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SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion

Xinling Wang¹, Wei Xu¹, Gaowei Hu¹, Shuai Xia¹, Zhiping Sun¹, Zezhong Liu¹, Youhua Xie¹, Rong Zhang¹, Shibo Jiang^{1,2} and Lu Lu¹ Cellular & Molecular Immunology _____; <https://doi.org/10.1038/s41423-020-0424-9>

COVID-19, the novel coronavirus disease caused by SARS-CoV-2 and outbreaked at the end of 2019 in Wuhan, China,¹ becomes a worldwide pandemic. SARS-CoV-2 belongs to the betacoronavirus genus and has 79.5% identity to SARS-CoV. SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) as its host entry receptor.² The clinical manifestations of COVID-19 include pneumonia, diarrhea, dyspnea, and multiple organ failure. Interestingly, lymphocytopenia, as a diagnostic indicator, is common in COVID-19 patients. Xiong et al. found upregulation of apoptosis, autophagy, and p53 pathways in PBMC of COVID-19 patients.³ Some studies reported that lymphocytopenia might be related to mortality, especially in patients with low levels of CD3⁺, CD4⁺, and CD8⁺ T lymphocytes.^{4,5} Lymphocytopenia was also found in the Middle East respiratory syndrome (MERS) cases. MERS-CoV can directly infect human primary T lymphocytes and induce T-cell apoptosis through extrinsic and intrinsic apoptosis pathways, but it cannot replicate in T lymphocytes.⁶ However, it is unclear whether SARS-CoV-2 can also infect T cells, resulting in lymphocytopenia.

To address this question, we evaluated the susceptibility of T lymphocytes to SARS-CoV-2 infection. To accomplish this, pseudotyped SARS-CoV and SARS-CoV-2 were packaged based on methods described previously.⁷ The pseudoviruses could infect permissive cells (293T/ACE2 and Huh7 cells) expressing the ACE2 receptor, but could not infect nonpermissive cells (HeLa cells) (Fig. 1a). We used pseudovirus with equal infectivity to 293T/ACE2 cells (Fig. 1c) to infect two T lymphocyte cell lines, MT-2 and A3.01, with very low, or close to negative, expression level of hACE2 mRNA (Fig. 1b). Surprisingly, over several replicates, we saw that the T-cell lines were significantly more sensitive to SARS-CoV-2 infection when compared with SARS-CoV (Fig. 1c). In other words, these results tell us that T lymphocytes may be more permissive to SARS-CoV-2 infection and less permissive for SARS-CoV infection, similar to the findings in a previous study.⁶ Therefore, it is plausible that the S protein of SARS-CoV-2 might mediate potent infectivity, even on cells expressing low hACE2, which would, in turn, explain why the transmission rate of SARS-CoV-2 is so high. It is also possible that other receptors mediate the entry of SARS-CoV-2 into T cells, such as CD147, present on the surface of T lymphocytes,⁸ which was recently reported to be a novel invasive route for SARS-CoV-2.⁹

To assess if SARS-CoV-2 enters T lymphocytes through non-receptor-mediated endocytosis, we used EK1 peptide which has been shown to inhibit SARS-CoV-2 spike protein (S) mediated cell-cell fusion and pseudovirus infection.^{7,10} Specifically, it

inhibits receptor-mediated infection by interacting with HR1 to block the formation of the six-helix bundle (6-HB), further inhibiting fusion between viral and target cell membranes. We found that the EK1 peptide had significant inhibitory activity against SARS-CoV-2 pseudoviruses on MT-2 cells (Fig. 1d), suggesting that virus entry depends on receptor-mediated fusion. However, only a high concentration (40 μ M) of EK1 had inhibitory activity on MT-2 cells. Meanwhile, the IC₅₀ value of EK1 was 2.38 μ M on 293T/ACE2 cells.¹⁰ These results suggest that SARS-CoV-2 can also enter T lymphocytes through the receptor-mediated endocytosis pathway. To clarify, we performed a SARS-CoV-2 S-mediated cell-cell fusion assay according to previous studies.^{7,10} After 48 h of coculture, 293T cells expressing SARS-CoV-2 S protein fused with MT-2 cells. Compared with unfused cells, the fused cells clustered together and appeared as a large faint green fluorescent mass. In contrast, no fused cells were found in the SARS-CoV coculture (Fig. 1e). Therefore, it can be concluded that SARS-CoV-2 might infect T cells through S protein-mediated membrane fusion.

To further determine the susceptibility of MT-2 cells to live virus, we used SARS-CoV-2 to infect MT-2 cells and detected the SARS-CoV-2 nucleoprotein (NP) in the cells as reported previously.⁶ Notably, several MT-2 cells were infected with SARS-CoV-2 (Fig. 1f). Quantitatively, the percentage of SARS-CoV-2 NP-positive MT-2 cells was 23.11% higher than that of uninfected cells at 24 h post infection, which is about 4.6-fold of the portion at 1 h (Fig. 1f). This result means that the virus penetrated MT-2 cells at 24 h and infected them.

Given that MERS-CoV can efficiently infect, but not replicate, in T lymphocytes,⁶ we further detected the number of viral genome copies at different time points post infection to explore the replication characteristics of SARS-CoV-2 in MT-2 cells. Similar to MERS-CoV, SARS-CoV-2 failed to replicate in MT-2 cells (Fig. 1g). The number of viral genome copies at 6 h was significantly higher than other time points in the cell lysate, but always remained steady at all time points in the supernatants. These results suggest that SARS-CoV-2 may enter MT-2 cells at 6 h post infection, but does not replicate, and then the viral RNA degrade. In supernatants, the detected viral copies might be the background of the residual virions, similar to the results of the previous MERS-CoV study (Fig. 1g).⁶

Based on the results of pseudovirus and live virus infection, here we proved that (1) SARS-CoV-2 could infect T cells, (2) SARS-CoV-2 infected T cells through receptor-dependent, S protein-mediated membrane fusion, and (3) infection could be inhibited by EK1

¹Key Laboratory of Medical Molecular Virology (MOE/NHC/CAMS), School of Basic Medical Sciences and Biosafety Level 3 Laboratory, Fudan University, Shanghai, China and

²Lindsley F. Kimball Research Institute, New York Blood Center, New York, NY, USA

Correspondence: Shibo Jiang (shibojiang@fudan.edu.cn) or Lu Lu (lul@fudan.edu.cn)

These authors contributed equally: Xinling Wang, Wei Xu, Gaowei Hu

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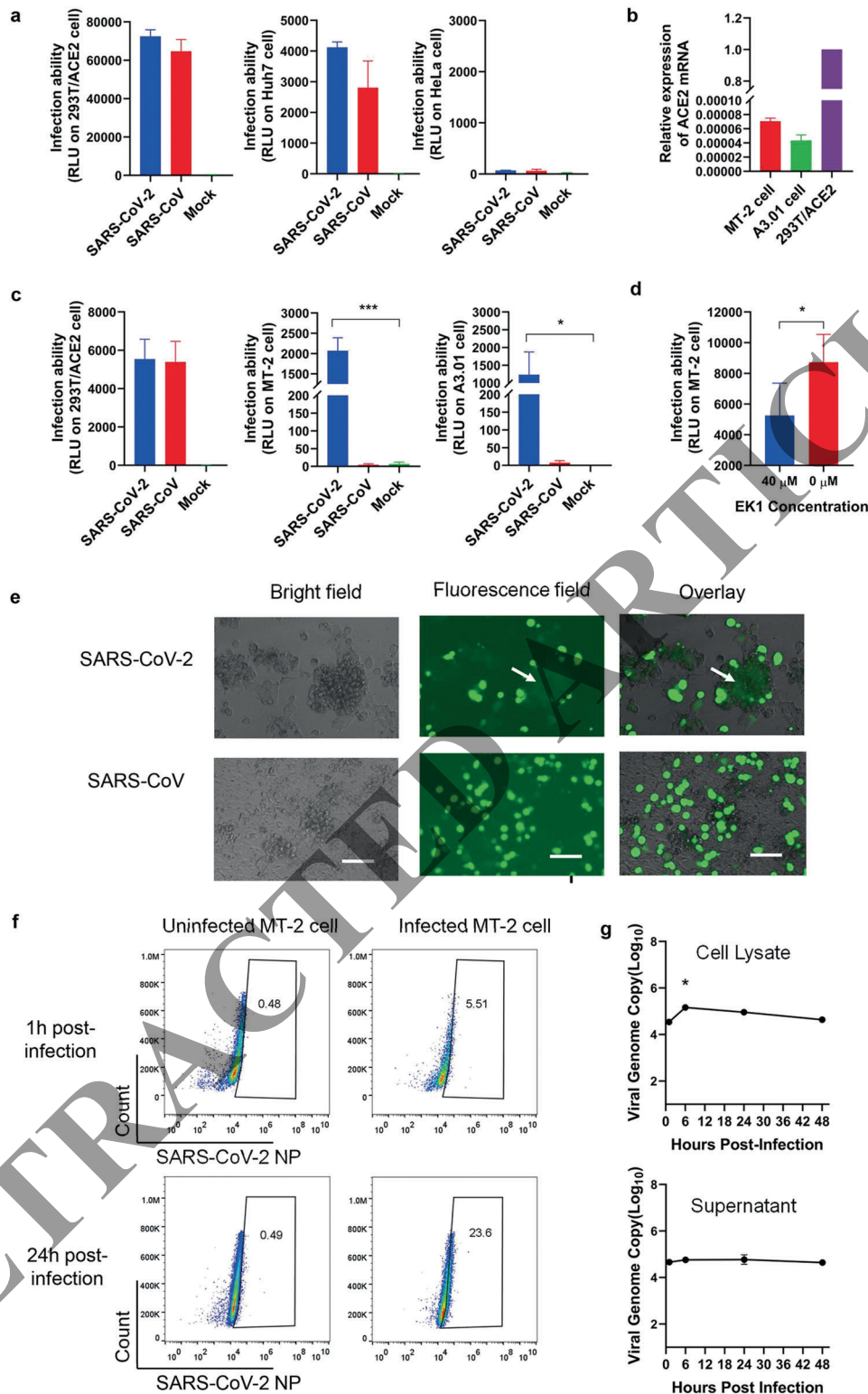


Fig. 1 Sensitivity of T lymphocytes to SARS-CoV-2 infection. **a** Infection of pseudotyped SARS-CoV-2 and SARS-CoV. 293T/ACE2 and Huh7 are permissive cells, while HeLa is a nonpermissive cell line. **b** Expression of ACE2 mRNA in T cells. 293T/ACE2 cells used as a control. **c** Infection of pseudotyped SARS-CoV-2 and SARS-CoV on T lymphoid cell lines. **d** Inhibition of EK1 peptide on pseudotyped SARS-CoV-2 on MT-2 cells. **e** SARS-CoV-2 S-mediated cell-cell fusion on MT-2 cells. MT-2 cells were cocultured with 293T/SARS-CoV-2/EGFP cells. Cell-cell fusion was photographed under an optical microscope with fluorescence or visible light. Fused cells were indicated with a white arrow. Scale bars 800 μ m. **f** Detection of SARS-CoV-2 NP-positive cells with flow cytometry. MT-2 cells were infected with SARS-CoV-2 at 1TCID₅₀ per cell, respectively. Cells were fixed and permeabilized at 24 and 48 h post infection and immunolabeled for detection of SARS-CoV-2 NP. **g** Infection of MT-2 cells by SARS-CoV-2 is abortive. Cells were inoculated with SARS-CoV-2 at 1TCID₅₀ per cell and harvested at 1, 6, 24, and 48 h post infection. Cell lysate (above) and supernatant (below) were collected to detect the viral N gene with RT-qPCR. In panels, bars and error bars represent means and standard deviations. Statistical analyses were performed using the unpaired *t* test. **p* < 0.05

peptide. However, we observed a very low expression level of hACE2 in T cells; therefore, we further proposed that a novel receptor might mediate SARS-CoV-2 entry into T cells. Similar to MERS-CoV, SARS-CoV-2 infection of T cells is abortive. A recent study reported that viral reads barely displayed in PBMC samples from COVID-19 patients through transcriptome sequencing of RNAs. Thus, it was inferred that SARS-CoV-2 could not infect PBMCs. However, the transcriptomic characteristics of PBMCs were detected and analyzed from three patients. Two SARS-CoV-2 reads were detected in one patient's PBMCs, and zero reads in another.³ This result could be attributed to nonproductive replication of SARS-CoV-2 in T lymphocytes, with little viral genome in PBMCs possibly degrading in the sample collection and RNA extraction process. Thus, the questions of SARS-CoV-2 infection and replication in primary T cells and whether the infection induces apoptosis in T cells still need further research, potentially evoking new ideas about pathogenic mechanisms and therapeutic interventions.

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ADDITIONAL INFORMATION

Competing interests: The authors declare no competing interests.

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