PSGL-1 blocks SARS-CoV-2 S protein-mediated virus attachment and infection of target cells

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Abstract

P-selectin glycoprotein ligand-1 (PSGL-1) is a cell surface glycoprotein that binds to P-, E-, and L-selectins to mediate the tethering and rolling of immune cells on the surface of the endothelium for cell migration into inflamed tissues. PSGL-1 has been identified as an interferon-γ (INF-γ)-regulated factor that restricts HIV-1 infectivity, and has recently been found to possess broad-spectrum antiviral activities. Here we report that virion incorporation of PSGL-1 on SARS-CoV and SARS-CoV-2 pseudovirions blocks S protein-mediated virus attachment and infection of target cells. These findings suggest that PSGL-1-imprinted non-infectious viral particles could serve as a live attenuated vaccine for SARS-CoV-2 infection.

The ongoing coronavirus disease 2019 (COVID-19) is a global pandemic afflicting more than 3 million people in over 200 countries and territories, resulting in more than 235,000 deaths as of May 1, 2020. Currently, there are no effective treatments or vaccines. Understanding virus-host interactions is critical for developing novel therapeutics and vaccines. P-selectin glycoprotein ligand-1 (PSGL-1, also known as SELPLG or CD162) is a human protein recently identified to possess broad-spectrum antiviral activity [1]. PSGL-1 binds to the selectin family of proteins, P-, E-, and L-selectin [2-4], and mediates immune cell tethering and rolling on the surface of endothelium to promote cell migration into inflamed tissues [5-8]. In the context of viral infection, PSGL-1 has been identified as an IFN-γ-regulated inhibitory factor involved in blocking HIV-1 infectivity [7,9], and was recently found to possess broad-spectrum antiviral activity [1], blocking viral infections through steric hindrance of particle attachment to target cells [1,10].

The coronavirus spike (S) proteins play an essential role in viral entry by binding the cell-surface receptor on target cells and mediating the fusion between viral and cellular membranes during virus entry [11]. The S protein is also the target of neutralizing antibodies generated by the infected host. Because of its central role in virus infection and adaptive immunity, the S protein is a prime target for the development of antiviral therapeutics and vaccines. In addition to the adaptive arm of the host immune response, viral infections trigger an innate immune response, largely induced by IFN, that sets up an antiviral state. Hundreds of IFN-stimulated genes (ISGs) are induced by viral infection [12-14]. While the role of some ISGs in blocking the replication of particular viruses has been well established, the vast majority of ISGs have not been characterized. Because of the significance of host innate immunity in viral transmission and replication within and between hosts, there is an unmet need to understand these antiviral inhibitory factors in detail.

Previous studies have demonstrated that PSGL-1 can be incorporated into HIV-1 virions, and its virion incorporation subsequently blocks viral infectivity [1,10]. To investigate the ability of PSGL-1 to restrict coronavirus infection, we first established a lentiviral vector-based coronavirus pseudovirus infection system [15], in which the S proteins from either SARS-CoV or SARS-CoV-2 were used to pseudotype lentiviral particles (**Fig. 1a** and **1d**). Using this system, we assembled particles in the presence or absence of PSGL-1 [1], and then used the particles to infect target Vero cells, which endogenously express the primary receptor, angiotensin converting enzyme 2 (ACE2) [16,17], for SARS-CoVs. For the production of SARS-CoV pseudoviruses, we found that expression of PSGL-1 in viral producer cells had a minor effect on virion release (**Fig. 1b**), consistent with the previous finding that PSGL-1 expression has minimal effects on viral release [1]. However, the infection of Vero cells by PSGL-1-imprinted particles was completely abrogated (**Fig. 1c**), demonstrating the abilities of PSGL-1 to block the infectivity of SARS-CoV S-bearing virions.

We further tested the effect of PSGL-1 on the infectivity of lentiviral particles pseudotyped with the SARS-Cov-2 S protein. As shown in **Fig. 1e**, PSGL-1 expression in viral producer cells had only a minor effect on virion release. In our initial infection assays in Vero cells, we found that particles pseudotyped with SARS-CoV-2 S protein had much lower infectivity than those pseudotyped with SARS-CoV S protein. To resolve this technical issue, we developed a more sensitive reporter system, in which a luciferase reporter (Luc) gene was expression from the HIV-1 LTR in the presence of co-expressed HIV-1 Tat protein [18] (**Fig. 1d**). A major advantage of this system is that high-level Luc expression can be achieved upon transactivation by co-expressed Tat protein following viral entry, which minimizes non-specific Luc background from

non-productive viral entry [19]. Using this system, we found that the infectivity of the SARS-Cov-2 pseudovirus is also potently inhibited by the expression of PSGL-1 in the virus-producer cells (**Fig. 1f**). Together, these results suggest that the presence of PSGL-1 on virion particles can inhibit the binding of S proteins to their cellular receptors.

To validate this conclusion, we performed a virion attachment assay, and observed that the lentiviral particles pseudotyped with SARS-CoV or SARS-CoV-2 S protein produced from PSGL-1-expressing cells were impaired in their ability to attach to target cells (**Fig. 2**), These results demonstrate that the presence of PSGL-1 on virus particles can structurally hinder S protein interaction with the target cells, consistent with our previous studies of PSGL-1 and HIV-1 infection [1,10].

In this report, we demonstrate that the expression of PSGL-1 in virus-producer cells can block the infectivity of virions bearing the S protein of either SARS-CoV or SARS-CoV-2, a phenotype shared among several other viruses (e.g., HIV-1, murine leukemia virus, and influenza virus) found to be sensitive to PSGL-1 restriction [1]. PSGL-1 has been suggested to be expressed in certain lung cancer cells [20]. However, it remains to be determined whether PSGL-1 is expressed in the target cells of SARS coronaviruses in the lungs, and, if so, whether its expression can impact viral infection. It also remains unknown whether coronaviruses possess a mechanism for antagonizing PSGL-1. In HIV-1 infection, the viral accessory protein Vpu and Nef have been shown to antagonize PSGL-1 on CD4 T cells through surface downregulation and intracellular degradation [1]. Interestingly, a recent study suggested that SARS-CoV-2 may also enter and infect human CD4 T cells, and express viral genes in T cells. Although a spreading viral infection was not observed in T cells [21], infection of T cells by SARS-CoVs could potentially occur in the scenario of PSGL-1-mediated binding of T cells to inflamed human airway endothelium [22,23]. Nevertheless, it remains to be determined whether there is a role for PSGL-1 in blocking SARS-CoV-2 spreading infection in human cells that express PSGL-1.

Our discovery of PSGL-1 inactivation of SARS-CoV and SARS-CoV-2 pseudoviruses suggest that virion incorporation of PSGL-1 could serve as a new approach for developing safer live-attenuated SARS-CoV-2 vaccines. In addition, PSGL-1-inactivated coronaviruses or virus-like particles can largely maintain the native structure of viral surface proteins, but are blocked for binding to cell receptors. Such particles may stimulate immune responses different from chemically inactivated coronaviruses [24,25]. It remains to be determined whether PSGL-1-imprinted particles can minimize the product of suboptimal antibodies that are frequently associated with vaccine-mediated immunopathology [25] and antibody-dependent enhancement of viral infection (ADE) [26].

Methods

Virus assembly

The SARS-CoV S protein expression vector was kindly provided by Dr. Gary R. Whittaker. The SARS-CoV-2 S protein expression vector was purchased from Sinobiological. For the production of GFP reporter lentiviral particles pseudotyped with SARS-CoV-S, SARS-CoV-S expression vector (0.5 μg), pCMVΔR8.2 (7.5 μg), and pLKO.1-puro-TurboGFP (10 μg) were cotransfected with either pCMV3-PSGL-1 (2 μg), or pCMV3-Empty (2 μg) as previously described [1]. For the production of luciferase reporter lentiviral particles pseudotyped with SARS-CoV-2-S, SARS-CoV-2-S expression vector (0.5 μg), pCMVΔR8.2 (7.5 μg), and pLTR-Tat-IRES-Luc (10 μg) were cotransfected with either pCMV3-PSGL-1 (2 μg), or pCMV3-Empty (2 μg). Both of SARS-CoV-S and SARS-CoV-2-S pseudotyped viral particles were produced in HEK293T cells. Virus supernatants were collected at 48 - 84 hours post transfection, concentrated with centrifugation, and stored at -80°C.

HIV-1 p24 ELISA

SARS-CoV-S and SARS-CoV-2-S pseudotyped lentiviral particles were quantified using an in-house p24 ELISA kit. Briefly, microtiter plates (Sigma-Aldrich) were coated with anti-HIV-1 p24 monoclonal antibody (183-H12-5C, NIH AIDS Reagent Program). Samples were incubated for 2 hours, followed by washing and incubating with biotinylated anti-HIV immune globulin (HIVIG from the NIH AIDS Reagent Program) for 1 hour. Plates were then washed and incubated with avidin-peroxidase conjugate (R&D Systems) for 1 hour, followed by washing and incubating with tetramethylbenzidine (TMB) substrate. Plates were kinetically read using an ELx808 automatic microplate reader (Bio-Tek Instruments) at 630 nm.

Viral infectivity assay

Virion particles produced in the presence of PSGL-1 or the empty vector were used to infect Vero or VeroE6 cells (ATCC). Cells were pretreated with Infectin II (kindly provided by Virongy) for 1 hour at 37°C, and then infected for 5 hours. Infected cells were cultured for 3 days. The percentage of GFP+ cells was quantified by using flow cytometry. For quantifying luciferase activity, cells were washed and lysed in Luciferase Assay Lysis Buffer (Promega). Luminescence was measured by using GloMax® Discover Microplate Reader (Promega).

Viral attachment assay

Virion particles produced in the presence of PSGL-1 or the empty vector were incubated with Vero or VeroE6 cells (pre-chilled at 4°C for 1 hour) at 4°C for 2 hours. The cells were then washed extensively with cold PBS buffer for 5 times, and then lysed in NuPAGE LDS Sample Buffer (Invitrogen). Cell lysates were analyzed by SDS-PAGE and Western blots using nitrocellulose membranes (Invitrogen), which were blocked for 30 min with 5% milk. The blot was incubated with a mouse anti-HIV-1 p24 monoclonal antibody (NIH AIDS Reagent Program, 183-H12-5C) (1:1000 dilution) or anti-GAPDH goat polyclonal antibody (Abcam) (1:1000 dilution) at 4˚C overnight, then washed and incubated with anti-mouse IgG, HRP-linked antibody (Cell Signaling) (1:2000 dilution) or anti-goat horseradish peroxidase-conjugated antibody (KPL) (1:2500 dilution) at room temperature for 30-60 minutes. Chemiluminescence signals were developed with Super Signal West Femto Maximum Sensitivity Substrate (Pierce).

Data availability All data generated or analyzed during this study are included in this article and its extended data file. Reagents are available from Y. W upon request.

References

1 Fu, Y. *et al.* PSGL-1 restricts HIV-1 infectivity by blocking virus particle attachment to target cells. *Proc Natl Acad Sci U S A*, doi:10.1073/pnas.1916054117 (2020).

2 Sako, D. *et al.* Expression cloning of a functional glycoprotein ligand for P-selectin. *Cell* **75**, 1179-1186 (1993).

3 Guyer, D. A. *et al.* P-selectin glycoprotein ligand-1 (PSGL-1) is a ligand for L-selectin in neutrophil aggregation. *Blood* **88**, 2415-2421 (1996).

4 Goetz, D. J. *et al.* Isolated P-selectin glycoprotein ligand-1 dynamic adhesion to P- and E-selectin. *J Cell Biol* **137**, 509-519 (1997).

5 Somers, W. S., Tang, J., Shaw, G. D. & Camphausen, R. T. Insights into the molecular basis of leukocyte tethering and rolling revealed by structures of P- and E-selectin bound to SLe(X) and PSGL-1. *Cell* **103**, 467-479 (2000).

6 Almulki, L. *et al.* Surprising up-regulation of P-selectin glycoprotein ligand-1 (PSGL-1) in endotoxin-induced uveitis. *FASEB J* **23**, 929-939, doi:10.1096/fj.08-118760 (2009).

7 Xu, H. *et al.* Recruitment of IFN-gamma-producing (Th1-like) cells into the inflamed retina in vivo is preferentially regulated by P-selectin glycoprotein ligand 1:P/E-selectin interactions. *J Immunol* **172**, 3215-3224 (2004).

8 Schumacher, A. *et al.* P-selectin glycoprotein ligand-1 (PSGL-1) is up-regulated on leucocytes from patients with chronic obstructive pulmonary disease. *Clin Exp Immunol* **142**, 370-376, doi:10.1111/j.1365-2249.2005.02920.x (2005).

9 Liu, Y. *et al.* Proteomic profiling of HIV-1 infection of human CD4(+) T cells identifies PSGL-1 as an HIV restriction factor. *Nat Microbiol* **4**, 813-825, doi:10.1038/s41564-019-0372-2 (2019).

10 Murakami, T., Carmona, N. & Ono, A. Virion-incorporated PSGL-1 and CD43 inhibit both cell-free infection and transinfection of HIV-1 by preventing virus-cell binding. *Proc Natl Acad Sci U S A* **117**, 8055-8063, doi:10.1073/pnas.1916055117 (2020).

11 Tang, T., Bidon, M., Jaimes, J. A., Whittaker, G. R. & Daniel, S. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. *Antiviral Res* **178**, 104792, doi:10.1016/j.antiviral.2020.104792 (2020).

12 Crosse, K. M., Monson, E. A., Beard, M. R. & Helbig, K. J. Interferon-Stimulated Genes as Enhancers of Antiviral Innate Immune Signaling. *J Innate Immun* **10**, 85-93, doi:10.1159/000484258 (2018).

13 Raniga, K. & Liang, C. Interferons: Reprogramming the Metabolic Network against Viral Infection. *Viruses* **10**, doi:10.3390/v10010036 (2018).

14 Schoggins, J. W. Interferon-Stimulated Genes: What Do They All Do? *Annu Rev Virol* **6**, 567-584, doi:10.1146/annurev-virology-092818-015756 (2019).

15 Belouzard, S., Chu, V. C. & Whittaker, G. R. Activation of the SARS coronavirus spike protein via sequential proteolytic cleavage at two distinct sites. *Proc Natl Acad Sci U S A* **106**, 5871-5876, doi:10.1073/pnas.0809524106 (2009).

16 Li, W. *et al.* Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. *Nature* **426**, 450-454, doi:10.1038/nature02145 (2003).

17 Hoffmann, M. *et al.* SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **181**, 271-280 e278, doi:10.1016/j.cell.2020.02.052 (2020).

18 Meltzer, B. *et al.* Tat controls transcriptional persistence of unintegrated HIV genome in primary human macrophages. *Virology* **518**, 241-252, doi:10.1016/j.virol.2018.03.006 (2018).

19 Wang, Z. *et al.* Development of a nonintegrating Rev-dependent lentiviral vector carrying diphtheria toxin A chain and human TRAF6 to target HIV reservoirs. *Gene Ther* **17**, 1063-1076 (2010).

20 Gong, L., Cai, Y., Zhou, X. & Yang, H. Activated platelets interact with lung cancer cells through P-selectin glycoprotein ligand-1. *Pathol Oncol Res* **18**, 989-996, doi:10.1007/s12253-012-9531-y (2012).

21 Wang, X. *et al.* SARS-CoV-2 infects T lymphocytes through its spike protein-mediated membrane fusion. *Cell Mol Immunol*, doi:10.1038/s41423-020-0424-9 (2020).

22 Symon, F. A., McNulty, C. A. & Wardlaw, A. J. P- and L-selectin mediate binding of T cells to chronically inflamed human airway endothelium. *Eur J Immunol* **29**, 1324-1333, doi:10.1002/(SICI)1521-4141(199904)29:04<1324::AID-IMMU1324>3.0.CO;2-2 (1999).

23 Ainslie, M. P., McNulty, C. A., Huynh, T., Symon, F. A. & Wardlaw, A. J. Characterisation of adhesion receptors mediating lymphocyte adhesion to bronchial endothelium provides evidence for a distinct lung homing pathway. *Thorax* **57**, 1054-1059, doi:10.1136/thorax.57.12.1054 (2002).

24 Tseng, C. T. *et al.* Immunization with SARS coronavirus vaccines leads to pulmonary immunopathology on challenge with the SARS virus. *PLoS One* **7**, e35421, doi:10.1371/journal.pone.0035421 (2012).

25 Bolles, M. *et al.* A double-inactivated severe acute respiratory syndrome coronavirus vaccine provides incomplete protection in mice and induces increased eosinophilic proinflammatory pulmonary response upon challenge. *J Virol* **85**, 12201-12215, doi:10.1128/JVI.06048-11 (2011).

26 Iwasaki, A. & Yang, Y. The potential danger of suboptimal antibody responses in COVID-19. *Nat Rev Immunol*, doi:10.1038/s41577-020-0321-6 (2020).

Acknowledgements The authors wish to thank the NIH AIDS Reagent Program for reagents; Dr. Gary R. Whittaker for providing SARS-CoV S protein expression vector. This work was funded in part by US Public Health Service grants 1R01AI148012 from NIAID to Y.W., Research in the Freed laboratory is supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, NIH.

Author contributions Experiments were designed by Y.W., E.O.F., and K.K.. Manuscript was written by Y.W., and edited by E.O.F. Experiments were performed by S.H., B.H,, D.D,, and I.V.A..

Competing interests A provisional patent applications pertaining to the results presented in this paper have been filed by George Mason University.

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Fig. 1

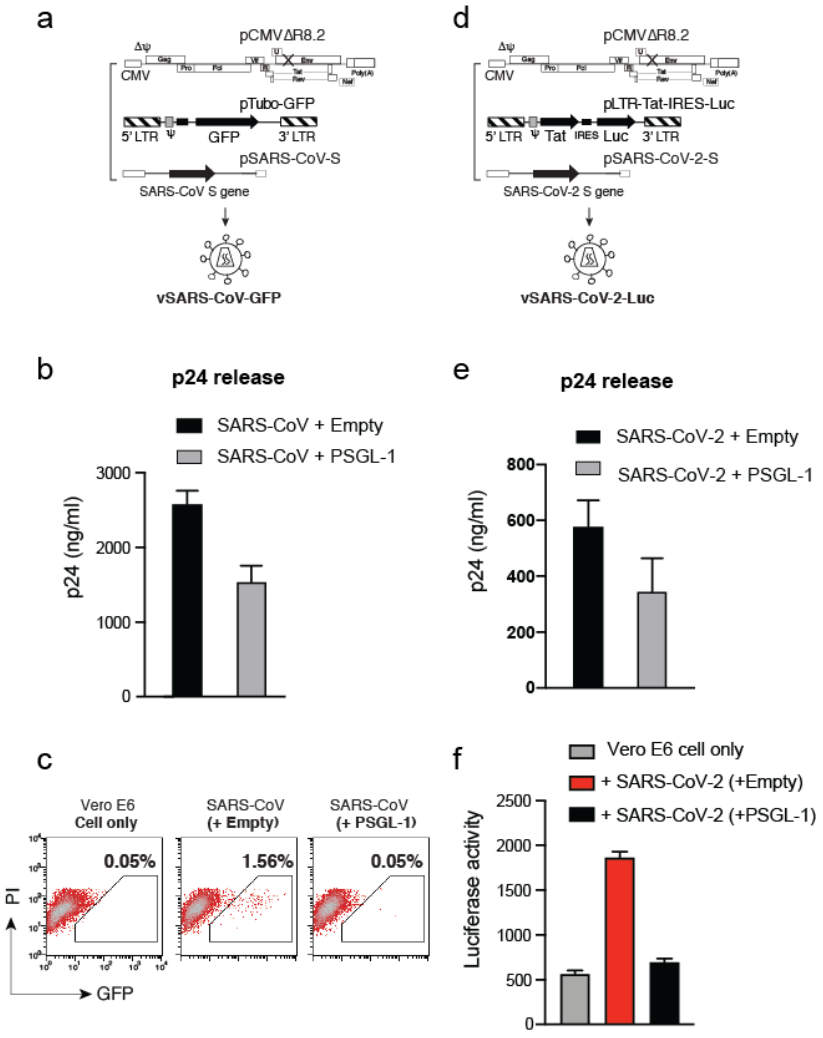

Fig. 1. PSGL-1 inactivates SARS-CoV and SARS-CoV2 pseudoviruses. a, Schematic of the assembly of lentiviral particles pseudotyped with SARS-CoV S protein. **b**, A PSGL-1 expression vector or a control empty vector was cotrasfected with the lentivirus packaging construct and GFP reporter plasmid, and viral release was quantified at 72 hours post co-transfection by HIV-1 p24 ELISA. **c**, The infectivity of virions released was further quantified by infecting Vero E6 cells. GFP expression was quantified at 72 hours post infection. The percentages of GFP+ cells are shown. **d**, Schematic of the assembly of lentiviral particles pseudotyped with SARS-CoV-2 S protein. **e**, A PSGL-1 expression vector or a control empty vector was cotransfected with the lentivirus packaging construct and luciferase reporter plasmid, and viral release was quantified at 72 hours post co-transfection by HIV-1 p24 ELISA. **f**, The infectivity of virions released was quantified by infecting Vero E6 cells. Luciferase activity in infected cells was quantified at 72 hours post infection.

Fig. 2

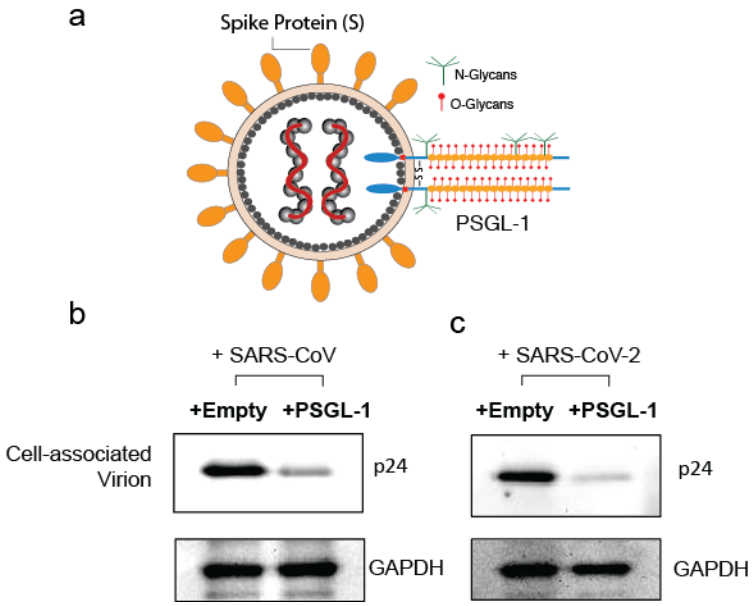

Fig. 2. **PSGL-1 blocks SARS-CoV and SARS-CoV pseudotype virus attachment to target cells. a**, Model of PSGL-1 virion incorporation and steric hindrance of S protein-mediated virion attachment to target cells. **b and c**, An equal amount of virions produced in the presence of a PSGL-1 vector or a empty vector were assayed for attachment to target Vero or Vero E6 cells at 4°C for 2 hours. Cells were extensively washed, and cell-associated virions were analyzed by western blot for HIV-1 p24. GAPDH was used for a loading control.