Western Diet and SIV Co-Infection Disrupt Immune Responses to SARS-CoV-2 and Heighten Pathogenesis

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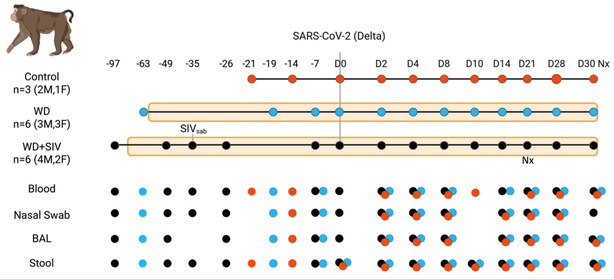
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BACKGROUND

Conditions such as diabetes, obesity, cardiopulmonary diseases, and HIV infection all increase the risk for severe COVID-19. Diet can contribute to the development of obesity and diabetes but its direct impact on SARS-CoV-2 infection has been understudied. We have examined the impact of a high-fat and high-sugar diet (Western diet, WD) on SARS-CoV-2 infection in pigtailed macaques (PTMs). We have additionally investigated the impact of SIV co-infection on SARS-CoV-2 in PTMs to better understand the risk of severe COVID-19 for people living with HIV.

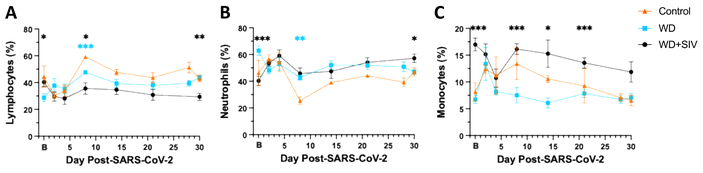
METHODS

- Control group: 3 PTMs were maintained on standard monkey chow prior to and during SARS-CoV-2 infection.
- WD group: 6 PTMs were fed standard monkey chow, then a high-fat and high-sugar chow (WD) approximately 2 months prior to SARS-CoV-2 infection. Macaques remained on the WD throughout infection.
- WD+SIV group: 6 PTMs were fed WD prior to SIV infection and subsequent SARS-CoV-2 infection, and maintained on the WD for the remainder of the study.
- All animals were monitored until euthanasia and necropsy (Nx) and sampled as below. One WD+SIV PTM was euthanized early (Day 21 SARS-CoV-2) due to emergent anemia and lethargy. All days are relative to SARS-CoV-2 infection. Yellow boxes represent WD feeding. BAL: bronchioalveolar lavage. sab: sabaeus.



RESULTS

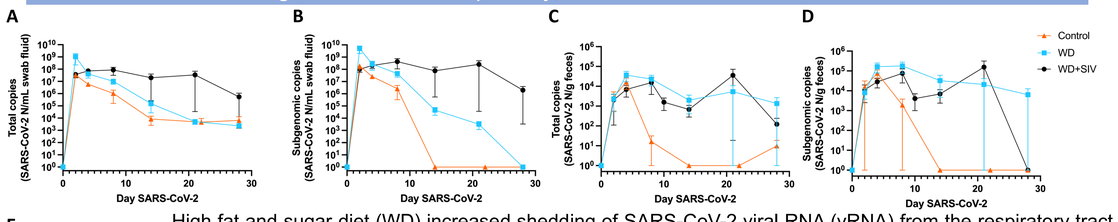
Immune Response to SARS-CoV-2 Altered with WD & SIV

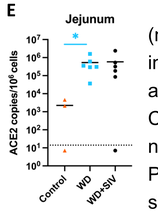


PTMs experienced an increase in lymphocyte frequency in the blood (**A**) at Day 8 SARS-CoV-2, but frequencies were significantly lower in WD PTMs compared to controls, suggesting a blunted adaptive immune response with WD. WD+SIV PTMs also had significantly lower lymphocyte frequencies than WD PTMs at Day 8 SARS-CoV-2 and demonstrated lower lymphocyte frequencies throughout SARS-CoV-2 infection, in keeping with expected CD4+ T cells loss resulting from SIV infection. WD also resulted in a significantly higher frequency of neutrophils at Day 8 SARS-CoV-2 (**B**) and monocyte frequencies (**C**) were significantly elevated in WD+SIV PTMs over WD PTMs at several time points following SARS-CoV-2 infection. Group means shown with error bars SEM. Significant differences between groups are shown in blue asterisks (control to WD) and black asterisks (WD to WD+SIV). *** p<0.002; ** p<0.01; * p<0.05

RESULTS

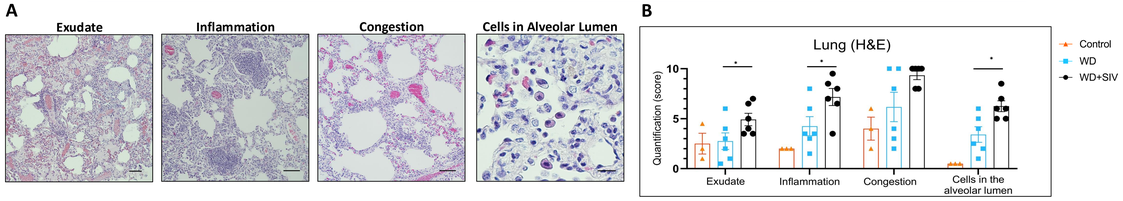
SARS-CoV-2 Shedding Increased in Respiratory, GI Tracts with WD and in Nasal Swabs with SIV

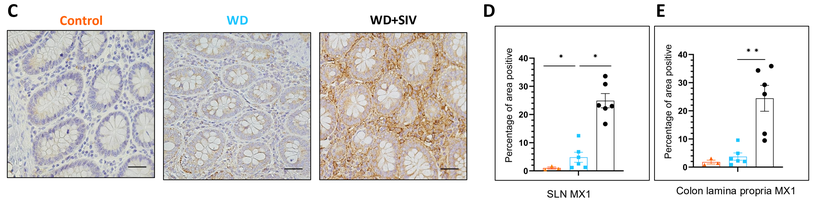




High fat and sugar diet (WD) increased shedding of SARS-CoV-2 viral RNA (vRNA) from the respiratory tract (nasal swabs, **A** and **B**) and GI tract (stool, **C** and **D**). Both total vRNA (**A** and **C**) and subgenomic vRNA indicating viral replication (sgRNA, **B** and **D**) were increased, with WD fed animals shedding higher quantities of all vRNA at peak and shedding sgRNA for longer duration than control animals. WD+SIV PTMs shed SARS-CoV-2 vRNA at lower peak levels than WD animals, but shedding of both total and sgRNA was prolonged in nasal swabs. WD PTMs also had significantly higher *ACE2* copies in jejunal tissue (small intestine) than control PTMs at approximately one month post-SARS-CoV-2 (**E**). Group means in bars in E, and limit of detection shown in the horizontal dashed line. *** p<0.002; ** p<0.01; * p<0.05 **A-D**: Undetected samples are graphed as 10^0 and calculated as 0 in means. Group means shown with error bars SEM.

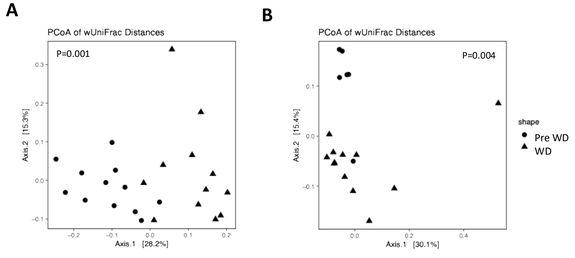
WD and SIV Enhance Lung Pathology, Inflammation in GI Tissue





Lung tissue from approximately one month post-SARS-CoV-2 infection was fixed and embedded for hematoxylin & eosin (H&E) staining and evaluated for pathology (**A**). Lung lesions were generally mild and suggestive of interstitial pneumonia. Clinical scoring of lung lesions was performed (**B**). More cells infiltrated the alveolar lumen in WD PTMs than controls, while SIV co-infection significantly enhanced cell infiltration and inflammation over WD PTMs. Immunohistochemistry for inflammatory marker MX1 in colon tissue (**C**) showed significantly increased MX1 in WD+SIV PTMs (**E**), with MX1 staining also significantly increased in superficial lymph nodes (**D**, SLNs) in both WD and WD+SIV PTMs. Group means with SEM in **B**, **D**, and **E**. *** p<0.002; ** p<0.01; * p<0.05

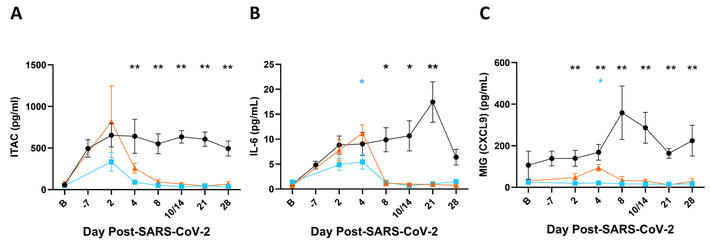
Bacterial Dysbiosis with Western Diet



Bacterial taxa from stool were identified by 16S sequencing. Beta diversity of stool bacterial communities was assessed with weighted UniFrac distances, and significantly different pre- vs. post-WD (Adonis testing, PCoA of weighted UniFrac distances) prior to infection in both the WD and WD+SIV groups. **A** is the WD group and **B** the WD+SIV group. All samples are pre-SIV infection.

RESULTS

WD and SIV Dysregulate Immune Response to SARS-CoV-2 Infection



Plasma cytokines were measured longitudinally for each group of animals. Baseline or B samples are prior to dietary change or SIV infection. ITAC (CXCL11, **A**), which is induced by IFNβ and IFNγ, was elevated with SIV infection and with SARS-CoV-2, but most notably declined following Day 2 of SARS-CoV-2 in control and WD PTMs while remaining elevated in the WD+SIV group. Control PTMs also had higher ITAC levels at Day 2 of SARS-CoV-2 than WD animals. IL-6 (**B**), a pro-inflammatory cytokine, was also elevated with SARS-CoV-2 infection, but significantly lower at peak in WD PTMs than controls, suggesting a blunted response to infection. This was further evidenced with MIG (CXCL9, **C**), which was not induced with SARS-CoV-2 in WD PTMs but was in controls and WD+SIV animals. WD+SIV PTMs also demonstrated a hyperinflammatory profile with significant elevations of ITAC, IL-6, and MIG over WD PTMs at several time points. Group means shown with error bars SEM. *** p<0.002; ** p<0.01; * p<0.05

CONCLUSIONS

A high-fat, high-sugar diet (WD) in macaques enhanced SARS-CoV-2 shedding, suggesting worsened viral control. Disruptions to the microbiome, lymphocyte response to SARS-CoV-2, and circulating cytokines also occurred with WD, indicating the diet impacts gut microbiota and may alter immunity, predisposing to enhanced disease. SIV co-infection also altered SARS-CoV-2 infection response, with worsened viral control and greater inflammation, and significantly enhanced lung pathology. Both WD and SIV enhance SARS-CoV-2 pathogenesis, but through distinct impacts on the immune system.

FUTURE DIRECTIONS

- Explore impact of diet and SIV infection on:
  - short chain fatty acids in GI tract
  - cellular and humoral immune responses to SARS-CoV-2

ACKNOWLEDGEMENTS

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