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LEADING EDGE TRANSLATIONAL RESEARCH

Human Cardiac Pericytes Are Susceptible to SARS-CoV-2 Infection

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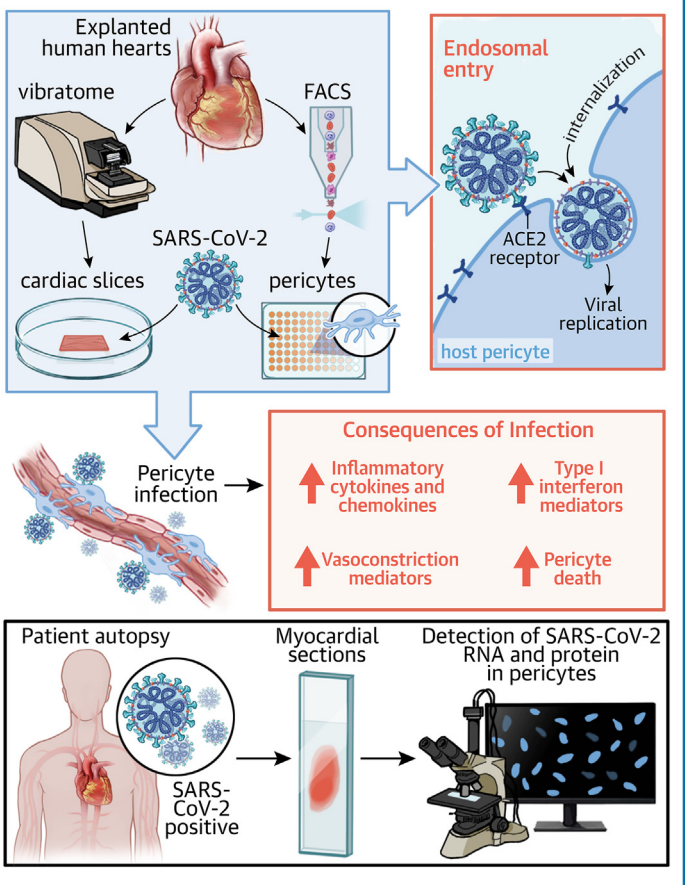
VISUAL ABSTRACT



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HIGHLIGHTS

- **SARS-CoV-2 infects and replicates within cardiac pericytes in human organotypic cardiac slices.**
- **SARS-CoV-2 infects human primary cardiac pericytes through an ACE2- and endosomal-dependent pathway.**
- **Infection of human primary cardiac pericytes leads to up-regulation of inflammatory chemokine and cytokine expression, type I IFN signaling, mediators of vasoreactivity, and NF-κB–dependent cell death.**
- **SARS-CoV-2 directly infects cardiac pericytes in patients with COVID-19 myocarditis.**

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ABBREVIATIONS AND ACRONYMS

ACE2 = angiotensin-converting enzyme 2

IFN = interferon

MOI = multiplicity of infection

PDGFRβ = platelet-derived growth factor receptor beta

SUMMARY

COVID-19 is associated with serious cardiovascular complications, with incompletely understood mechanism(s). Pericytes have key functions in supporting endothelial cells and maintaining vascular integrity. We demonstrate that human cardiac pericytes are permissive to SARS-CoV-2 infection in organotypic slice and primary cell cultures. Viral entry into pericytes is mediated by endosomal proteases, and infection leads to up-regulation of inflammatory markers, vasoactive mediators, and nuclear factor kappa-B–dependent cell death. Furthermore, we present evidence of cardiac pericyte infection in COVID-19 myocarditis patients. These data demonstrate that human cardiac pericytes are susceptible to SARS-CoV-2 infection and suggest a role for pericyte infection in COVID-19. (J Am Coll Cardiol Basic Trans Science 2022;■:■–■) © 2022 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

SARS-CoV-2 infection leads to a constellation of serious cardiovascular manifestations in subsets of patients, including myocardial damage, myocarditis, arrhythmias, heart failure, acute coronary syndromes, thrombosis, and hemodynamic dysregulation.[1] A recent population-based study demonstrated an increased risk of serious vascular complications, including acute myocardial infarction and ischemic stroke, in COVID-19 patients.[2] COVID-19 also is associated with endothelial inflammation and dysfunction,[3] marked by up-regulation of proinflammatory mediators and endothelial damage.[4] Postmortem analyses of hearts from 41 COVID-19 patients revealed capillary, arteriole, and small artery microthrombi in 20% of cases.[5]

The mechanisms underlying cardiovascular complications of COVID-19 are incompletely understood. Evaluation of infection in the heart has suggested that cardiomyocytes and cardiac interstitial cells may be targets of SARS-CoV-2 infection.[6-9] In contrast, several studies have reported that SARS-CoV-2 is unable to infect cultured endothelial cells.[6,10] Intriguingly, cardiac pericytes express angiotensin-converting enzyme 2 (ACE2), a host receptor for SARS-CoV-2, at high levels.[11] Pericytes have a critical role in maintaining endothelial integrity and vascular homeostasis. Conceptually, SARS-CoV-2 infection of pericytes could contribute to the vascular manifestations of COVID-19 including thrombosis, inflammation, and hemodynamic derangements.[12] Recent work has shown that pericyte-like cells generated from human pluripotent stem cell-derived neural crest stem cells can be productively infected by SARS-CoV-2.[13] To date, no studies have evaluated whether cardiac pericytes, which are thought to be derived from epicardium,[14] are targets of SARS-CoV-2 infection. Here, we demonstrate that human cardiac pericytes are susceptible to SARS-CoV-2 infection, reveal that the production of inflammatory and vasoactive mediators and cell death are consequences of pericyte SARS-CoV-2 infection, and show evidence of pericyte infection in cardiac tissue from COVID-19 patients.

METHODS

A detailed methods section, including Supplemental Table S1, can be found in the Supplemental Appendix.

BIOSAFETY. All aspects of this study were approved by the Office of Environmental Health and Safety at Washington University School of Medicine before the initiation of this study. Work with SARS-CoV-2 was performed in a BSL-3 laboratory by personnel equipped with powered air-purifying respirators.

VIRUSES. The USA_WA1/2020 isolate of SARS-CoV-2 (WA1/2020) was obtained from the U.S. Centers for Disease Control and Prevention. The NeonGreen SARS-CoV-2 virus stock was obtained from P-Y. Shi (University of Texas Medical Branch).[15] B.1.1.7 (Alpha), B.1.351 (Beta), and B.1.617.2 (Delta) strains of SARS-CoV-2 were obtained from infected individuals.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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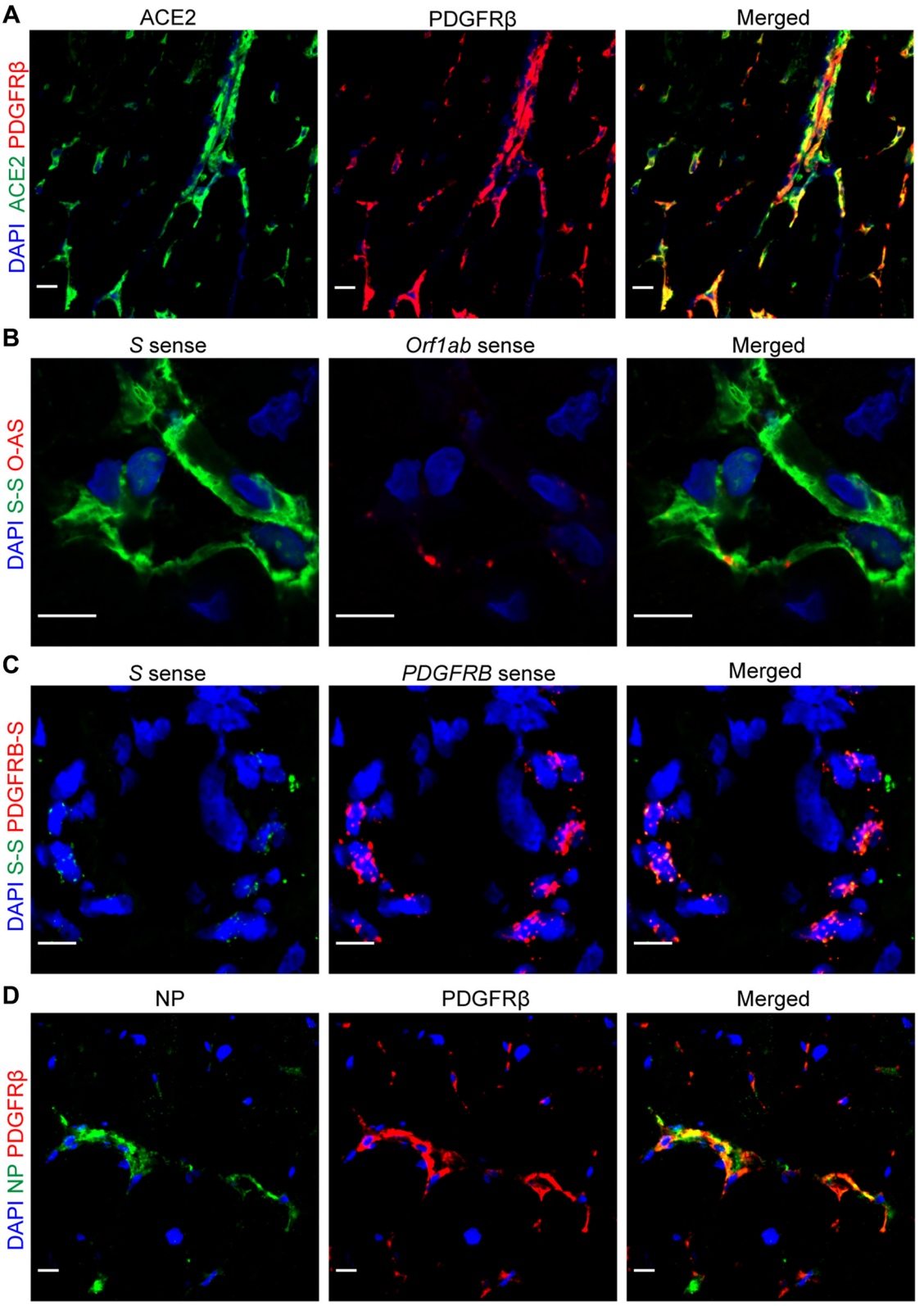
FIGURE 1 **Detection of SARS-CoV-2 in Pericytes of Inoculated Ventricular Organotypic Slices**



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All viral stocks were propagated in Vero-TMPRSS2 cells[16] (a gift of S. Ding, Washington University), deep-sequenced with the ARTIC protocol for Illumina sequencing,[17] and found to have no sequence variationss in the S gene furin cleavage site (positions 23606-23608 of NC_045512.2).

HUMAN TISSUE ACQUISITION. Nonfailing human hearts were obtained from Mid-America Transplant Services. Failing human heart tissue (anterior wall of the left ventricle) was obtained through the Translational Cardiovascular Biobank and Repository at Washington University. Control and COVID-19 myocarditis autopsy specimens were obtained from Barnes Jewish Hospital. Cardiac pericytes were isolated from both failing and nonfailing heart sources. Detailed subject information is available in Supplemental Table S1. Experimental protocols were approved by the Washington University in St. Louis Institutional Review Board (IRB). Nonfailing human heart tissue and autopsy specimens were exempt from IRB, and failing human heart tissue was procured under the Translational Cardiovascular Biobank and Repository IRB Protocol #201104172. Informed consent was obtained for all tissue used in this study, and all experiments were performed in accordance with all human research guidelines.

RNA SEQUENCING. The complete data set has been submitted to the National Center for Biotechnology Information's Gene Expression Omnibus database with the accession number GSE183850 and was released to the public on August 31, 2022.

STATISTICAL ANALYSIS. All data are expressed as means ± SEM. All data were normally distributed based on the Shapiro-Wilk test and were thus analyzed with parametric tests. For comparison of more than 2 experimental groups, the statistical significance of observed differences in mean values was evaluated using 1- or 2-way analysis of variance (ANOVA), followed by a post hoc Tukey's or Dunnett's multiple comparison test. For comparison of 2 experimental groups, an equal or unequal variance Student's *t*-test, as determined by an F test for equality of 2 variances, was used, and a 2-tailed *P* value <0.05 was considered statistically significant. All analyses were performed using Prism Version 9 (GraphPad).

RESULTS

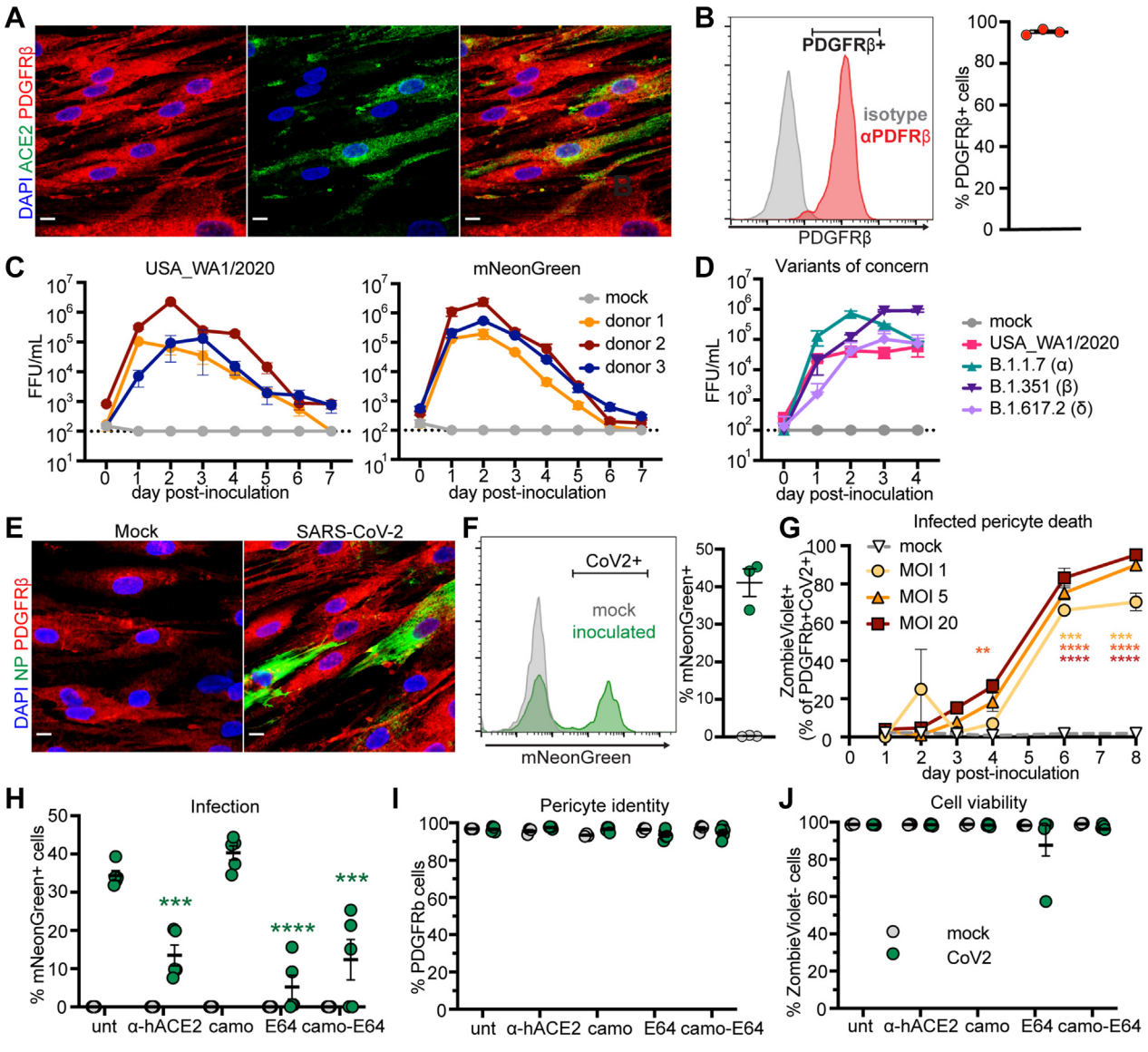
ACE2 IS EXPRESSED IN HUMAN CARDIAC PERICYTES IN SITU. Single-cell RNA sequencing of the human heart established that *ACE2* mRNA is expressed in cardiac pericytes.[18] To confirm ACE2 protein expression in pericytes, we performed immunohistochemistry on human ventricular tissue from nonfailing donor hearts rejected from transplantation (Supplemental Table S1). In vascular regions, we observed ACE2 staining surrounding $CD31^+$ endothelial cells (Supplemental Figure S1A). Costaining for platelet-derived growth factor receptor beta (PDGFRβ) (pericyte marker) showed that pericytes express ACE2 (**Figure 1A** and Supplemental Figure S1B). We also observed ACE2 expression in some cardiomyocytes, as previously reported (Supplemental Figure S1C).[6,8] These data demonstrate that cardiac pericytes and cardiomyocytes both express ACE2.

HUMAN CARDIAC PERICYTES ARE PERMISSIVE TO SARS-CoV-2 INFECTION. To evaluate SARS-CoV-2 tropism in human heart tissue, we utilized an ex vivo organotypic slice culture system.[19] Briefly, 400-μm slices of left ventricular myocardium from nonfailing donor hearts were cultured at a liquid-air interface on transwell inserts. Slices were inoculated with 5×10^5 focus forming units of SARS-CoV-2 (USA_WA1/2020 strain). Slices then were cultured for 24 or 48 hours. In situ hybridization revealed the presence of SARS-CoV-2 *S* RNA (**Figure 1B**) with increased signal over time (Supplemental Figures S2A and S2B). We identified the presence of the replication intermediate (antisense RNA) for both *ORF1ab* and *S* genes in cells where genomic *S* gene RNA was present 48 hours after inoculation (**Figure 1B** and Supplemental Figure S2C). SARS-CoV-2 RNAs colocalized with *PDGFRB* mRNA in perivasculature regions (**Figure 1C**). Immunostaining for SARS-CoV-2 nucleocapsid protein (NP) revealed viral protein in PDGFRβ+ cells surrounding CD31+ endothelial cells,

FIGURE 1 Continued

(A) Representative immunostaining for angiotensin-converting enzyme 2 (ACE2) **(green)** and platelet-derived growth factor receptor beta (PDGFRβ) **(red)** in human left ventricular tissue (n = 3 independent donor hearts). **(B to D)** Cardiac slices were inoculated with 5×10^5 focus forming units of SARS-CoV-2 WA1/2020 (approximately MOI of 1) and harvested 48 hours later (n = 3). **(B)** Representative RNA in situ hybridization of SARS-CoV-2 *S* sense (S-S) **(green)** and SARS-CoV-2 *ORF1ab antisense* (O-AS) **(red)** at 48 hours postinoculation. **(C)** Representative RNA in situ hybridization of SARS-CoV-2 *S* sense (S-S) **(green)** and pericyte marker *PDGFRB* sense **(red),** and **(D)** immunostaining for the SARS-CoV-2 NP **(green)** with pericyte marker PDGFRβ **(red).** All scale bars: 10 μm. All images representative from 3 technical slice replicates from each of the 3 independent donor hearts. DAPI = 4′,6-diamidino-2-phenylindole.

FIGURE 2 **SARS-CoV-2 Productively Infects Human Primary Cardiac Pericytes**



(A) Immunostaining for ACE2 **(green)** and PDGFRβ **(red)** of cardiac pericytes isolated from left ventricle (n = 3 donors). **(B)** Representative flow cytometry histogram and quantification of PDGFRβ in pericytes (n = 3). **(C and D)** Focus-forming assay time course of pericyte infection with SARS-CoV-2 WA1/2020 and SARS-CoV-2 mNeonGreen (n = 3) **(C)** and Alpha (B.1.1.7), Beta (B.1.351), and Delta (B.1.617.2) SARS-CoV-2 variants (representative of n = 2) **(D); dotted line** indicates limit of detection. **(E)** Immunostaining for the SARS-CoV-2 nucleocapsid protein (NP) **(green)** and PDGFRβ **(red)** (n = 3). **(F)** Representative flow cytometry histogram and quantification of cardiac pericyte infection (mNeonGreen) at day 1 postinoculation with multiplicity of infection (MOI) of 20 (n = 3). **(G)** Flow cytometry-based cell viability (ZombieViolet+ = dead) time course of inoculated pericytes. Statistical comparison to mock at corresponding time point. **(H to J)** Pericytes were pretreated with vehicle (unt), 10 μg/mL of human anti-ACE2 antibody (α-hACE2), 100 μmol/L of serine protease inhibitor camostat methylate (camo), 100 μmol/L of cysteine protease inhibitor E-64 (E64), or a combination of camostat methylate and E-64 (camo-E64) for 1 hour, inoculated with mock or SARS-CoV-2 mNeonGreen at MOI of 20 for 20 hours (n = 2). Quantification of infection (mNeonGreen+) **(D),** pericyte identity (PDGFRβ+) **(E),** and cell viability (Zombie-Violet-) **(F). (D to F) Points** represent separate wells; statistical comparison to untreated inoculated group. Repeated in 2 donors. 2-way analysis of variance with Dunnet's post-test: $^{**}P < 0.01$; $^{***}P < 0.001$. Scale bars: 20 μm. Abbreviations as in Figure 1.

suggesting that SARS-CoV-2 can infect cardiac pericytes productively in a native tissue environment (**Figure 1D** and Supplemental Figure S3). We further demonstrated that PDGFRβ colocalizes with another pericyte marker NG2 (Supplemental Figure S4A) and found colocalization of NG2 with NP in infected cardiac slices (Supplemental Figure S4B). We also observed SARS-CoV-2 RNA within the myocardium, consistent with previous studies demonstrating cardiomyocyte infection (Supplemental Figure S3).[7,8]

SARS-CoV-2 INFECTS HUMAN PRIMARY CARDIAC PERICYTES. To elucidate mechanisms of viral entry and explore consequences of cardiac pericyte infection, we isolated cardiac pericytes ($CD146^+CD45^-CD56^-CD34^-CD117^-$ cells) from nonfailing and failing human left ventricular tissue using fluorescence-activated cell sorting and established primary cultures by modifying a published protocol[20] (Supplemental Figures S5A and S5B and Supplemental Table S1). Cultured cardiac pericytes displayed a morphology consistent with pericytes in vivo[21] and expressed pericyte-specific markers (PDGFRβ, NG2, and NOTCH3) (Supplemental Figures S5D and S5E). Primary cardiac pericytes retained PDGFRβ and ACE2 protein expression in culture (**Figures 2A and 2B**).

Pericytes isolated from 3 independent donors were inoculated with SARS-CoV-2 WA1/2020 or SARS-CoV-2 mNeonGreen (WA1/2020 background)[15] at a multiplicity of infection (MOI) of 1 and cultured for up to 7 days. Measurement of infectious virus by focus-forming assay revealed robust viral replication, which peaked at 2 to 3 days postinoculation (**Figure 2C**). Similar kinetics were observed for the Alpha (B.1.1.7), Beta (B.1.351), or Delta (B.1.617.2) SARS-CoV-2 variants (**Figure 2D**). SARS-CoV-2 infection of cultured cardiac pericytes was corroborated by immunostaining (**Figure 2E**) and flow cytometry (**Figure 2F**). Approximately 40% of cells were $mNeonGreen^+$ on day 1 postinoculation (**Figure 2F**). We observed virus-induced pericyte cell death beginning after day 4 postinoculation (**Figure 2G**).

To determine whether SARS-CoV-2 could infect human endothelial cells or pericytes from other organs, we inoculated primary cardiac microvascular endothelial cells, brain pericytes, and placental pericytes under the same conditions but did not detect productive infection (Supplemental Figures S6A). Each of these cell types expressed lower levels of *ACE2* mRNA compared with cardiac pericytes (Supplemental Figure S6B). These data suggest that not all pericytes are permissive to SARS-CoV-2 infection (Supplemental Figures S6A and S6B). To assess if coculture of pericytes and endothelial cells made endothelial cells susceptible to infection, we inoculated primary cardiac pericytes, endothelial cells, or a 1:1 mixture of pericytes and endothelial cells with reporter mNeonGreen virus. Pericytes remained the only cell type with detectable viral replication, suggesting that coculture does not alter the permissiveness of at least some endothelial cells to SARS-CoV-2 infection (Supplemental Figure S6C).

SARS-CoV-2 ENTERS CARDIAC PERICYTES THROUGH AN ENDOSOMAL PATHWAY. After binding to ACE2, the SARS-CoV-2 spike protein undergoes a proteolytic event that is necessary for fusion of the viral and host membranes and entry into the cell. Activation of spike by TMPRSS proteases mediates entry at the plasma membrane. Alternatively, spike activation via endosomal cathepsins enables cell entry through fusion with endo-lysosomal membranes.[22,23] Human cardiac pericytes express ACE2, as well as genes encoding cathepsin B (*CTSB*) and L (*CTSL)* and furin (*FURIN*) proteins, but have undetectable levels of *TMPRSS2* or *TMPRSS4* (Supplemental Figure S7A). To define the mechanism of viral cell entry, we treated cardiac pericytes with anti-ACE2 neutralizing antibody, camostat mesylate (TMPRSS2 and TMPRSS4 inhibitor), and/or E-64 (endosomal cysteine protease inhibitor). We then inoculated cells with SARS-CoV-2 mNeonGreen at a high MOI of 20 to ensure that a high percentage of cells was infected in culture, which allowed us to focus on cell entry rather than other parts of the replication cycle and avoid prolonged exposure to inhibitors. After 20 hours, we used flow cytometry to assess the percentage of cardiac pericytes that expressed mNeonGreen (mNeonGreen+; PDGFRβ+) as an indicator of viral infection[15] (Supplemental Figures S7B and S7C). Neutralizing anti-ACE2 antibody and E-64 treatment significantly reduced the number of infected pericytes (**Figure 2H**). However, camostat treatment did not suppress pericyte infection (**Figure 2H**). Next-generation sequencing confirmed that the polybasic furin cleavage motif, which enhances spike cleavage by TMPRSS2 and TMPRSS4 proteases, was intact and present at 100% of SARS-CoV-2 mNeonGreen and USA_WA1/2020 viral stocks (see Methods section). Combining E-64 and camostat did not further reduce pericyte infection (**Figure 2H**). In all drug treatment conditions, cardiac pericytes maintained their identity as evidenced by PDGFRβ+ expression (**Figure 2I**), and minimal cell death was observed indicating that cell viability was not affected by either inhibitor (**Figure 2J**). Inhibition of entry was dose-dependent for E-64 (Supplemental Figure S7D), and drug

toxicity was not detected at the doses used (Supplemental Figures S7E and S7F). We observed similar responses to anti-ACE2 antibody, E64, and camostat treatment when cardiac pericytes were infected at an MOI of 1 (Figure S8).

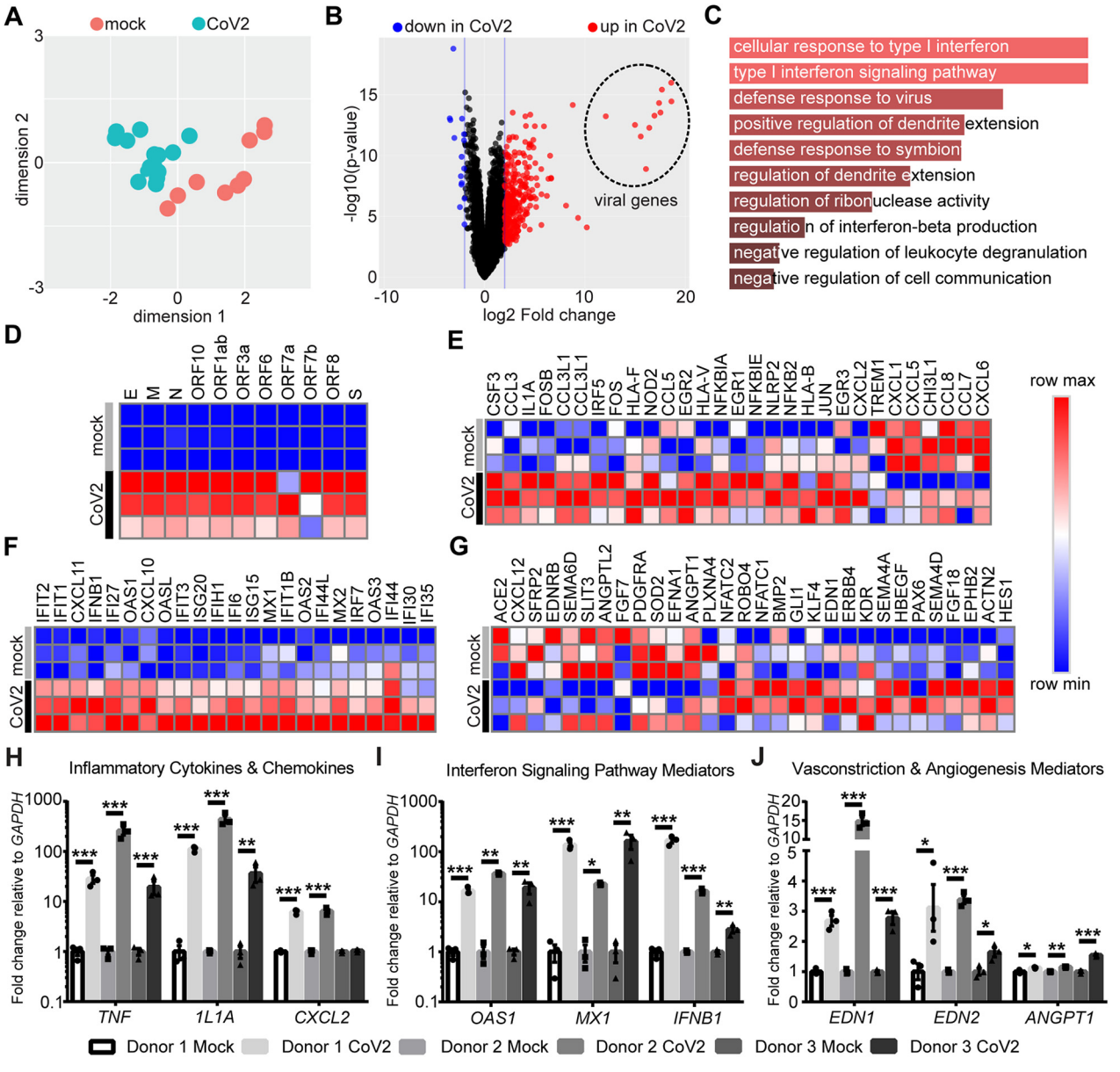
To further test the requirement for endosomal proteases in viral entry, we used bafilomycin-A1, a potent H^+-ATPase inhibitor that prevents endosomal acidification required for the activity of cysteine proteases. Similar to previous entry inhibitor experiments, pericytes were pretreated with bafilomycin for 2 hours, inoculated with high MOI 20 of SARS-CoV-2 mNeonGreen, and collected for flow cytometry 20 hours later. Bafilomycin decreased infection at and above 10 nmol/L (Supplemental Figure S9A) and did not cause loss of pericyte identity (Supplemental Figure S9B) or cell death (Supplemental Figure S9C) at tested concentrations. To focus on early stages of viral entry, we treated pericytes with an anti-SARS-CoV-2 neutralizing monoclonal antibody (mAb SARS2-38, 20 μg/mL)[24] after initial infection. This method allowed us to isolate entry from subsequent rounds of infection and spread in the culture. Primary cardiac pericytes were pretreated with 10 nmol/L bafilomycin for 2 hours, infected with SARS-CoV-2 mNeonGreen, and then treated with anti-SARS-CoV-2 neutralizing mAb or vehicle 4 hours later. Cells were harvested for flow cytometry 8 hours after initial virus exposure (Supplemental Figure S9D). Pericyte infection was diminished in the presence of bafilomycin but not by treatment with the neutralizing antibody (Supplemental Figures S9E and S9F). Together, these results demonstrate that SARS-CoV-2 entry into cardiac pericytes is at least partially dependent on ACE2 and endosomal proteases.

CARDIAC PERICYTE INFECTION ELICITS INFLAMMATORY AND VASOACTIVE RESPONSES AND ALTERS ENDOTHELIAL CELL TRANSCRIPTION. To examine the transcriptomic changes induced by SARS-CoV-2 infection, we inoculated cardiac pericytes from all 3 donors with SARS-CoV-2 mNeonGreen (MOI of 1) and performed RNA sequencing 4 days postinoculation. We observed high levels of infection (**Figure 2C**) and minimal cell death (**Figure 2G** and Supplemental Figure S10) at this time point. Principal component analysis demonstrated independent clustering of mock and SARS-CoV-2-infected cardiac pericytes (**Figures 3A and 3B**). Gene ontology pathway analysis revealed increases in the innate immune response to pathogens, type I interferon (IFN) signaling, and leukocyte degranulation (**Figure 3C**). We observed expression of SARS-CoV-2 viral genes in infected samples from all 3 donors (**Figure 3D**), along with several inflammatory chemokines and cytokines (**Figure 3E**). Similar findings have been reported in other cell types infected with SARS-CoV-2.[25] Of note, chemokine genes, such as *CXCL1*, *CXCL5*, *CXCL6*, *CCL7*, and *CCL8,* were down-regulated in infected cardiac pericytes, suggesting a distinct pattern of immune activation in these cells (**Figure 3E**). Type I IFNs and interferon-stimulated genes (ISGs) showed uniform up-regulation after infection (**Figure 3F**). We also observed up-regulation of vasoactive genes (*EDN1*, *EDN2*) and down-regulation of *ACE2*, a hallmark of SARS-CoV-2 infection.[26] Differential expression of key genes was validated by reverse transcription quantitative polymerase chain reaction (RT-qPCR) (**Figures 3H and 3J**). Given that interleukin (IL)-6 and -8 are elevated in COVID-19 patients,[27] we performed targeted RT-qPCR for the genes encoding these cytokines and demonstrated up-regulation of *IL6* in all 3 donors, whereas *IL8* was only significantly up-regulated in one of the 3 donors (Supplemental Figure S11).

Given that robust up-regulation of IFN signaling and cytokines can lead to cell death, we examined whether inhibiting the inflammatory response in infected pericytes would reduce death of infected cells (**Figure 2G**). TPCA-1, a potent and selective inhibitor of I-kB kinase signaling, has been shown to blunt IFN signaling pathway and reduce production of pro-inflammatory cytokines.[28,29] TPCA-1 treatment led to dose-dependent inhibition of type I IFN response genes and cytokine expression (Supplemental Figure S12A) in infected pericytes and caused no significant cell death in uninfected cells (Supplemental Figure S12B). Nuclear factor kappa-B (NF-κB) inhibition did not inhibit pericyte infection or influence death of infected cells within the first 2 days (Supplemental Figure S12C). However, NF-κB inhibition resulted in a dose-dependent increase in survival and abundance of infected cells 6 days after virus inoculation (Supplemental Figure S12D). These data suggest that NF-κB signaling contributes to the death of infected cardiac pericytes possibly through the generation of inflammatory mediators.

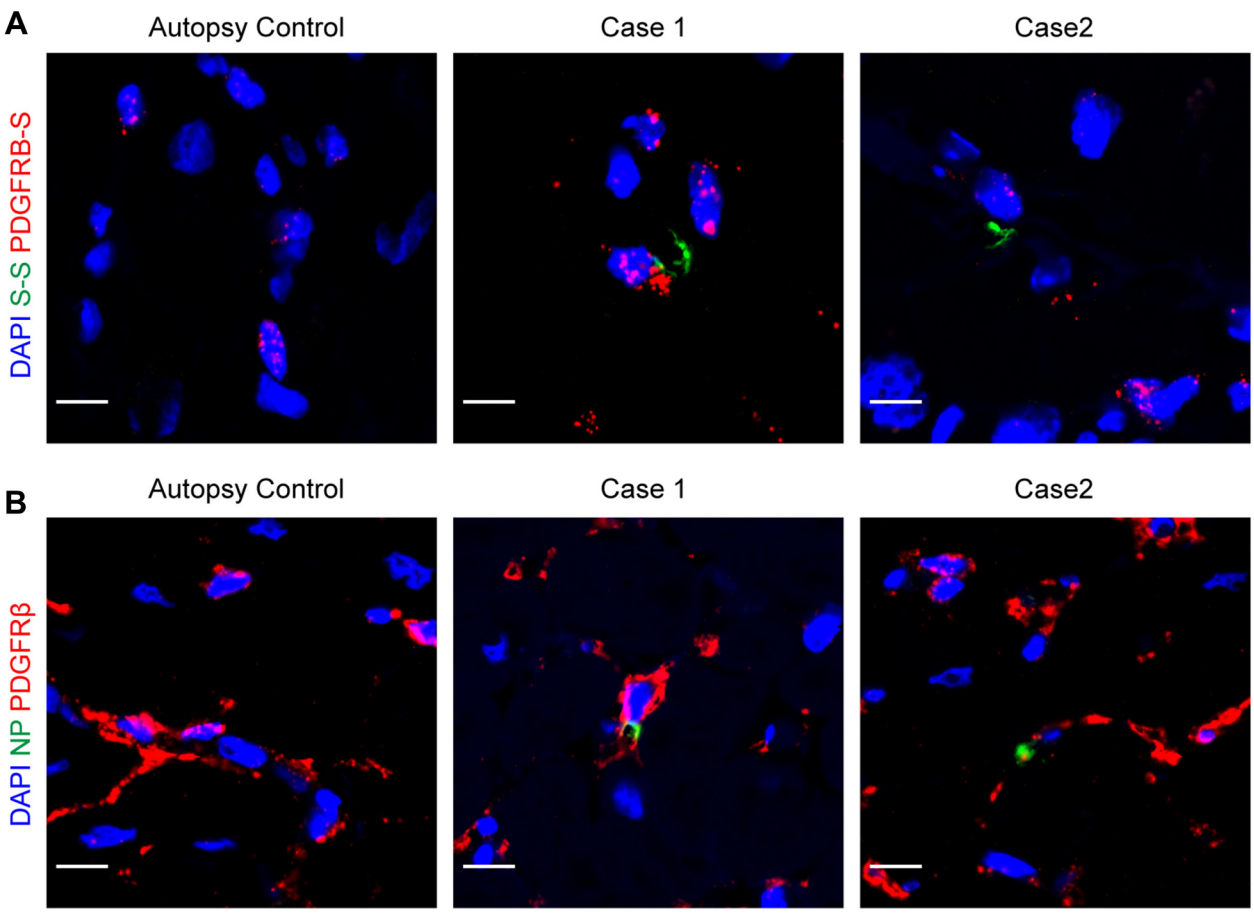
Because pericytes reside in proximity to endothelial cells and are crucial for vascular homeostasis, we next tested the effects of pericyte infection on endothelial cell activation. Pericyte conditioned medium was collected from mock-infected pericytes (Mock Cond Media) or SARS-CoV-2 infected pericytes (CoV2 Cond Media). Fresh pericyte culture medium with supernatant from uninfected Vero-TMPRSS cells was introduced to control for effects of pericyte

FIGURE 3 SARS-CoV-2 Infection Up-regulates Type I IFN Signaling, Inflammatory Pathways, and Vasoactive Genes in Cardiac Pericytes



(A to G) RNA sequencing of pericytes at 4 dpi. Cardiac pericytes from 3 donors were inoculated with SARS-CoV-2 mNeonGreen (5 technical replicates/donor) or mock (3 technical replicates/donor). **(A)** Multidimensional scaling plot with mock **(red)** and SARS-CoV-2 infected (CoV2) **(green)** samples; **dots** represent technical replicates. **(B)** Volcano plot showing differentially expressed genes between mock and infected samples. Each **dot** represents a gene, with **blue** denoting down-regulated genes, **black** denoting genes nonsignificant changes, and **red** denoting up-regulated genes. **(C)** Gene ontology pathway analysis with top 10 up-regulated pathways in infected pericytes. **(D to G)** Heat maps of selected differentially expressed genes of viral origin **(D),** pericyte genes involved in inflammatory response **(E);** response to IFN signaling **(F);** and vascular development, homeostasis, and function **(G). (H to J)** Reverse transcription quantitative polymerase chain reaction analyses of genes encoding inflammatory cytokines and chemokines **(H),** type I interferon signaling pathway components **(I),** and vasoconstriction and angiogenesis mediators **(J); data points** denote separate wells (3 technical replicates/donor) with statistical comparison to mock group for each donor; an equal or unequal variance Student's *t*-test, as determined by an F test for equality of 2 variances: $*P < 0.05$; $**P < 0.01$; $***P < 0.001$, nonsignificant comparisons not shown. GAPDH = glyceraldehyde 3-phosphate dehydrogenase; other abbreviations as in Figure 1.

FIGURE 4 **Detection of SARS-CoV-2 in Pericytes of COVID-19 Cardiac Autopsy Specimens**



(A) RNA in situ hybridization of SARS-CoV-2 *S* sense (S-S) **(green)** with pericyte marker *PDGFRB* sense (PDGFRB-S) **(red)** in the perivascular region of autopsy control (n = 1) and COVID-19–positive formalin-fixed paraffin-embedded specimens (n = 2). **(B)** Immunostaining for the SARS-CoV-2 NP **(green)** and pericyte marker PDGFRβ **(red)** within the perivascular region of autopsy control (n = 1) and COVID-19–positive formalin-fixed paraffin-embedded specimens (n = 2). Abbreviations as in **Figure 1**.

culture media and cellular components of Vero-TMPRSS cells, which are used to propagate virus (Mock). Fresh pericyte culture medium spiked with the same amount of virus as in infected conditioned medium (6.5×10^4 focus forming units/mL) was used to control for effects of SARS-CoV-2 virus on endothelial cells (CoV2). Human umbilical vein endothelial cells were treated with a mix of endothelial cell medium and 1 of the 4 experimental conditions (Mock, CoV2, Mock Cond Media, CoV2 Cond Media) for 24 hours, at which point RNA was harvested for RT-qPCR. We observed an increase in type I IFN response (Supplemental Figure S13A) and chemokine expression (CXCL2 and CCL5) in endothelial cells exposed to CoV2 conditioned media (Supplemental Figure S13B), suggesting that infected pericytes produce soluble mediators that trigger inflammatory responses in cultured endothelial cells. Thrombomodulin (*THBD*) was down-regulated following exposure to CoV2 and CoV2 conditioned media (Supplemental Figure S13C), highlighting that viral exposure may elicit changes in endothelial cell biology in the absence of productive infection. THBD protein expression also appeared to be reduced in infected cardiac slices (Supplemental Figure S13E). These results demonstrate that infection of cardiac pericytes

leads to inflammatory and vasoactive responses in infected pericytes and uninfected endothelial cells that might be relevant to the clinical manifestations and pathology of COVID-19.

SARS-CoV-2 INFECTION IN COVID-19 PATIENTS. We obtained autopsy specimens from 2 myocarditis subjects with confirmed SARS-CoV-2 infection and 1 autopsy heart sample from a subject who did not have COVID-19 as a negative control, which underwent similar time to procurement and preservation procedures (Supplemental Table S1). In situ hybridization targeting the SARS-CoV-2 *S* gene revealed evidence of viral RNA within perivascular regions of both COVID-19 cases that colocalized with *PDGFRB* mRNA (**Figure 4A**). Immunostaining for the SARS-CoV-2 nucleocapsid protein also revealed evidence of viral protein expression in PDGFRβ^{+} cells (**Figure 4B**), suggesting that cardiac pericytes are targets of SARS-CoV-2 infection in humans.

DISCUSSION

We provide multiple lines of evidence that cardiac pericytes are targets of SARS-CoV-2 infection. Infected cardiac pericytes remain viable for several days in culture, suggesting they may indeed serve as a previously hypothesized site of replication and reservoir of virus.[18] Infected pericytes display an inflammatory signature and express vasoactive mediators that could affect vascular function and vasoreactivity and release soluble mediators capable of activating endothelial cells in vitro. Cardiac pericyte cell death at later stages of infection combined with an inflammatory response in endothelial cells could lead to loss of endothelial integrity, endothelial cell dysfunction, basement membrane exposure, and thrombosis. Although pericyte-like cells derived from human neural crest stem cells have been shown to be productively infected by SARS-CoV-2,[13] commercially available pericytes from the brain and placenta were not productively infected by SARS-CoV-2 in this study. It remains unknown whether pericytes from other organs, such as the lungs and kidneys, can be infected and elicit a similar response. Although human autopsy specimens suggest that cardiac pericyte infection can occur in human disease, these studies represent the tissue state at the time of collection, typically late in disease progression, which precludes insights into in vivo viral kinetics and downstream sequelae. Future studies are needed to elucidate the pathophysiological consequences of pericyte infection in vivo and their collective contribution to cardiovascular manifestations of COVID-19.

STUDY LIMITATIONS. While the use of primary human cardiac pericytes allowed us to mechanistically dissect consequences of pericyte infection by SARS-CoV-2 in a relevant human model, in vivo studies are required to address how pericyte infection relates to the systemic, cardiac, and vascular manifestations of COVID-19. Further animal studies are also necessary to define relevant signaling mechanisms between infected pericytes and endothelial cells, including the role of contact-mediated signaling.

CONCLUSIONS

This study identifies human cardiac pericytes as a novel target of SARS-CoV-2. We demonstrate that pericyte infection leads to innate inflammatory response, NF-kB-dependent pericyte cell death, and production of soluble factors that activate endothelial cells. These findings position mural cell infection as a new mechanism contributing to the cardiovascular manifestations of COVID-19.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Elucidating SARS-CoV-2 tropism in the human heart is important for understanding the pathogenesis of COVID-19 cardiovascular manifestations. Additional knowledge and increased awareness of pericyte infection, mechanisms of SARS-CoV-2 cell entry, and outcomes of infected pericytes will provide information necessary for modeling and developing therapeutics for COVID-19.

TRANSLATIONAL OUTLOOK: Human organotypic cardiac slice culture combined with primary pericyte isolation and culture techniques provide an integrated human translational platform for studying cardiac pathology in COVID-19. Future studies can leverage these models to test the cardiac effects of viral inhibitors and potential therapeutics for COVID-19 disease, which can inform the development and use of therapeutics in patients.

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APPENDIX For a supplemental methods, figure and tables, please see the online version of this paper.