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SARS-CoV-2 interaction with Siglec-1 mediates *trans*-infection by dendritic cells

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Antigen-presenting cells (APCs) may be resistant to SARS-CoV-2 infection but still contribute to viral pathogenesis. Lectins such as sialic acid-binding Ig-like lectin 1 (Siglec-1/CD169) mediate the attachment of viruses to APCs. Here, we show that APCs effectively capture SARS-CoV-2 within compartments via recognition of Siglec-1. This receptor interacts with sialylated gangliosides on membranes of SARS-CoV-2 variants, as previously shown for retroviruses or filoviruses [1]. Blockage of Siglec-1 on monocyte-derived dendritic cells (MDDCs) decreased SARS-CoV-2 viral transfer or *trans*-infection to bystander target cells. However, monocyte-derived macrophages (MDMs) capturing SARS-CoV-2 via Siglec-1 did not transmit infectious particles. The presence of pulmonary APCs co-expressing Siglec-1 and SARS-CoV-2 corroborated these findings *in vivo*.

We used the methodology described in the Supplementary Methods to dissect the contribution of Siglec-1 to SARS-CoV-2 pathogenesis. Siglec-1 expression is induced on APCs upon IFN- α or LPS exposure and increased in myeloid cells of COVID-19 patients [2]. Here, we tested whether this lectin could bind SARS-CoV-2 via recognition of sialylated gangliosides on viral membranes. APCs captured incoming SARS-CoV-2 in cellular compartments, eventually leading to viral degradation (Supplementary Fig. 1,2). Raji B cell lines transfected with different lectins (Supplementary Fig. 3A) were pulsed with SARS-CoV-2, washed and assessed by ELISA to measure cell-associated viral nucleocapsid content (Fig. 1A). While Raji cells transfected with wild-type Siglec-1 captured SARS-CoV-2, cells transfected with DC-SIGN, Siglec-5, Siglec-7 or devoid of these lectins did not (Fig. 1A). Viral uptake via Siglec-1 relied on the recognition of sialylated ligands, as observed with Raji cells transfected with the Siglec-1 mutant R116A, which lacks sialic acid recognition capacity and did not trap SARS-CoV-2 (Fig. 1A). Raji cells pretreated with the α -Siglec-1

monoclonal antibody (mAb) 7-239 reduced SARS-CoV-2 uptake (Supplementary Fig. 3B). SARS-CoV-2 variants were equally trapped via Siglec-1 but not by the mutated Siglec-1 R116A, indicating that sialic acid recognition is critical (Fig. 1B). Super-resolution microscopy of SARS-CoV-2 confirmed that GM1, one of the sialyllactose-containing gangliosides interacting with Siglec-1 [1], was detectable on 74% of virus particles (Fig. 1C). MDMs and MDDCs treated with IFN- α to induce Siglec-1 expression displayed higher SARS-CoV-2 uptake than nonactivated cells (Fig. 1D), and uptake was blocked by the α -Siglec-1 mAb 7-239 (Fig. 1E). These complementary approaches identified Siglec-1 as a central molecule mediating SARS-CoV-2 uptake via sialic acid recognition.

Once Siglec-1 binds to HIV-1 or Ebola viruses, receptors polarize and engulf particles within viral-containing compartments (VCCs) that are continuous with the plasma membrane and connected to the extracellular space [1]. To elucidate whether Siglec-1 also recruits SARS-CoV-2 to VCCs, we used confocal and electron microscopy and found Siglec-1-positive VCCs containing viral particles on activated MDDCs (Fig. 1F, G and Movie 1). Siglec-1 has a dual role in enhancing infectivity, either facilitating fusion on APCs, as is the case for the Ebola virus, or mediating transmission to other target cells in *trans*, as is the case for retroviruses [1]. Since SARS-CoV-2 fusion on APCs is limited (Supplementary Fig. 1B), we explored the relevance of viral *trans*-infection. Raji cells were exposed to SARS-CoV-2, washed and cocultured with targets expressing or not expressing ACE2 (Fig. 1H). Raji Siglec-1 cells cocultured with ACE2-expressing cells released higher amounts of SARS-CoV-2 than cells expressing the R116A mutant (Fig. 1H). A minimal viral amount was detected in cocultures with cells lacking ACE2, excluding SARS-CoV-2 replication on Raji cells or the release of initially trapped viruses (Fig. 1H). IFN- α -treated MDDCs cocultured

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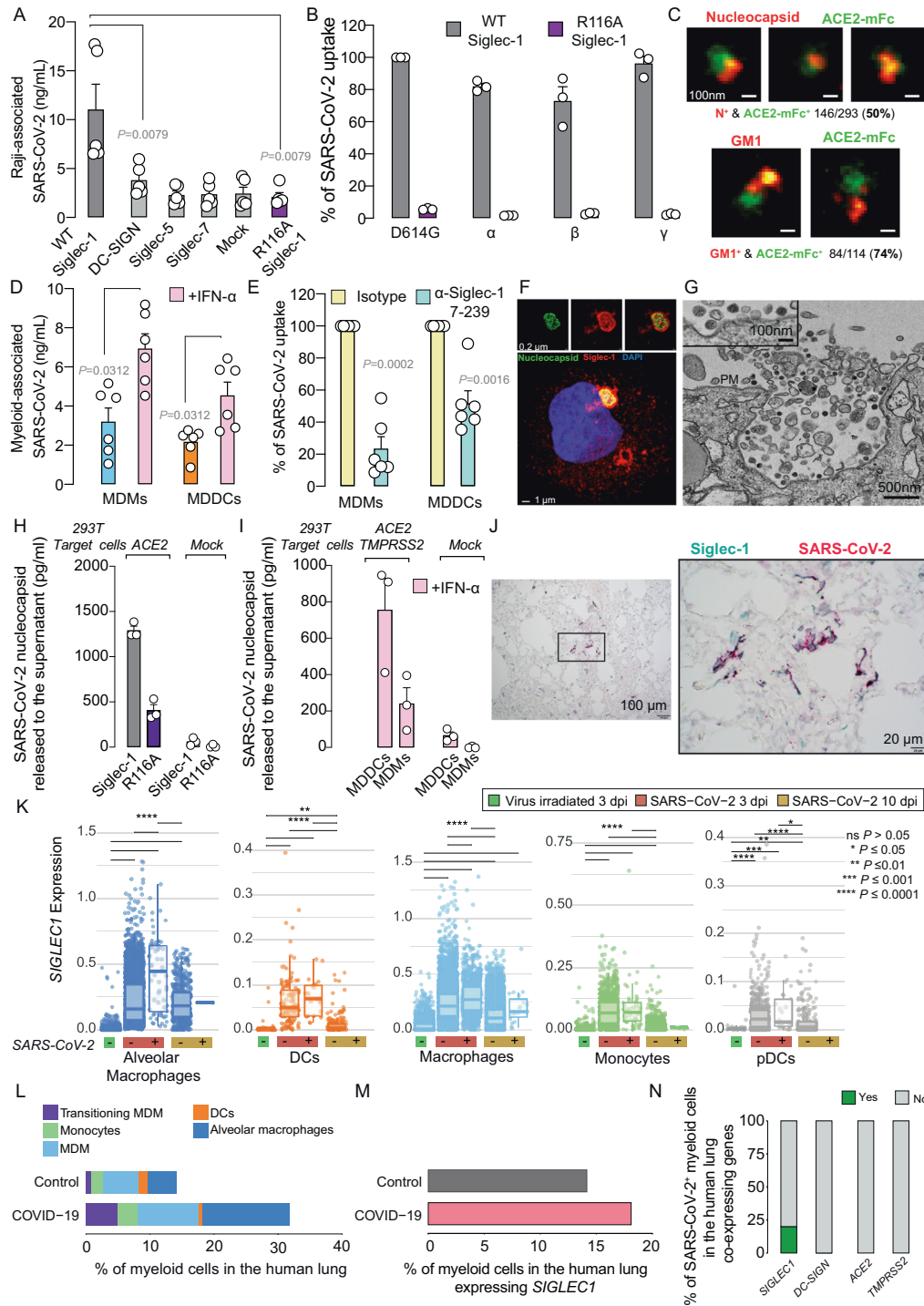


Fig. 1 SARS-CoV-2 trapped via Siglec-1 on MDDCs mediates viral *trans*-infection of target cells, and in pulmonary tissues, SARS-CoV-2 is detected on APCs expressing Siglec-1. **A** Uptake of SARS-CoV-2 by Raji B cells measured by ELISA from two experiments. **B** Percentage of SARS-CoV-2 variant uptake from one experiment. Values are normalized to D614G strain uptake by Siglec-1 cells, set at 100%. **C** Superresolution microscopy of SARS-CoV-2 stained with α -nucleocapsid (red) and ACE2-mFc (green). The percentage of costaining is shown. **D** Uptake of SARS-CoV-2 by MDMs and MDDCs activated or not with IFN- α for 48 h from two experiments. **E** Percentage of viral uptake inhibition of IFN- α -treated APCs preincubated with mAbs from two experiments. Values are normalized to viral uptake by isotype-treated cells, set at 100%. **F**, **G** Confocal and electron microscopy of VCCs in LPS- and IFN- α -activated MDDCs exposed to SARS-CoV-2. See Movie 1. PM, plasma membrane. **H**, **I** Transmission of SARS-CoV-2 by indicated cells. APCs exposed to SARS-CoV-2 were cocultured with HEK-293T cells expressing ACE2 or not. Viral release from one experiment was measured with an ELISA. **J** Costaining with α -Siglec-1 and α -SARS-CoV-2 nucleocapsid antibodies in the lungs of an infected rhesus macaque. **K** *SIGLEC1* expression on AGM APCs containing or not containing SARS-CoV-2 RNA. Boxplots show the median, the first and third quartiles and whiskers. **L**, **M** Single-cell analysis of pulmonary myeloid cells of 16 COVID-19 patients and 3 controls and APCs expressing *SIGLEC1*. **N** Myeloid cells of COVID-19 patients containing viral RNA and coexpressing *SIGLEC1*, *DC-SIGN*, *ACE2* or *TMPRSS2*. **A**, **B**, **D**, **E**, **H**, and **I** show mean values and SEMs. Statistical differences were assessed with the Mann-Whitney *t* test (**A**), Wilcoxon-matched paired *t* test (**D**), one-sample *t* test (**E**), Mann-Whitney-Wilcoxon test with Bonferroni correction (**K**), scCODA (**L**), chi-square test (**M**; $P = 1.045e^{-11}$) or Bonferroni-adjusted chi-square test (**N**; $P = 0.0272624$)

with ACE2- and TMPRSS2-expressing target cells released higher amounts of SARS-CoV-2 than MDMs (Fig. 1I). Experiments with nonreplicative HIV-1 pseudotyped with SARS-CoV-2 spike confirmed Siglec-1 transmission of SARS-CoV-2 via ACE2-dependent fusion on target cells (Supplementary Fig. 4). Hence, SARS-CoV-2 retention on MDDCs via Siglec-1 allows *trans*-infection.

Lung immunohistochemistry analysis from a SARS-CoV-2-infected rhesus macaque used in a previous study [3] confirmed the codetection of Siglec-1 and viral nucleocapsid in cells with myeloid morphology (Fig. 1J). Single-cell RNA sequencing data previously collected in African green monkeys (AGMs) allowed us to focus on pulmonary APCs (Supplementary Fig. 5), where SARS-CoV-2 infection upregulated *SIGLEC1* expression 3 days post-inoculation (dpi) (Supplementary Fig. 6A). Viral RNA was detected in high numbers at 3 dpi, but the levels decreased at 10 dpi (Supplementary Fig. 6B), consistent with the resolution of infection in this mild COVID-19 model. When we compared the expression of *SIGLEC1* on APCs without or with associated viral RNA, only alveolar and interstitial macrophages containing viral RNA significantly increased *SIGLEC1* expression (Fig. 1K). Thus, infection triggers *SIGLEC1* expression on all APCs, but this phenomenon is not linked to the detection of cell-associated viral RNA. We next analyzed the most detailed single-cell atlas [4] of human lungs of COVID-19 patients with advanced disease and controls (Supplementary Fig. 7A–B). Here, APCs were enriched for viral RNA but lacked ACE2 and TMPRSS2 viral entry receptors (Supplementary Fig. 7C). Pulmonary myeloid cells were enriched in COVID-19 patients as compared to those in controls (Fig. 1L; Supplementary Fig. 8A–E), and *SIGLEC1*-expressing APCs were also enriched (Fig. 1M; Supplementary Fig. 8F). Twenty percent of pulmonary APCs expressing viral RNA also coexpressed *SIGLEC1*, whereas none coexpressed other lectins or viral receptors (Fig. 1N). These results corroborated the presence of the Siglec-1 receptor or transcripts on APCs containing SARS-CoV-2 *in vivo*. Targeting Siglec-1 or *trans*-infection [5] could offer cross-protection against SARS-CoV-2 and other enveloped viruses that exploit APCs for viral dissemination and lead to the development of new broad-spectrum antivirals for future outbreaks.

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AUTHOR CONTRIBUTIONS

Conceived and designed the experiments: B.C., J.M.-P., J.V.-A., and N.I.-U. Performed experiments and provided key reagents: D.P.-Z., J.M.-B., J.R., M.E., D.R.-R., C.R., M.S., M.P., S.G., M.Pa., J.C., E.B., E.R., I.E., X.M., M.N., B.T., F.T.-F., J.B., J.S., J.V.-A., and N.I.-U. Analyzed and interpreted the data: D.P.-Z., J.M.-B., J.R., M.E., D.R.-R., C.R., M.S., M.S., M.P., S.G., J.C., E.B., E.R., I.E., X.M.-T., M.N., R.P., B.T., F.T.-F., I.B., V.G., J.C., J.B., A.T., H.H., J.S., B.C., J.M.-P., J.V.-A., and N.I.-U. Wrote the paper: D.P.-Z., J.M.-B., J.R., M.E., D.R.-R., J.V.-A., and N.I.-U.

COMPETING INTERESTS

A patent application related to this work has been filed (US 63/152,346). Unrelated to the submitted work, J.C., J.B., and B.C. are founders and shareholders of Albaluna Therapeutics, S.L.; B.C. is a founder and shareholder of AELIX Therapeutics, S.L.; H.H. is cofounder and shareholder of OmniScope Limited; J.M.-P. reports institutional grants and educational/consultancy fees outside the submitted work from AbiVax, Astra-Zeneca, Gilead Sciences, Grifols, Janssen, Merck and ViiV Healthcare; and N.I.-U. reports institutional grants from Pharma Mar, Dentaïd, Hipra, Grifols and Palobiofarma. The authors declare that no other competing financial interests exist.

ADDITIONAL INFORMATION

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