SARS-CoV-2 leads to myocardial injury in rhesus macaque

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Dear Editor,

Coronavirus disease 2019 (COVID-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, which induces multiple cardiovascular complications including acute myocardial injury resulting from acute coronary syndrome, myocarditis, stress-cardiomyopathy, arrhythmias, cardiogenic shock, and cardiac arrest. The incidence of myocardial injury in COVID-19 patients was up to 20% or higher in the subpopulation with existing cardiovascular diseases, accounting for 69.4% of mortality. Although the presence of angiotensin-converting enzyme 2 (ACE2) in cardiomyocytes could result in viral myocarditis in COVID-19 patients, the cardiopathology involved in COVID-19 is still unclear.

We aimed to explore the mechanism of myocardial injury in COVID-19 patients using a rhesus macaque model of SARS-CoV-2 respiratory tract infection. First, we analyzed previously published single-nucleus RNA sequencing data of the normal atrial tissue of 16 monkeys. As shown in Supplementary Fig. S1, the main cell types were defined based on published marker genes. Furthermore, the expression of ACE2 and the serine protease TMPRSS2 were investigated. ACE2 was expressed in cell types including pericytes, epicardial cells, and cardiomyocytes. TMPRSS2 was expressed in cells of the cardiac conduction system, endothelial cells, and cardiomyocytes (Fig. 1a). Next, we performed KEGG enrichment analysis using the differentially expressed genes between cells with and without TMPRSS2 expression. Interestingly, among the enriched KEGG pathways, we found that genes up-regulated in the TMPRSS2-expressing cells (e.g., MX1, MX2, IFIH1, DDX58, EIF2AK2) were enriched in the Coronavirus-COVID-19 pathway (Fig. 1b), which includes the genes that are known to be involved in the invasion of SARS-CoV-2 into alveolar epithelial cells (https://www.kegg.jp/keggbin/show_pathway?hsa05171/TMPRSS2/MX1/PK3R1/IFIH1/DDX58/EIF2AK2/ADAM17/MX2/JAK1). These results suggested that TMPRSS2-expressing cells, including cardiomyocytes, may be directly attacked by the virus, potentially causing viral myocarditis.

To explore the possibility of viral myocarditis, six rhesus macaques (3–5 kg, 3–5 years of age) were intratracheally challenged on the first day with 1 × 10^6 TCID50 (50% tissue-culture infectious dose) of SARS-CoV-2, and six age- and body weight-matched macaques served as healthy controls. Seven days post initial infection, the left ventricles were collected. As shown in Fig. 1c, SARS-CoV-2 positive- and negative-strand RNA was found in the infected macaques by RNA in-situ hybridization, indicating that SARS-CoV-2 infection and replication occurred in the ventricular tissue. Viral RNA copies were present at 1 × 10^1.2 copies/g in the SARS-CoV-2 group. The presence of myocarditis was demonstrated by the higher numbers of invaded inflammatory cells in the virus-exposed group (Fig. 1d). Importantly, the typical histopathology of myocarditis with increased interstitial infiltration of CD68^+ macrophages and CD3^+ T lymphocytes was observed in the virus-exposed group, along with increased levels of inflammatory cytokines (Fig. 1e; Supplementary Fig. S2). In addition, cardiac inflammation was accompanied with sporadic cardiac fibrosis in SARS-CoV-2-infected rhesus macaques (Fig. 1f). These results demonstrated the occurrence of viral myocarditis after SARS-CoV-2 infection.

In addition to myocarditis, other etiologies may lead to cardiac injury. Endothelial dysfunction in response to viral infection can lead to coagulation disorder. The activation of the coagulation pathway with the possible development of disseminated intravascular coagulation (DIC) may result in thrombus formation in severe COVID-19 patients. Consistently, we found micro-thrombi in SARS-CoV-2-infected macaques (Fig. 1g) and decreased thrombomodulin (TM) and VE-cadherin (Fig. 1h, i), which promoted coagulation and increased endothelial permeability respectively. We also found increased expression of ICAM-1 and VCAM-1 in the SARS-CoV-2 group, suggesting the propensity for extravasation of inflammatory cells and subsequent myocarditis (Supplementary Fig. S3). Taken together, these results indicated that endothelial dysfunction was associated with thrombotic events and cardiac injury in COVID-19.

Besides cardiomyocytes and endothelial cells, our results showed that T cells, pericytes, and fibroblasts also express ACE2 and TMPRSS2 (Fig. 1a) and may participate in cardiac injury in COVID-19. Activated inflammatory response by T cells may contribute to myocardial dysfunction. ACE2 is highly expressed on pericytes, allowing SARS-CoV-2 to attack these cells and contribute to cardiac damage. Fibroblasts secret collagens and extracellular matrix proteins to promote cardiac fibrosis. In addition, our results showed that some cells express ACE2 but not TMPRSS2, including M1 macrophages and epicardial cells. M1 macrophages mediate inflammatory response via producing pro-inflammatory cytokines in heart failure. Epicardial cells participate in the development of coronary artery disease and atrial fibrillation via structural and electrical remodeling of the atria. This suggests multiple cell types with or without TMPRSS2 may contribute to the cardiac damage in COVID-19.

However, the molecular mechanism of myocardial injury in COVID-19 remains to be elucidated. MAPK, NF-kB, and PI3K signaling pathways induce a myocardial inflammatory response and cardiomyocytes apoptosis in viral myocarditis. Inhibition of MAPK alleviates cardiac function in mice with viral myocarditis. The augmented inflammation of viral myocarditis is mainly associated with NF-kB signaling. PI3K signaling participates in cardiomyocyte autophagy in CVB3 (Coxsackievirus B3) infection. Taken together, these findings indicate that MAPK, NF-kB, and PI3K signaling are involved in myocardial injury and deserve further investigation in SARS-CoV-2-induced myocardial injury.

Previous studies have demonstrated that cardiomyocytes are directly infected by SARS-CoV-2 in vitro. Human induced pluripotent stem cell-derived cardiomyocytes (iPS-CMs) are susceptible to SARS-CoV-2 infection via ACE2, and pathologic responses including apoptosis, oxidative stress, and immune response were identified in iPS-CMs challenged with SARS-CoV-2. Furthermore, SARS-CoV-2 can infect cardiomyocytes in 3D cardiac...
In this study, using SARS-CoV-2-infected rhesus macaques, we demonstrated the occurrence of viral myocarditis in vivo for the first time. This particular animal model and time point were chosen to mimic the clinical signs of COVID-19 patients. At seven days post infection, viral RNA copies (~1 × 10^4 copies/mL) were detected in the lungs, which also showed ground-glass opacity and obscure markings in the upper lobe of the right lung by X-ray. Our findings on SARS-CoV-2-induced cardiac injury are particularly important, given that this animal model is used for vaccine testing and drug development.
In conclusion, we found that SARS-CoV-2-infected rhesus macaques showed viral myocarditis, where inflammation and endothelial injury jointly resulted in cardiac damage. Our results suggest that the possibility of cardiac injury requires close monitoring in COVID-19 patients, particularly in those with pre-existing cardiovascular conditions. The potential myocardial damage caused by future therapeutic agents deserves careful investigation.

DATA AVAILABILITY
All data in this article and supplementary materials are publicly available to authorized users.

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AUTHOR CONTRIBUTIONS
J.W., J.N.L., and W.M.T. initiated project, Y.F.F., X.M.S., Y.H., M.L., and X.X.G. performed experiments and analyzed the data. Z.W.L. analyzed the single-nucleus RNA sequencing data. W.D., C.Q., and J.N.L. provided rhesus macaques tissue samples, X.M.S., Y.F.F., and J.W. wrote the manuscript.

ADDITIONAL INFORMATION
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REFERENCES
1. Guo, T. et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). JAMA Cardiol. 5, 811–818 (2020).
2. Ma, S. et al. Single-cell transcriptomic atlas of primate cardiopulmonary aging. Cell Res. 31, 415–432 (2021).
3. Deng, W. et al. Ocular conjunctival inoculation of SARS-CoV-2 can cause mild COVID-19 in rhesus macaques. Nat. Commun. 11, 4400 (2020).
4. Chang, H. et al. The PI3K/Akt/mTOR pathway is involved in CVB3-induced autophagy of HeLa cells. Int. J. Mol. Med. 40, 182–192 (2017).
5. Bojkova, D. et al. SARS-CoV-2 infects and induces cytotoxic effects in human cardiomyocytes. Cardiovasc Res. 116, 2207–2215 (2020).