Galectin-9 Induces SARS-CoV-2 Replication and Inflammation in Airway Epithelial Cells

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BACKGROUND

- SARS-CoV-2 is the etiologic agent of Coronavirus Disease 2019 (COVID-19).
- The major cause of death in COVID-19 patients is acute respiratory distress syndrome (ARDS) accompanied by a cytokine storm.
- Plasma levels of Galectin-9 (Gal-9), a β-galactoside-binding lectin involved in immune regulation and viral immunopathogenesis, are highly elevated in the setting of severe COVID-19 disease.
- Plasma Gal-9 levels are highly correlated with levels of the IL-6, IL-8, and TNFα pro-inflammatory cytokines during COVID-19 disease.

OBJECTIVE

- To elucidate the molecular and immunologic mechanisms linking Gal-9 expression to SARS-CoV-2 pathology.

METHODS

- Calu-3 airway epithelial cells (AECs) were infected with SARS-CoV-2 isolate USA-WA1/2020 (MOI=0.01).
- Primary AECs were isolated from healthy donor lung transplant tissue, cultured at air liquid interface (ALI), and infected with SARS-CoV-2 lineage P.1 (MOI=0.1).
- SARS-CoV-2 replication was assessed by RT-PCR quantitation of the nucleocapsid (N) gene, immunofluorescence assay (IFA) of N protein, and titration of supernatant infectious virus ($TCID_{50}$).
- Viral entry was measured using luciferase activity of VSV-SARS-CoV-2 S-ΔG-luciferase reporter pseudovirus.
- ACE2 and TMPRSS2 cell-surface expression levels were measured by flow cytometry.
- Pro-inflammatory factors (IL-6, IL-8, and TNFα) were detected by RT-PCR and a bead-based immunoassay.
- Total RNA-seq was used to evaluate Gal-9 effects on the host transcriptome.

Gal-9 facilitates SARS-CoV-2 replication and promotes virus-associated immunopathology in the human airway, motivating exploration into Gal-9 manipulation as a therapeutic strategy for COVID-19 disease.

RESULTS

1) Gal-9 treatment potently enhances SARS-CoV-2 replication in human airway epithelial cells (AECs), including primary AECs in air-liquid interface (ALI) culture.

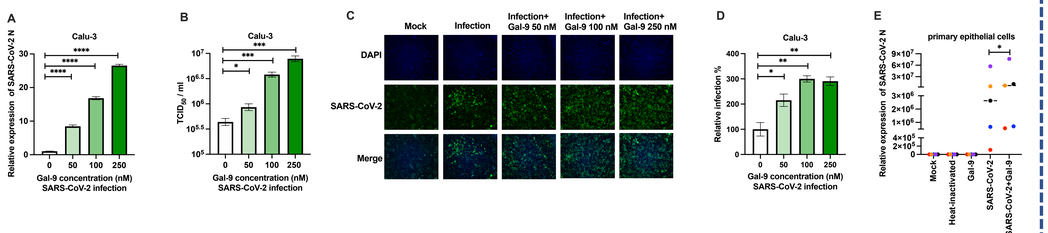


Figure 1. Gal-9 potently enhances SARS-CoV-2 replication in AECs. A. The effect of Gal-9 on viral N gene expression in Calu-3 cells was measured by RT-PCR. B. Viral titer ($TCID_{50}$/ml) of supernatants collected after SARS-CoV-2 infection in the presence of Gal-9 at the indicated concentrations. C. Calu-3 cells were stained with DAPI (blue) and anti-SARS-CoV-2 N protein (green) to detect SARS-CoV-2 replication and the effect of Gal-9 on virus infection. Scale bar, 500 μM. D. Quantification of SARS-CoV-2 infected cells in Calu-3 cells (shown in panel C). Data are normalized to Gal-9-untreated cells. E. RT-PCR analysis of SARS-CoV-2 N gene expression in ALI-cultured primary epithelial cells from five different donors. Heatin=heat-inactivated SARS-CoV-2 infection. Each color represents data from one donor. Groups were compared by Student's *t*-test or paired Wilcoxon test. Bar = mean ± SEM. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$.

2) Gal-9 promotes SARS-CoV-2 attachment and entry into AECs by enhancing the binding affinity of the viral spike protein to the angiotensin-converting enzyme 2 (ACE2) viral entry receptor on the cell surface.

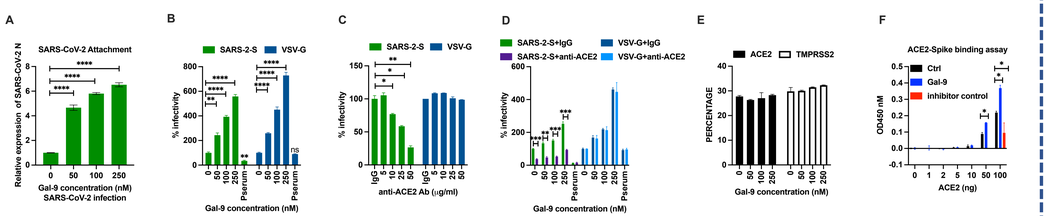


Figure 2. Gal-9 facilitates the cellular attachment and entry of SARS-CoV-2 by enhancing the binding of spike to ACE2. A. Attached SARS-CoV-2 virions on the cell surface were detected by RT-PCR. B. Relative infectivity of SARS-2-S pseudotyped virus and VSV-G pseudotyped virus in Calu-3 cells treated with Gal-9 at the indicated concentrations. C. Relative infectivity of SARS-2-S pseudotyped virus and VSV-G pseudotyped virus in Calu-3 cells treated with anti-ACE2 Ab at the indicated concentrations. D. The effect of anti-ACE2 Ab on Gal-9-enhanced cell entry of SARS-2-S was evaluated by measuring luciferase activity. E. Percentages of cells expressing ACE2 or TMPRSS2 at the cell surface, measured using flow cytometry. F. The dose-response of SARS-CoV-2 spike-ACE2 binding activity measured by reading the absorbance at the wavelength of 450 nm. Groups were compared by Student's *t*-test. Bar = mean ± SEM. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$.

3) Transcriptomic analysis revealed that Gal-9 and SARS-CoV-2 infection synergistically induce the expression of key proinflammatory programs in AECs including the IL-6, IL-8, IL-17, EIF2, and TNFα signaling pathways.

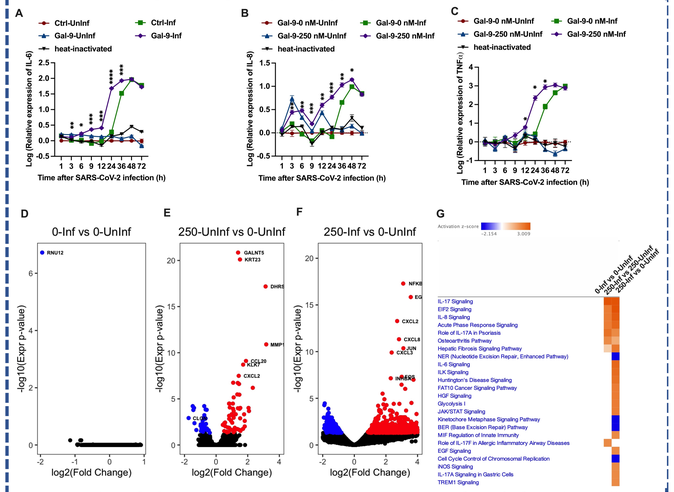


Figure 3. Gal-9 accelerates and increases SARS-CoV-2-mediated inflammatory response. A-C. mRNA levels of proinflammatory cytokines IL-6 (A), IL-8 (B), and TNFα (C) were detected by RT-PCR at the indicated time points. UnInf = uninfected cells, Inf = infected cells, heat-inactivated = infected with heat-inactivated SARS-CoV-2. D-F. Volcano plots showing the proportion of differentially-expressed genes (DEGs) in the setting of SARS-CoV-2 infection (0-Inf vs 0-UnInf) (D), Gal-9 treatment (250-UnInf vs 0-UnInf) (E), and SARS-CoV-2 infection in the presence of Gal-9 (250-Inf vs 0-UnInf) (F). DEGs (FDR<0.05) with $\log_2$(fold change) > 0 are indicated in red. DEGs (FDR<0.05) with log2(fold change) < 0 are indicated in blue. Non-significant DEGs are indicated in black. G. Top enriched canonical pathways identified using IPA. The orange and blue-colored bars in the bar chart indicate predicted pathway activation or predicted inhibition, respectively, based on z-score. Groups were compared by Student's *t*-test. Differential expression analyses were performed using DESeq2. Bar = mean ± SEM. *, $P < 0.05$, **, $P < 0.01$, ***, $P < 0.001$, ****, $P < 0.0001$.

CONCLUSIONS

- Gal-9 facilitates SARS-CoV-2 entry, replication, and virus-induced pro-inflammatory signaling in AECs *ex vivo*.
- Our data suggest that pharmacologic manipulation of Gal-9 should be explored as a SARS-CoV-2 therapeutic strategy.

ADDITIONAL KEY INFORMATION

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