PROLONGED SARS-COV-2 VIRAL BURDEN IN SIV-SARS-COV-2 CO-INFECTED MACAQUES

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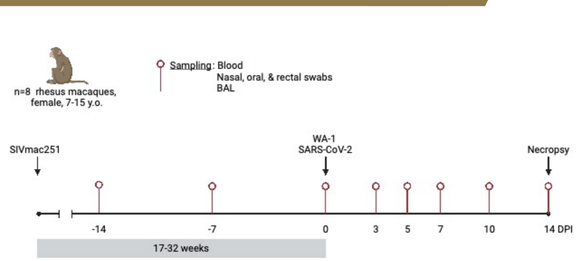
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BACKGROUND

Individuals living with HIV are at increased risk of morbidity and mortality from COVID-19. In addition, there may be increased risk of transmission due to prolonged infection and viral shedding. Here we utilize rhesus macaques to model SARS-CoV-2 co-infection during HIV-induced immunosuppression.

We hypothesize that immune dysregulation during untreated simian immunodeficiency virus (SIV) infection may contribute to inferior SARS-CoV-2 viral clearance and disease pathogenesis in rhesus macaques.

STUDY DESIGN & METHODS



Eight female rhesus macaques (aged 7-15 years, 5.5-9.9 kg) were infected with SIVmac251 via low dose intravaginal challenge. 17-34 weeks post SIV infection, animals were inoculated with 6.5x105 TCID50/mL SARS-CoV-2, isolate USA-WA1/2020 via combined intranasal and intratracheal routes and followed for 14 days post infection (DPI). Blood, bronchoalveolar lavage (BAL), stool, and nasal, oral, and rectal swabs were collected pre-infection and every 2-4 days thereafter.

ELISAs, ELISPOT, qRT-PCR, lung pathology, cytokine multiplex, and virus neutralization assays were performed to examine pathogenesis and immune responses to SARS-CoV-2.

ACKNOWLEDGEMENTS

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RESULTS

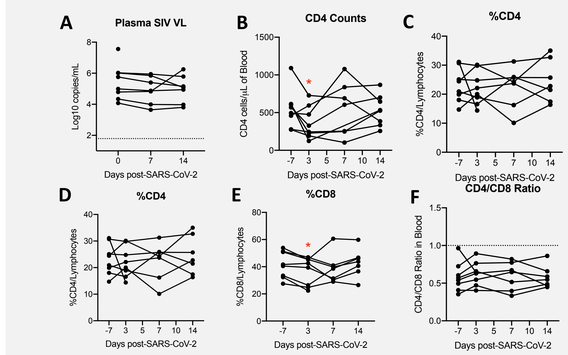


Figure 1. Impact of SARS-CoV-2 on SIV disease progression. (A) Quantification of SIV viral loads in plasma determined by RT-PCR. The dotted line indicates the lower limit of detection (1.79 log10 copies/mL). Measurements of blood (B) CD4 counts, (C) %CD4 of total lymphocytes, (D) CD8 counts, (E) %CD8 of total lymphocytes, and (F) CD4/CD8 Ratio. (A-F) Holm-Sidak's multiple comparisons test versus baseline, * $p < 0.05$.

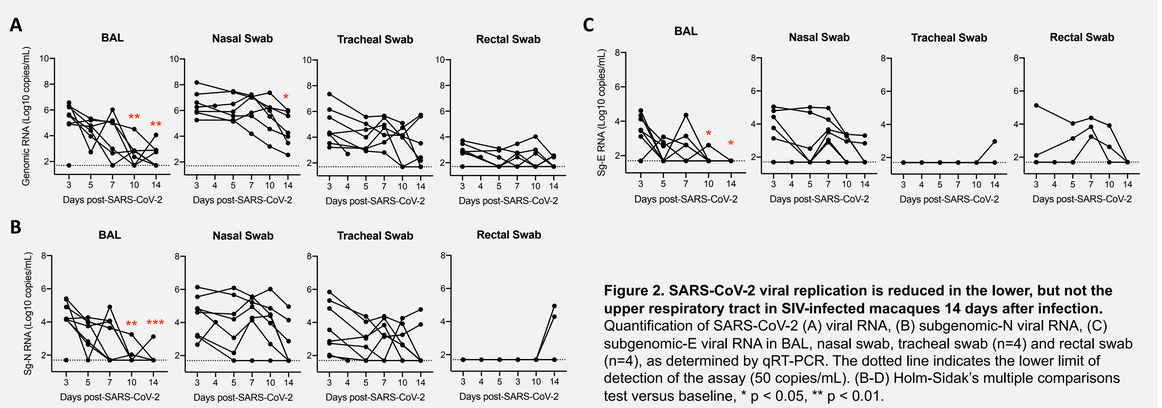


Figure 2. SARS-CoV-2 viral replication is reduced in the lower, but not the upper respiratory tract in SIV-infected macaques 14 days after infection. Quantification of SARS-CoV-2 (A) viral RNA, (B) subgenomic-N viral RNA, (C) subgenomic-E viral RNA in BAL, nasal swab, tracheal swab (n=4) and rectal swab (n=4), as determined by qRT-PCR. The dotted line indicates the lower limit of detection of the assay (50 copies/mL). (B-D) Holm-Sidak's multiple comparisons test versus baseline, * $p < 0.05$, ** $p < 0.01$.

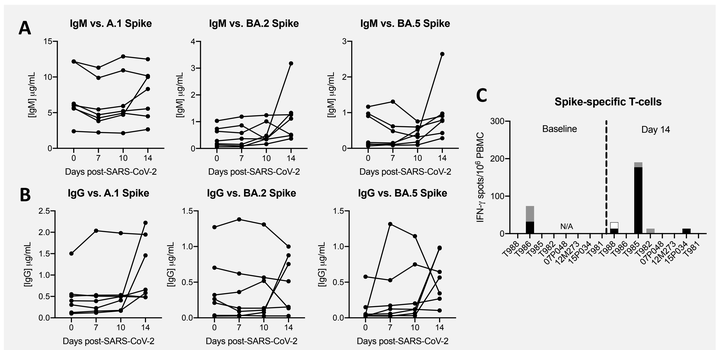


Figure 3. SIV infection impairs the generation of anti-SARS-CoV-2 immunity. Serum (A) anti-IgM and (B) anti-IgG enzyme linked immunosorbent assays (ELISAs) against A.1, BA.2, and BA.5 Spike proteins. (C) Magnitude of IFN-γ T-cell responses were measured by ELISpot assay in PBMCs following 48-hour stimulation with overlapping peptide pools encompassing the WA.1 SARS-CoV-2 spike (S), membrane (M), nucleocapsid (N) and envelope (E) proteins. (A-B) Holm-Sidak's multiple comparisons test versus baseline, * $p < 0.05$, ** $p < 0.01$.

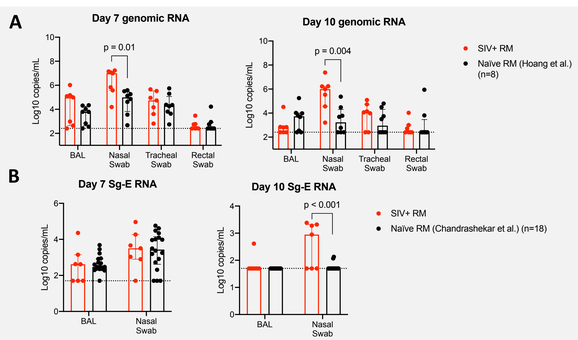


Figure 4. SARS-CoV-2 virus persists in the upper respiratory tract of SIV+ SARS-CoV-2+ rhesus macaques relative to published studies using SIV- SARS-CoV-2+ rhesus macaques. Comparison of (A) viral and (B) subgenomic-E SARS-CoV-2 RNA at days 7 and 10 in BAL, nasal and/or tracheal swab and rectal swab between SIV+ rhesus macaques (RM) from this study and from naïve RM from two published studies: (A) Hoang et al., PMCID: PMC7654323 and (B) Chandrashekar et al., PMCID: PMC8829873. T-test versus baseline, $p < 0.05$ is considered significant.

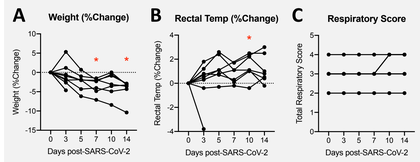


Figure 5. SIV infected rhesus macaques develop mild SARS-CoV-2 disease. On days of clinical exams (A) body weight, (B) body temperature, and (C) sedated clinical scores (discharges, respiratory rate, and respiratory character) were recorded. (A-B) The dotted line indicates no change from pre-SARS-CoV-2 coinfection. (A-C) Medians with interquartile are indicated. Holm-Sidak's multiple comparisons test versus baseline, * $p < 0.05$.

CONCLUSIONS

- We provide evidence for the utility of the rhesus macaque in modeling SARS-CoV-2 co-infection during HIV-induced immunosuppression.
- Our results suggest that SARS-CoV-2 infection does not further promote SIV disease progression.
- These findings suggest evidence of prolonged SARS-CoV-2 viral burden during co-infection.

MAIN TAKE-AWAY

Immunosuppression during SIV infection impairs de novo generation of anti-SARS-CoV-2 immunity, that may contribute to prolonged SARS-CoV-2 viral shedding, increased transmission windows, altered disease pathogenesis, and lower protection against subsequent SARS-CoV-2 exposures and transmission.