PROLONGED SARS-COV-2 VIRAL BURDEN AND IMPAIRED IMMUNITY IN SIV/SARS-COV-2 CO-INFECTED MACAQUES

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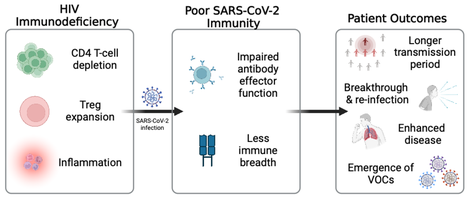
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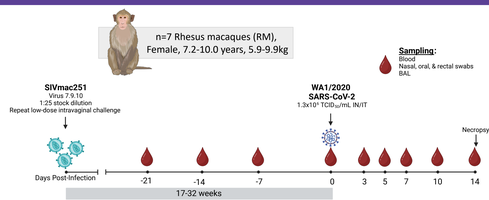
BACKGROUND

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



SIV- RM controls (reference):
- Hoang TN et al. 2021
- Chandrashekar A et al. 2021.

Controls (SIV- serum):
- Chandrashekar A et al. 2021.
- Fears AC et al. 2022

Seven female rhesus macaques (**RM**) (aged 7.2-10.0 years, 5.9-9.9 kg) were infected with SIVmac251 via low dose intravaginal challenge. 17-32 weeks post SIV infection, animals were inoculated with 1.3x10^6 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.

Serum from day 14 DPI from SIV- RM, from the studies indicated above, were utilized as SIV-/SARS-CoV-2+ controls against our SIV+/SARS-CoV-2+ RM.

ACKNOWLEDGEMENTS

Thank you to Dr. Nancy Miller for providing us with SIV+ animals and to Dr. Dan Barouch and Dr. Chad Roy for providing us with serum from SIV- RM.
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FINDINGS

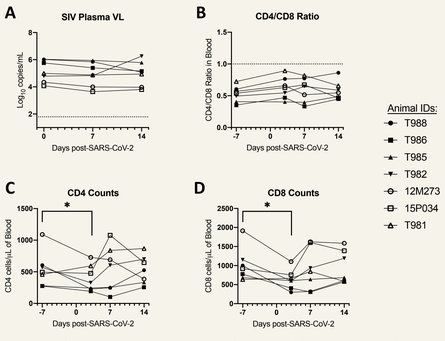


Figure 1. SIV disease progression is transiently impacted by SARS-CoV-2 infection. (A) Quantification of SIV viral loads in plasma determined by RT-PCR. The dotted line indicates the lower limit of detection (1.79 log10 copies/mL). Measurements of blood (B) CD4/CD8 T cell ratio, (C) CD4 T cell counts, and (D) CD8 T cell counts. (A-D) Holm-Sidak's multiple comparisons test versus baseline, *p<0.05. The dotted line in (B) represents a normal CD4/CD8 T cell ratio of 1.0.

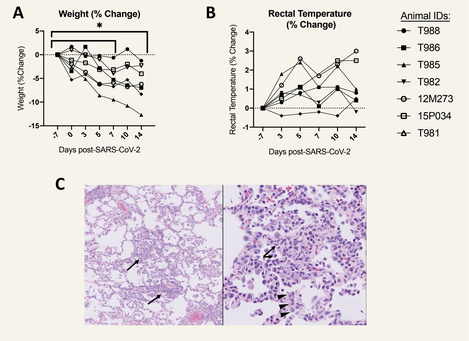


Figure 2. SIV-infected RM develop mild COVID-19 disease. On days of clinical exams (A) body weight and (B) rectal temperature were recorded. (C) Histopathology of SARS-CoV-2 infection in SIV+ RM. Consistent with histopathology of SARS-CoV-2 infection in SIV- RM. (A-B) Medians with interquartile are indicated. Holm-Sidak's multiple comparisons test versus baseline, * p < 0.05. The dotted line indicates no change from pre- SARS-CoV-2 coinfection.

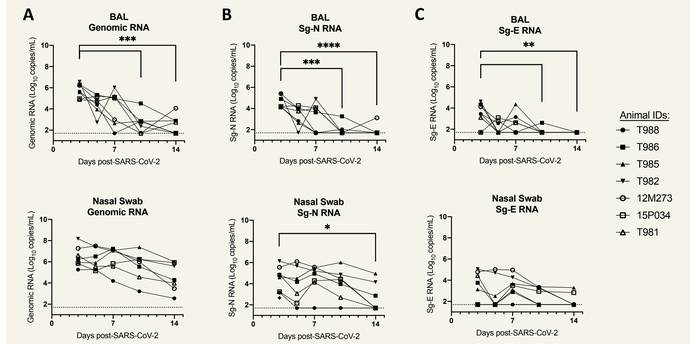


Figure 3. Persistence of SARS-CoV-2 in the upper respiratory tract in SIV+ RM. Quantification of SARS-CoV-2 (A) viral RNA, (B) subgenomic-N viral RNA, and (C) subgenomic-E viral RNA in BAL and nasal swabs, as determined by qRT-PCR. The dotted line indicates the lower limit of detection of the assay (50 copies/mL). (A-C) Holm-Sidak's multiple comparisons test versus baseline, * p < 0.05, ** p < 0.01, ***p<0.001, ****p<00001.

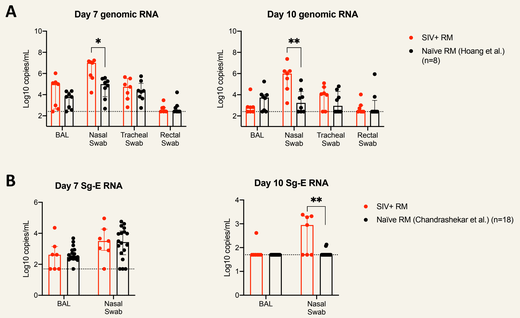


Figure 4. SARS-CoV-2 persists in the upper respiratory tract of SIV+ RM relative to published studies in SIV- RM. Comparison of (A) viral and (B) subgenomic-E SARS-CoV-2 RNA at days 7 and 10 in BAL, nasal and/or tracheal swab and rectal swab between SIV+ rhesus macaques (RM) from this study and from 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.

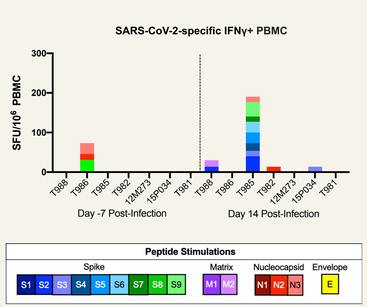


Figure 5. SIV+ RM generate limited anti-SARS-CoV-2 cellular immunity. Magnitude of IFN-γ T-cell responses were measured by ELISpot assay in PBMCs following 48-hour stimulation with overlapping peptide pools encompassing the WA.1 SARS-CoV-2 spike (S), membrane (M), nucleocapsid (N) and envelope (E) proteins. x

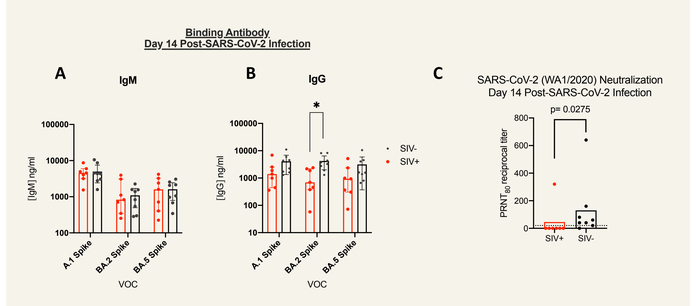


Figure 6. SIV infection impairs the generation of anti-SARS-CoV-2 humoral immunity. Serum (A) anti-IgM and (B) anti-IgG enzyme linked immunosorbent assays (ELISAs) against WA-1, BA.2, and BA.5 Spike proteins. (C) SARS-CoV-2 (WA1) neutralization titers using PRNT80. SIV-/SARS-CoV-2+ serum was provided by Chandrashekar A et al. 2021. Sci Transl Med PMCID: PMC8829873 (challenge dose 5×1010 TCID50 WA1/2020, IN/IT). (A-C) 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.
- These findings suggest evidence of prolonged SARS-CoV-2 viral burden during HIV/SARS-CoV-2 co-infection.
- Untreated SIV infection may impair the generation of anti-SARS-CoV-2 immunity.

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 re-exposure to SARS-CoV-2.