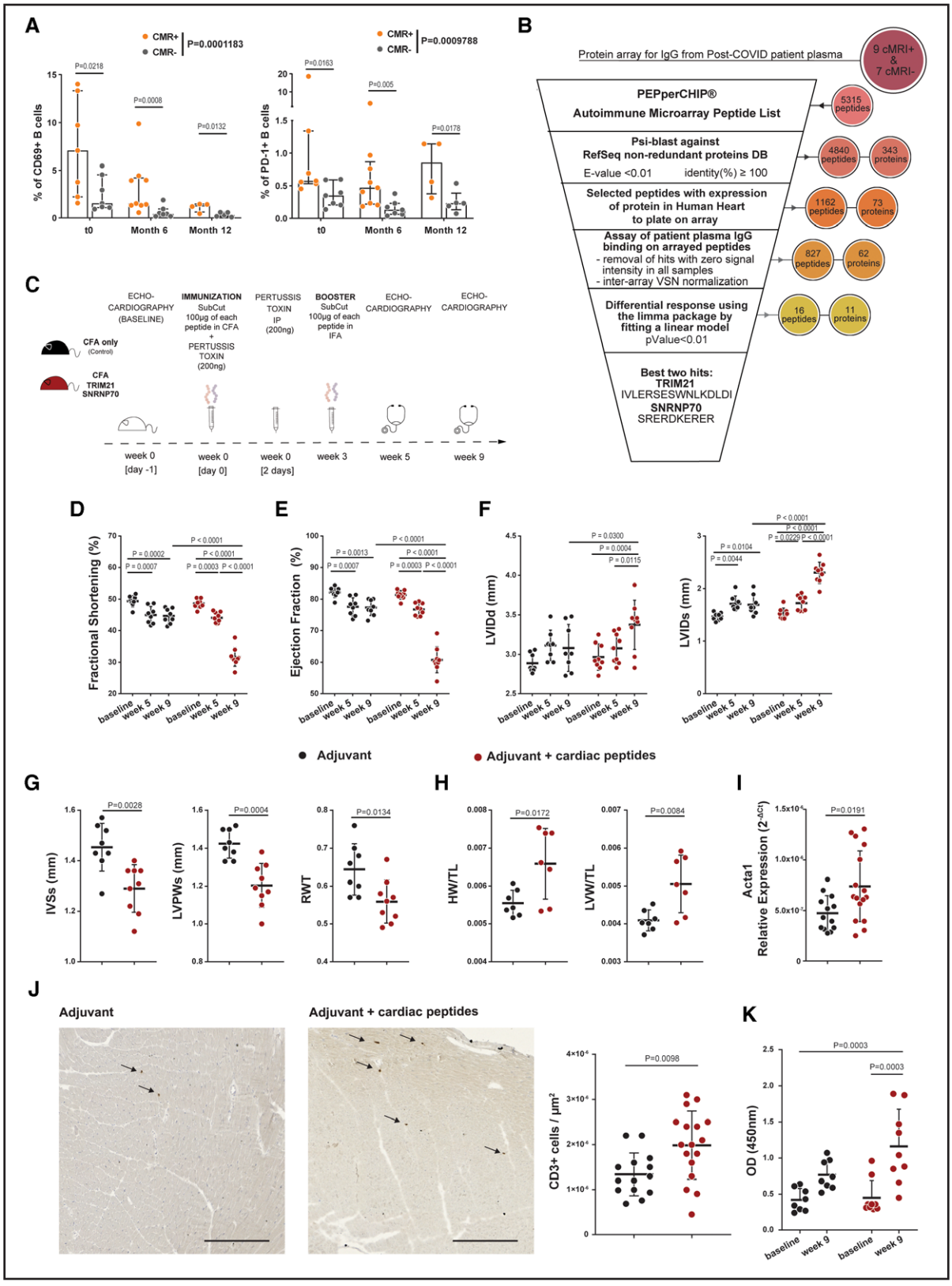

Figure. Patients with abnormal CMR showed late gadolinium enhancement areas or higher values of T1 mapping value (1017 milliseconds (ms) [1003–1033] vs 990 ms [985–996], *P*=0.019) than those with normal CMR, and were older (71 [64–73] vs 50 [47–55] y, *P*=0.013), while having comparable cardiovascular risk profile, coronavirus disease 2019 (COVID-19) severity, inflammatory and cardiac biomarkers, T2 mapping value, left and right ventricular ejection fraction (left: 63 [58–65]% vs 61 [57–63]%; right: 57 [55–63]% vs 57 [54–64]%) and volumes.

Medians (interquartile range) are shown for categorical (chi-square test) and continuous variables (Student *t*- or Wilcoxon test). (*Continued*)

Figure Continued. The nonischemic injury pattern was myocarditis-like in 3, diffuse fibrosis in 2, and nonischemic, nonspecific scar in 2 patients. Patients were unvaccinated. **A**, FACS of patient PBMCs. Percentage of live single B cells ($CD19^+$) expressing activation markers CD69 and PD-1 (medians and interquartile ranges). Each dot is 1 patient. Linear mixed-effects model (LMM): percentage of $CD69^+$ B cells (ANOVA [Type II Wald chi-square tests] table: condition main effect, P=0.0001; time main effect, P=2.8e-13; interaction, P=0.47); percentage of $PD1^+$ B cells (ANOVA [Type II Wald chi-square tests] table: condition main effect, P=0.001; time main effect, P=0.0001; interaction, P=0.88). **B**, Epitope discovery workflow to identify self-peptide hits. Lowest box shows names of protein from which the epitopes derive (bold), and the epitope amino acid sequence. Note the TRIM21 epitope is outside the IgG-binding PRYSPRY domain. **C**, Immunization scheme: healthy male mice were immunized with a mixture of the 2 cardiac epitopes (100 µg each) or PBS in Complete Freund's Adjuvant (CFA) (day 0). Mice were also injected intraperitoneally (days 0 and 2) with pertussis toxin and boosted at week 3 with peptides or PBS in Incomplete Freund's Adjuvant (IFA). Heart functionality was assessed at baseline and 2 and 6 weeks after boost. **D** and **E**, FS and EF of immunized mice at indicated time points analyzed by echocardiography (mean±SD, each dot represents 1 mouse—likewise for all subsequent panels). LMM with Sidak multiple comparisons test for simple-effects analysis: FS (ANOVA [Type II Wald chi-square tests] table: condition main effect, P=0.082; time main effect, P<2e-16; interaction, P<2e-16): control basal-week 5 P=0.0007, basal-week 9 P=0.0002, week 5-week 9 P=0.99; peptides basal-week 5 P=0.0003, basal-week 9 P<0.0001, week 5-week 9 P<0.0001; control-peptides basal P=1.00, control-peptides week 5 P=0.80, control-peptides week 9 P<0.0001. EF (ANOVA [Type II Wald chi-square tests] table: condition main effect, P=0.21; time main effect, P<2e-16; interaction, P<2e-16): control basal-week 5 P=0.0007, basal-week 9 P=0.0013, week 5-week 9 P=1.00; peptides basal-week 5 P=0.0003, basal-week 9 P<0.0001, week 5-week 9 P<0.0001; control-peptides basal P=0.30, control-peptides week 5 P=0.48, control-peptides week 9 P<0.0001. **F**, Left ventricle (LV) internal diastolic dimension (LVIDd) and LV internal systolic dimension (LVIDs) at baseline, week 5, and week 9. Two-way ANOVA with Sidak multiple comparison test. **G**, Interventricular septum in systole (IVSs), LV posterior wall in systole (LVPWs), and relative wall thickening (RWT) at week 9. Unpaired *t*-test, after normality testing. **H**, heart weight/tibia length (HW/TL) and LV weight/TL (LVW/TL) at week 9. Unpaired *t*-test, after normality testing. **I**, Acta1 mRNA (quantitative polymerase chain reaction) at week 9. Unpaired *t*-test, after normality testing. **J**, Representative image of $CD3^+$ (T) cells infiltrating the myocardium; arrows highlight positive signal. Scale bar: 200 µm. Total $CD3^+$ cells from LV were counted and normalized to the LV tissue area. Unpaired *t*-test, after normality testing. **K**, Antibodies against the 2 identified self-antigens at baseline and week 9. Two-way ANOVA with Sidak multiple comparison test. ANOVA indicates analysis of variance; CMR, cardiovascular magnetic resonance; EF, ejection fraction; FACS, fluorescence-activated cell sorting; FS, fractional shortening; PBMC, peripheral blood mononuclear cells; and TRIM21, tripartite motif containing 21.

Nonstandard Abbreviations and Acronyms

CMR	cardiovascular magnetic resonance
SNRNP70	small nuclear ribonucleoprotein U1 subunit 70
TRIM21	tripartite motif containing 21

drawn during COVID-19 hospitalization, and at 6- and 12-month follow-up after hospital discharge. Comparing immune cell subtypes in patients with normal versus abnormal CMR, only B cells showed significant differences: patients with abnormal CMR (CMR+) possessed more activated B cells, demonstrated by significantly higher levels of activation markers CD69 and PD-1, regardless of sampling time (Figure [A]). Hence, patients with a demonstrated myocardial injury after COVID-19 at CMR assessment show a selective, abnormal, and persistent activation of B cells. To test the hypothesis of a "bystander loss-of-tolerance"–triggered B cell autoimmunity, we analyzed the 6-month plasma samples of each patient, using an oligopeptide array containing known autoantigens demonstrated to be expressed in the heart, to detect IgG autoantibodies able to bind potential cardiac autoantigens. We performed a differential analysis and identified 2 autoantigens, from TRIM21 (tripartite motif containing 21) and SNRNP70 (small nuclear ribonucleoprotein U1 subunit 70), with no similarity to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens, which are expressed in the heart, and were targeted by antibodies that were significantly differentially enriched in the CMR+ but not the CMR- subgroup (Figure [B]). We then tested whether these antigens could drive anticardiac immune responses in mouse immunization experiments, using a standard Complete Freund's Adjuvant–based immunization protocol (Figure [C]). This confirmed our hypothesis, because immunized mice showed a significant reduction of left ventricular systolic function assessed by echocardiography compared with adjuvant-only control, by 9 weeks after the immunization (Figure [D and E]), a dilated phenotype with eccentric hypertrophy (Figure [F and G]), increased heart dimension (Figure [H]), upregulation of stress marker Acta1 (Figure [I]), and—expected for an immunization—increased T cell heart infiltration (Figure [J]) and autoantigen-specific antibody production (Figure [K]).

We show that in a small cohort of survivors of COVID-19, with high-sensitivity troponin elevation during COVID-19 hospitalization, with a clinically referred CMR at 6-month follow-up, B cells were activated and produced antibodies against 2 cardiac antigens (TRIM21 and SNRNP70) only in patients with abnormal CMR. These cardiac antigens were sufficient to induce cardiac dysfunction when used as drivers of autoimmune responses in mice. It is impossible to claim causality between COVID-19 and myocardial injury in these patients, because pre–COVID-19 CMR data are unavailable. Yet our study goes beyond previous analyses of autoimmunity in COVID-19 by demonstrating that the autoantigens involved may induce cardiac complications.

The pathogenesis of post–COVID-19 sequelae has many potential causal factors, including inflammation.[4] Our data suggest self-reactive B cells may be significantly involved. It is interesting that corticosteroids, which block T and B cells, are an effective therapy in late-stage COVID-19, when tissue-specific pathology has commenced, but not early-stage, when there is still virus targeted by T/B cells.[5] Further investigation is needed to clarify whether autoimmunity is a key factor of post–COVID-19 cardiovascular sequelae.

All data are available on reasonable request. The human study had Institutional Ethics Board approval and patient informed consent. The mouse study had National Board authorization.

ARTICLE INFORMATION

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Disclosures

The authors report no conflicts. J.J.P.v.B. became an employee of Janssen (Netherlands) after the completion of the work included in this article.

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