Characterization of a VSVΔG S (SARS-CoV-2 original variant) hybrid replicating virus as a possible model of mild COVID-19 disease

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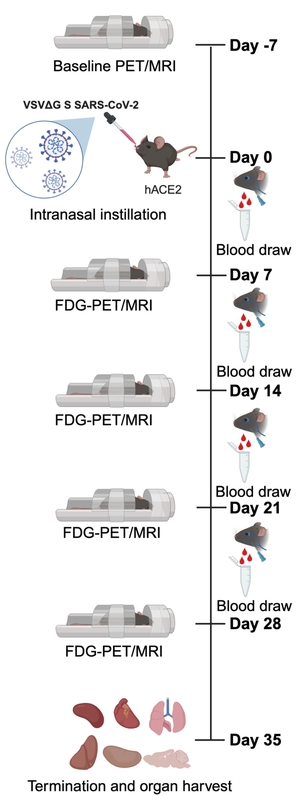
No conflicts of interest.

1 Introduction

- SARS-CoV-2 has led to 776 million infections and ~7 million deaths (Aug 4th, 2024)[1]
- The COVID-19 Immunity Task Force reports a Canadian seroprevalence of infection acquired antibodies of 81.4% as of December 2023
- Post-acute sequalae of COVID-19 (PASC) is estimated to occur in 10% of SARS-CoV-2 cases and has highly variable pathology[2]
- Currently no animal models fully recapitulate PASC[3]
- Molecular imaging can be used to track viral associated inflammation in the body
- Fluoro-2-deoxy-D-glucose (FDG) is preferentially taken up by cells with increased glucose metabolism[4,5]
- COVID-19 is a CL3 pathogen
- `Expensive and difficult to work with
- VSVΔG S expresses the spike protein of COVID-19 to facilitate early research
- ***Goal: Characterize imaging and biological features of infection with a hybrid replicating VSVΔG S (SARS-CoV-2). Compare pathology between variants.***

2 Methods



Mouse model:

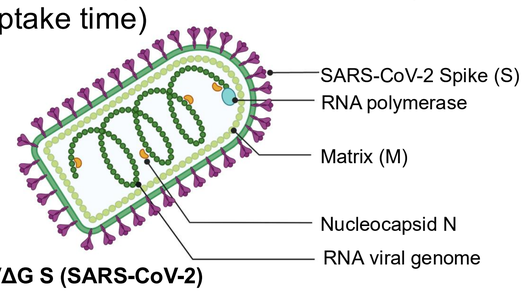
- K-18-hACE2 mice (C57BL/6 background)
- Directed expression to epithelial tissues, mimics human ACE2 distribution

Biological analysis:

- Samples: weekly and terminal blood collection, terminal organ collection
- Flow cytometry for immune phenotyping

FDG-PET/ MRI:

- Sequential MRI and PET scans
- Injection of 500 µCi FDG before MRI (70 mins uptake time)



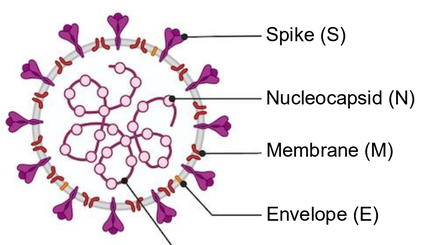
VSVΔG S (SARS-CoV-2)

Groups:

- Low titre: 5X10^4 PFU
- High titre: 1X10^5 PFU
- "Empty" VSVΔG 1X10^6 PFU
- Naïve control

Virus model:

- VSVΔG S SARS-CoV-2 (GFP+)
- Pseudotype virus expressing the S protein of COVID-19 (original variant)
- Intranasal administration to mimic human route of infection



SARS-CoV-2

3 Results

Fig 1.

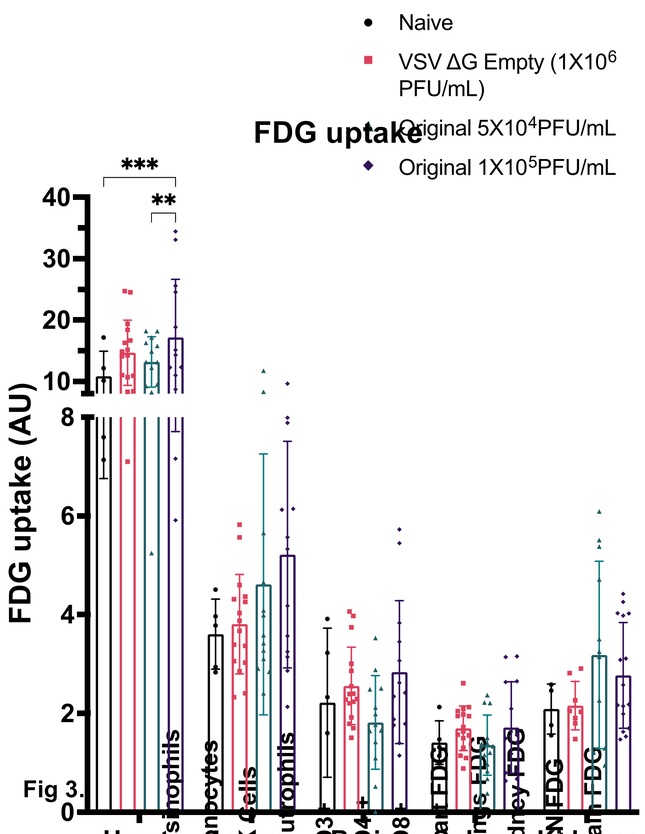


Figure 1 Normalized FDG uptake values for organs of interest compared between experimental groups. Raw FDG uptake values (MBq/mm^3) for each organ were divided by the FDG uptake values of the muscle in the same scan for internal normalization. Increased FDG uptake in the heart is expected due to its high metabolic requirements. Increased uptake in the lungs was observed for both titres of VSVΔG S SARS-CoV-2 infected groups. Titre-specific FDG increases were observed in the brain and kidneys as well. Overall, the 1x10^5 PFU/mL infected group showed increased FDG uptake compared to other groups ***** P<0.001, ****P<0.0001**

Figure 2 Representative FDG-PET/MRI images from empty VSV 1X10^6 PFU/mL control group, and groups infected with 5X10^4 PFU/mL or 1X10^5 PFU/mL VSVΔG S (SARS-CoV-2 original variant). All images are taken from week 1 of the experimental timeline, with all mice having had appropriate virus instilled intranasally 7 days prior. Colour bars on the side denote the non-normalized FDG concentration in MBq/mm^3. Note that the scale is different for each image due to variations in FDG activity.

Fig 2.

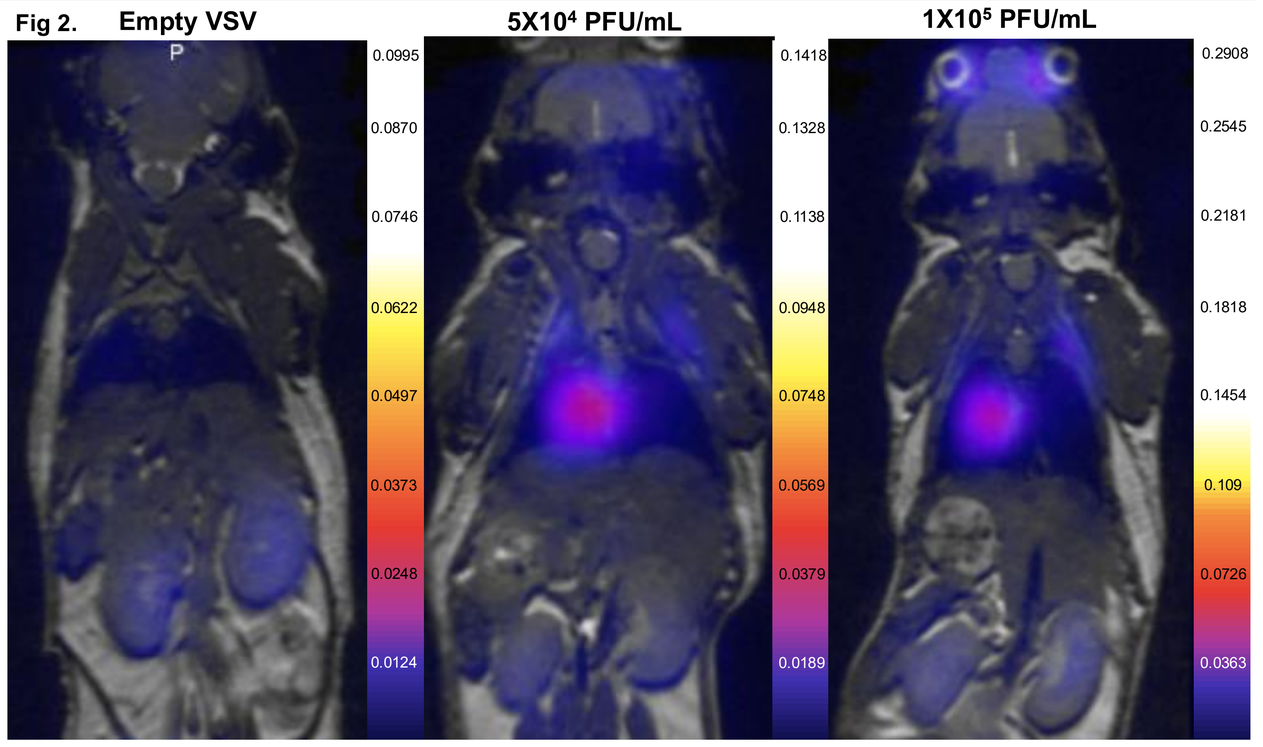


Fig 3.

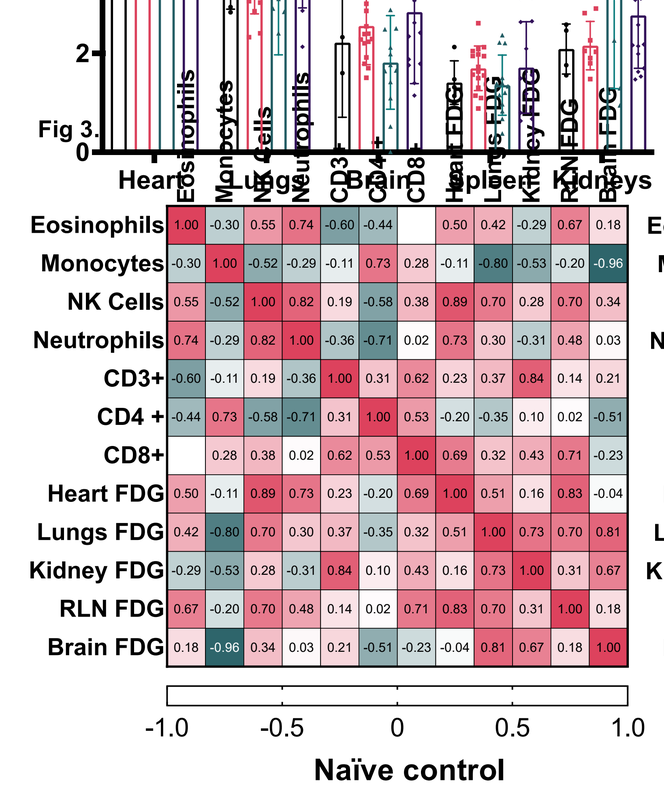


Figure 3 Cell type and FDG uptake correlations are determined by infection type. Pearson correlation matrices of various immune cell types and FDG uptake for naïve control mice and VSVΔG S (SARS-CoV-2 original variant) 1X10^5 PFU/mL infected mice. Immunophenotyping was performed using the BD Celesta flow cytometer.

Fig 4A.

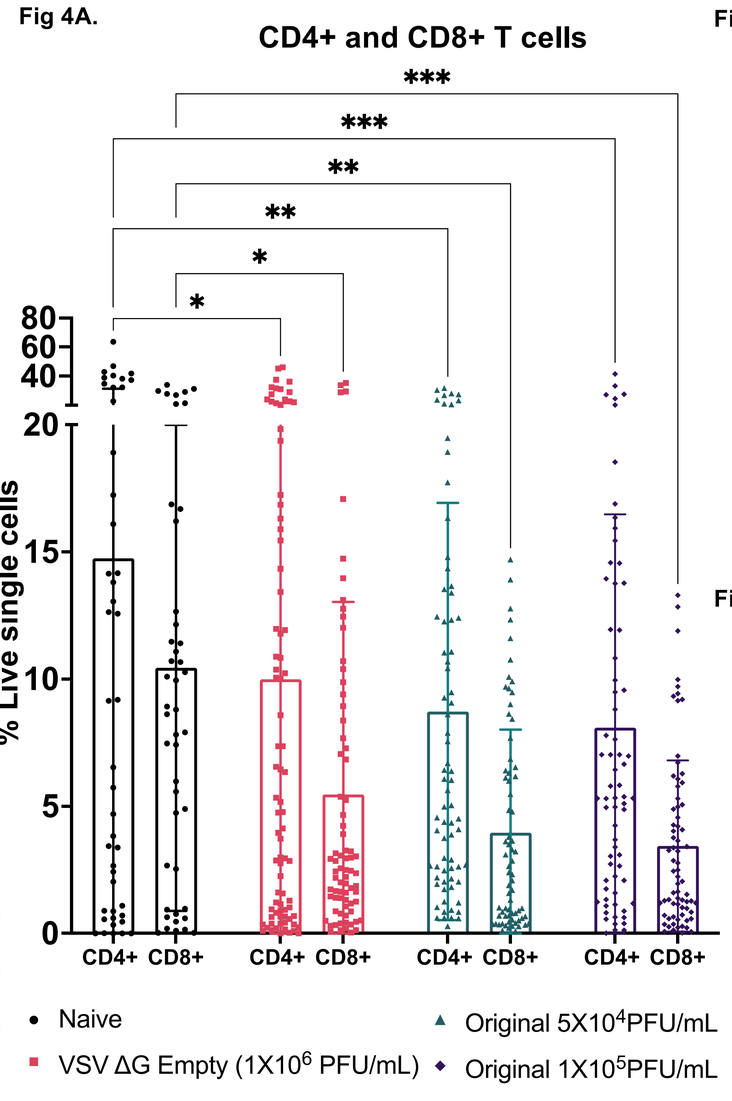


Fig 4B.

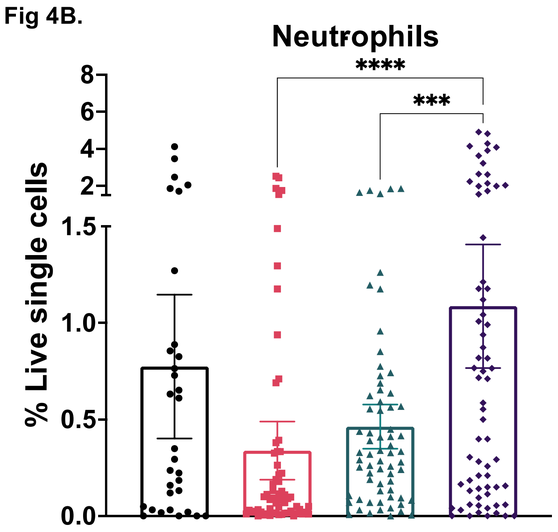


Fig 4C.

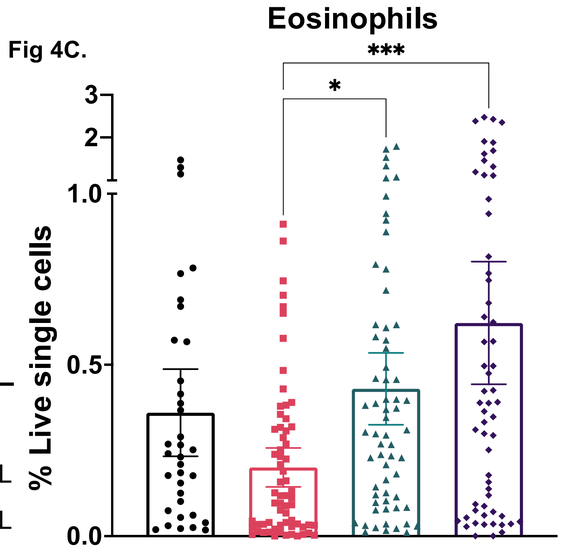


Figure 4 Immune cell changes between experimental groups. A) Comparison of CD4+ and CD8+ T cells between experimental groups. Infected groups showed decreased levels of both cell types when compared to control groups, with cell levels decreasing in a dose-dependant manner. **B)** Comparison of neutrophil (CD11b+, Gr1+) levels between experimental groups. The highest levels were observed in VSVΔG S 1X10^5 PFU/mL, while a slight decrease compared to naïve controls was observed for VSVΔG S 5X10^4 PFU/mL mice. **C)** Comparison of eosinophil (CD11b+, Siglec-F +) levels between experimental groups. Infected groups showed increased cell levels compared to control mice.
*** P>0.5, ** P>0.01, *** P>0.001, **** P>0.0001**

4 Conclusions

- Kidneys and lungs of infected mice showed increased FDG uptake compared to controls
- Infected mice have a correlation in FDG uptake between organs, but this is not the case for uninfected controls
- Infected groups show increased levels of eosinophils, NK cells, and basophils compared to controls
- Infected groups had consistent decreases in CD4+ and CD8+ T cells

5 Future Directions

- Investigate immune differences produced by different SARS-CoV-2 S variants
- Analyze immune phenotypes as a function of time and organ
- Terminate at different points in the study to collect temporal data on organ-level immune populations

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Coinfection of VSVΔG S (SARS-CoV-2 omicron) & E0771.lmb breast cancer results in novel immune phenotypes and increased tumour volumes

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No conflicts of interest.

1 Introduction

- SARS-CoV-2 has led to 776 million infections and ~7 million deaths (August, 2024)[1]
- Fluoro-2-deoxy-D-glucose (FDG) is a metabolic radio tracer used in PET. Its uptake is increased in cells with high glucose metabolism[2,3]
  - COVID-19 infection has been reported to cause increased FDG uptake, confounding imaging data in oncological scans[4]
- Early indications that COVID-19 immune changes can promote cancer metastasis and/or recurrence[5,6]

Goals:

- ***Characterize imaging and biological features of infection with VSV expressing SARS-CoV-2 spike in breast cancer model.***
- ***Investigate if timing of COVID-19 infection has different immunological outcomes***

2 Methods

Biological analysis:

- Samples: weekly and terminal blood, terminal organ collection

FDG-PET/MRI:

- Sequential MRI and PET scans
- Injection of 500 µCi FDG before MRI (70 mins uptake time)

Virus model:

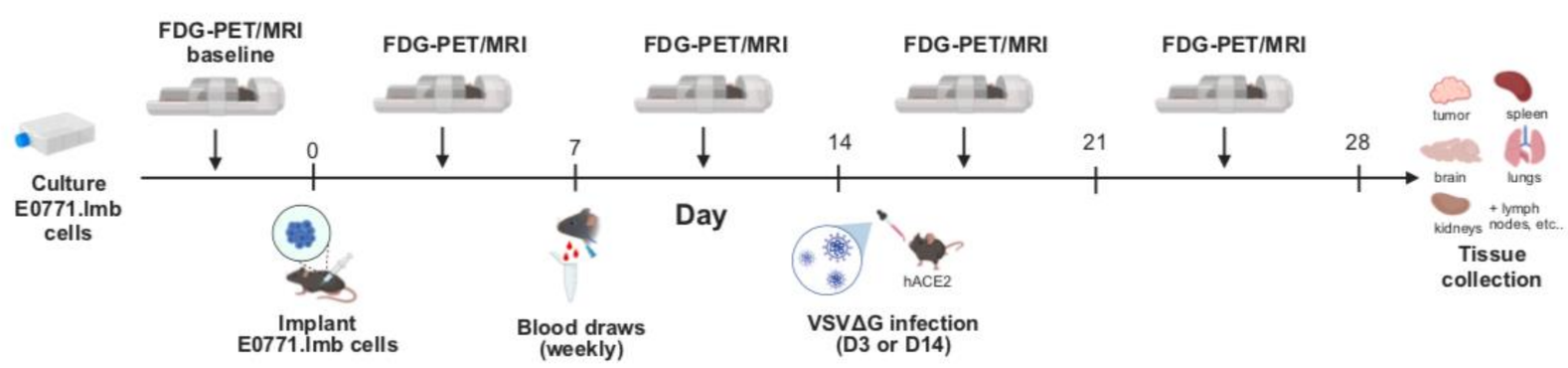
- VSVΔG S is a hybrid replicating virus expressing the S protein of COVID-19

Mouse model:

- K-18-hACE2 mice (C57BL/6 background)

Groups:

- Cancer (5X10^5 E0771.lmb cells/mouse)
- Cancer + Day 3 VSVΔG S
- Cancer + Day 14 VSVΔG S
- VSVΔG S (SARS-CoV-2 omicron 1X10^6 PFU/mL)
- "Empty" VSVΔG



3 Results

Fig 1A

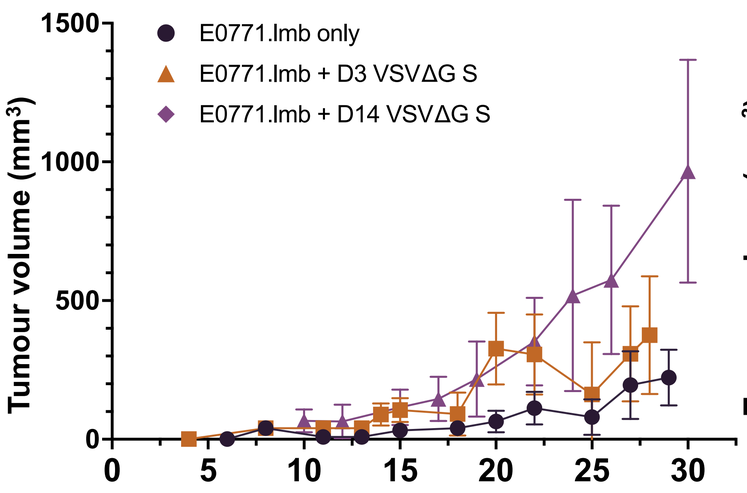


Fig 1B.

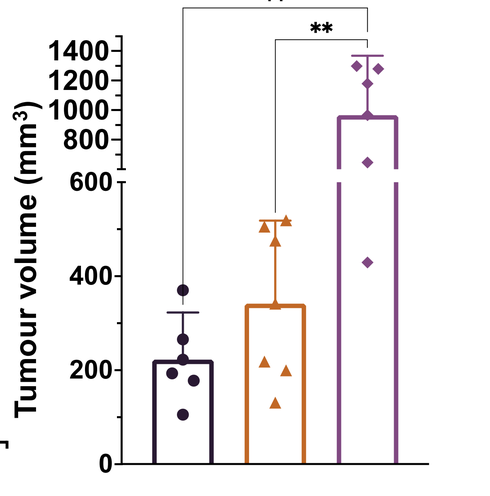
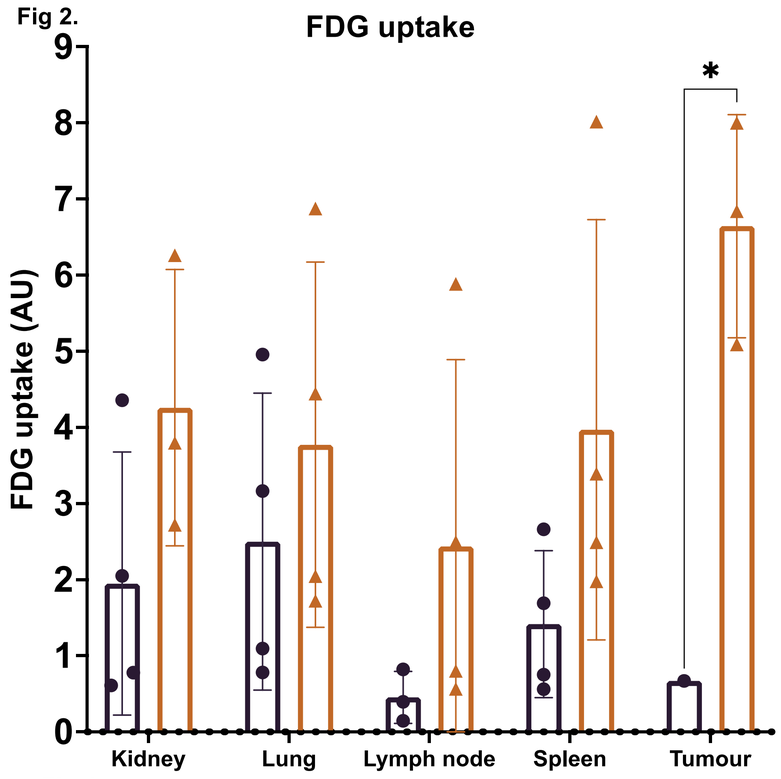


Figure 1 Cancer and VSVΔG S (SARS-CoV-2 Omicron variant) coinfected groups had larger tumor volumes compared to cancer only. Mice were implanted with E0771.lmb cells followed by an intranasal instillation of VSVΔG S on day 3 or 14. Tumor volumes were determined by *in vivo* tumor caliper measurements inputted into the equation *tumor volume (mm^3)* = $\frac{(short)^2 \times (long)}{2}$. **A)** Caliper measurements over time **B)** Tumor volumes upon termination (day 28, 29, or 30). Measurements were taken using the same formula as Fig 1A. **** P>0.001**

Fig 2.



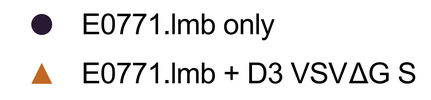


Figure 2 Normalized FDG uptake in organs of interest is increased in E0771.lmb coinfection with VSVΔG S (SARS-CoV-2 Omicron variant). Raw FDG uptake values for each organ (MBq/mm^3) were divided by the uptake values of upper right leg muscle in the same scan. This provided internal normalization of the data. In all tested organs, FDG uptake was increased in the coinfected group compared to mice with E0771.lmb alone. Increased FDG uptake indicates higher levels of pro-inflammatory cells present in an organ, or in the case of the tumours, could indicate a faster rate of malignant growth. Increased immune cell percentages seen in Figures 3 and 4 suggest that FDG increase in the tumour is due to higher levels of immune infiltration. *** P>0.05**

Fig 3A.

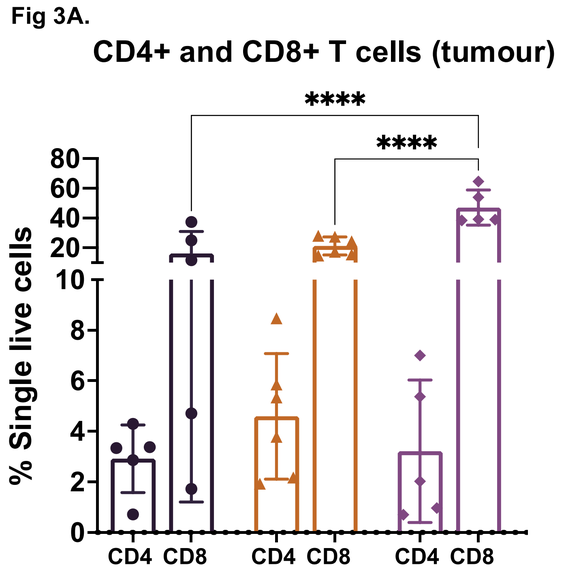


Fig 3B.

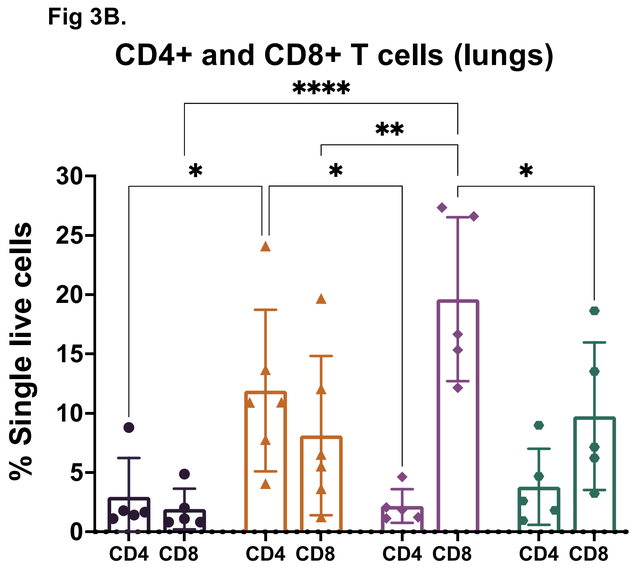


Fig 3C.

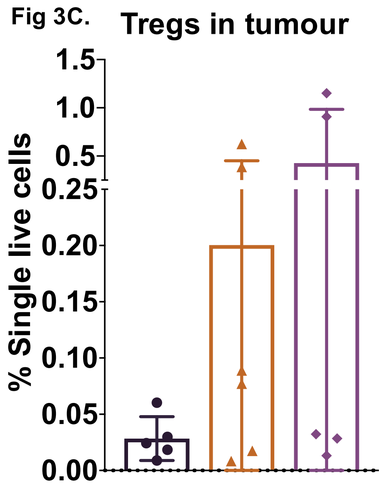


Figure 3 E0771.lmb alone and VSVΔG S (SARS-CoV-2 Omicron variant) co-infected groups show different levels of lymphocyte subsets. A) CD4+ and CD8+ T cells (CD3+) showed consistent group level differences in the tumour. For both cell types, E0771.lmb only groups had decreased levels of both CD4+ and CD8+ cells, with this difference being more pronounced for CD8+ T cells. Interestingly, the ratio of CD4:CD8 cells is disrupted in the tumour, with CD8+ T cells being more abundant in all groups. **B)** CD4+ and CD8+ T cells also showed group differences in the lungs. Notably, coinfected groups displayed phenotypes that differed from both the E0771.lmb and VSVΔG S alone groups. Furthermore, the Day 3 co-infected group is the only subset of mice that showed the expected relative levels of CD4 and CD8 cells. **C)** Levels of regulatory T cells (Tregs, defined as CD3+, CD4+, FoxP3+) were notably increasedin coinfected groups. Similar increases were observed in blood as well (data not shown). Studies have found that increased Treg infiltration into breast cancer tumours, as well as increased levels in peripheral blood are markers of poor prognosis[7]. *** P>0.5, ** P>0.01, *** P>0.001, **** P>0.0001**

Fig 4.

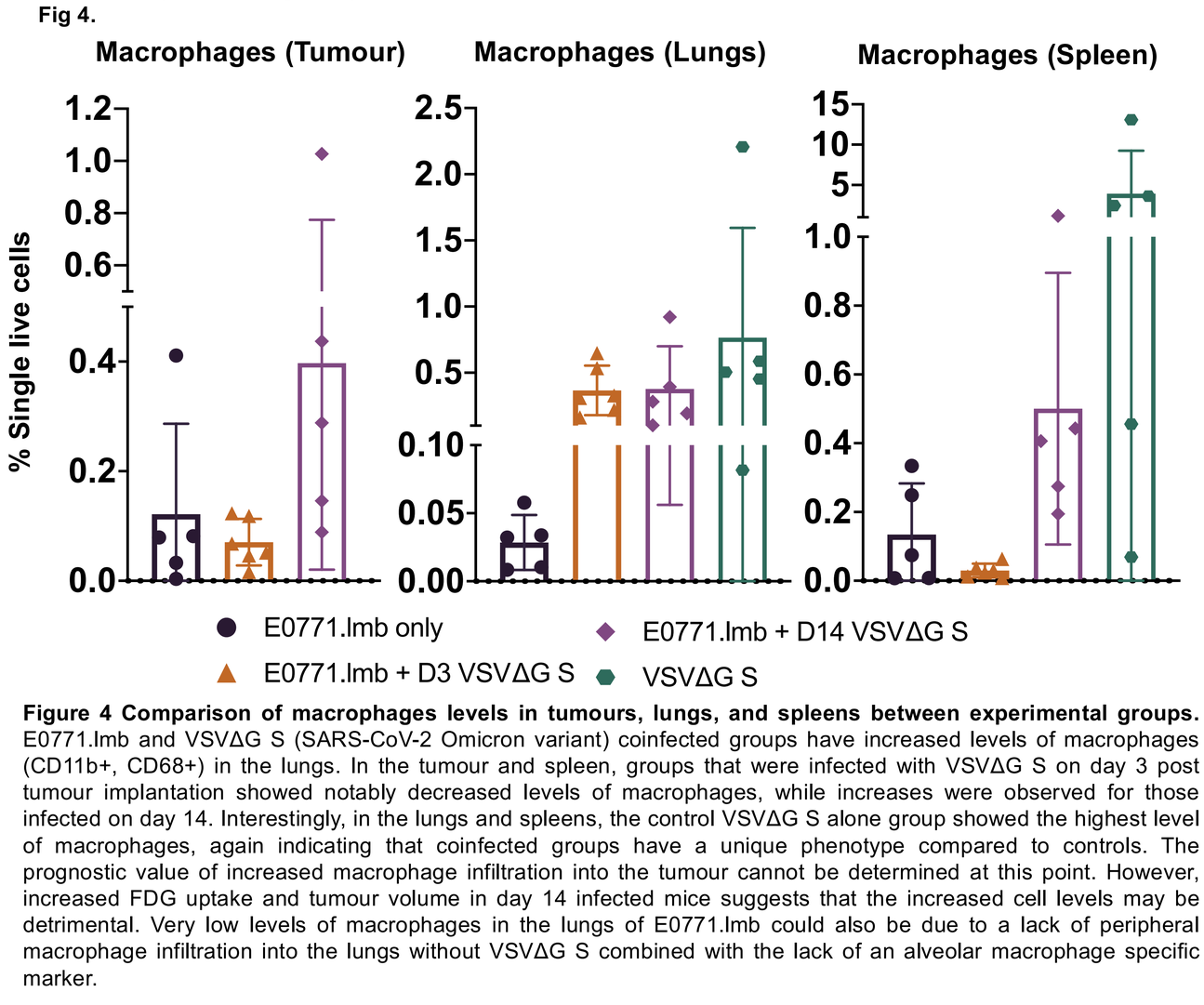


Figure 4 Comparison of macrophages levels in tumours, lungs, and spleens between experimental groups. E0771.lmb and VSVΔG S (SARS-CoV-2 Omicron variant) coinfected groups have increased levels of macrophages (CD11b+, CD68+) in the lungs. In the tumour and spleen, groups that were infected with VSVΔG S on day 3 post tumour implantation showed notably decreased levels of macrophages, while increases were observed for those infected on day 14. Interestingly, in the lungs and spleens, the control VSVΔG S alone group showed the highest level of macrophages, again indicating that coinfected groups have a unique phenotype compared to controls. The prognostic value of increased macrophage infiltration into the tumour cannot be determined at this point. However, increased FDG uptake and tumour volume in day 14 infected mice suggests that the increased cell levels may be detrimental. Very low levels of macrophages in the lungs of E0771.lmb could also be due to a lack of peripheral macrophage infiltration into the lungs without VSVΔG S combined with the lack of an alveolar macrophage specific marker.

4 Conclusions

- Combination of E0771.lmb and VSVΔG S (SARS-CoV-2 Omicron variant) results in increased tumour volumes over time and increased overall FDG uptake.
- Combination groups also show increases in CD4 and CD8+ T cells in the tumour, while there are infection day specific differences in the lungs
- Combined groups show increased macrophages in the tumour, lungs, and spleen

5 Future Directions

- Analyze blood immune phenotype data by time
- Correlation analysis of FDG and immune phenotype data
- Perform IHC on tumour samples to investigate spatial dynamics of immune infiltration
- Terminations at different time points to assess organ level changes over time
- Data analysis separated by time and organ

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