Contribution of neutrophils to disease progression in a murine coronavirus model of neurotropic disease

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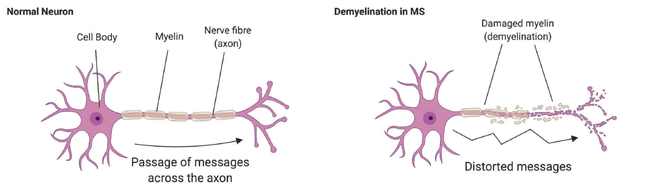
Introduction

Multiple sclerosis (MS)

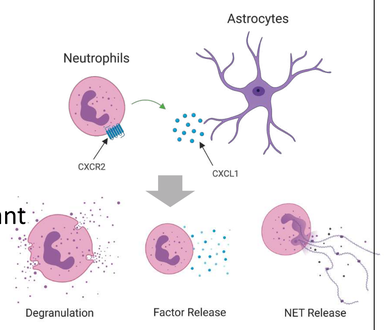
MS is a chronic inflammatory neurodegenerative disease characterized by CNS neuroinflammation, demyelination, and axonal loss that ultimately results in extensive neurologic disability.



JHMV Mouse Model. Intracranial inoculation of C57BL/6 mice with the neuroadapted JHM strain of mouse hepatitis virus (JHMV) results in an acute encephalomyelitis followed by viral persistence in white matter tracts resulting in an immune-mediated demyelinating disease. JHMV is related to other coronavirus strains including SARS-CoV, MERS-CoV, and the recently described SARS-CoV-2 making it an important model for coronavirus induced neurologic disease

Neutrophil and ELR+ Chemokines

- Neutrophils are short lived phagocytes equipped with a variety of anti-microbial responses.
- CXCL1 is a potent chemoattractant of neutrophils to sites of inflammation by high affinity binding with CXCR2



Rationale

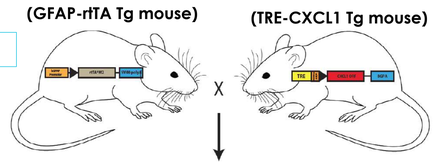
The present study was undertaken to better understand the mechanisms of action by which sustained neutrophil infiltration into the CNS increases white matter damage in a coronavirus model of neurologic disease

Methods

Mouse Model

Doxycycline (dox) treatment overexpresses CXCL1 in the CNS

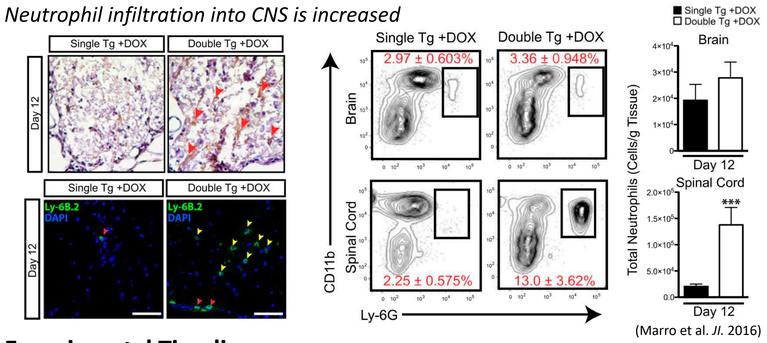
Single Tg (SG) = Tre only



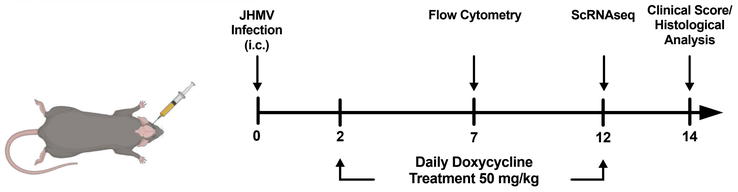
Double Tg (DG)= rTTA and Tre

rtTA x Tre Double Tg

Neutrophil infiltration into CNS is increased



Experimental Timeline

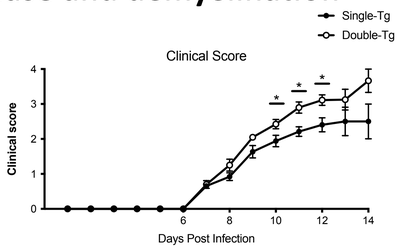


Results

Sustained CNS neutrophil infiltration increases clinical disease and demyelination

A.

JHMV score	Symptoms
0	No clinical disease.
1	Loss of tail tone; may be able to flip mouse over
2	Can flip over but mouse rights itself in less than 30 sec
3	Complete hind limb paralysis; mouse lays on its back
4	Dead



B.

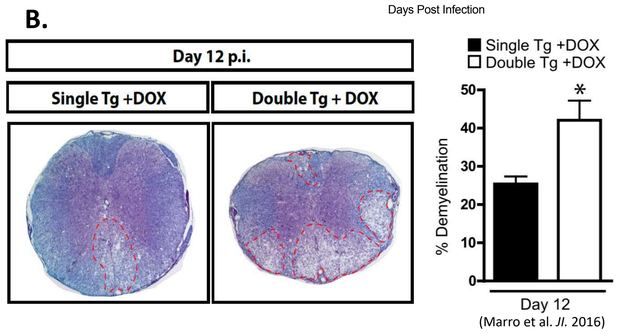


Figure 1: CXCL1 overproduction amplifies clinical disease and demyelination (**A**) Clinical disease was assessed until 14 dpi using a 4 point scale. Dg mice treated with Dox had more severe clinical score compared to Sg mice (**B**) Representative Luxol fast blue stained spinal cord sections with demyelination outlined in red. Quantification of demyelination in defined spinal cord sections indicates increased white matter damage in DG mice. NOTE: Neutrophil depletion in JHMV-infected Dg mice decreases demyelination.

Single Cell RNA sequencing (scRNA-Seq) reveals immunogenic landscape following neutrophil infiltration

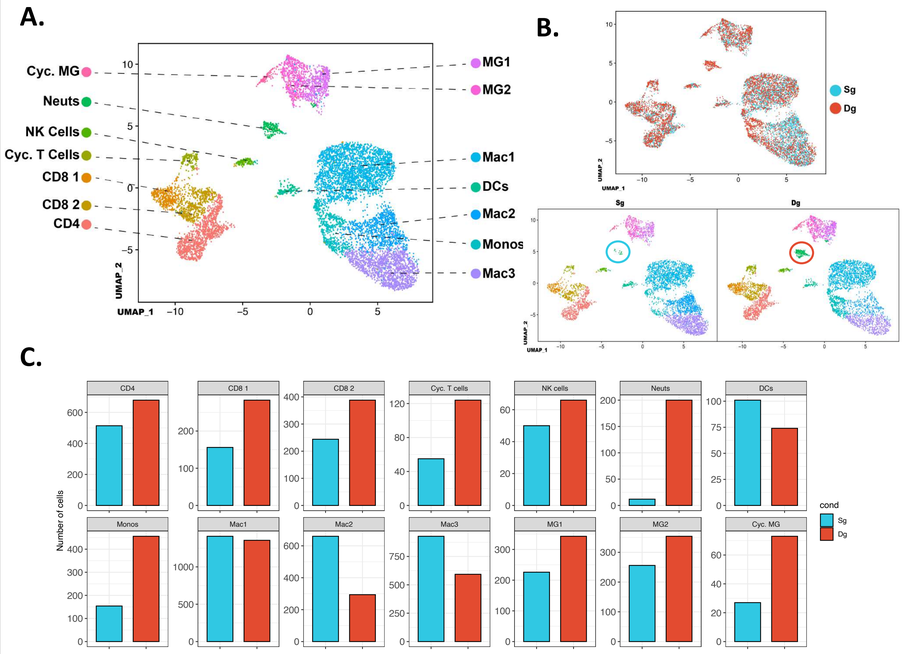


Figure 2: scRNA-Seq of CD45+ cells isolated from spinal cords of JHMV-infected mice treated with Dox at 12 dpi. (**A**) uMAP plot of scRNAseq data revealing 14 distinct cell clusters, aggregate data from DG and SG conditions at 12 days p.i. (**B**) Overlay of DG and SG uMAP plots, Neutrophil (Neuts) clusters circled in both conditions respectively. (**C**) Quantification of cell counts for each cluster in DG (red) vs SG (blue) conditions.

Neutrophils cluster based on known markers

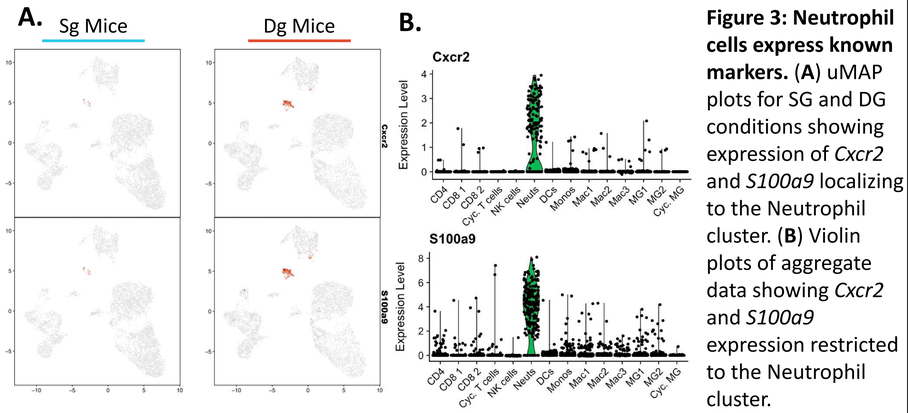


Figure 3: Neutrophil cells express known markers. (**A**) uMAP plots for SG and DG conditions showing expression of *Cxcr2* and *S100a9* localizing to the Neutrophil cluster. (**B**) Violin plots of aggregate data showing *Cxcr2* and *S100a9* expression restricted to the Neutrophil cluster.

Neutrophil cluster express disease-associated markers

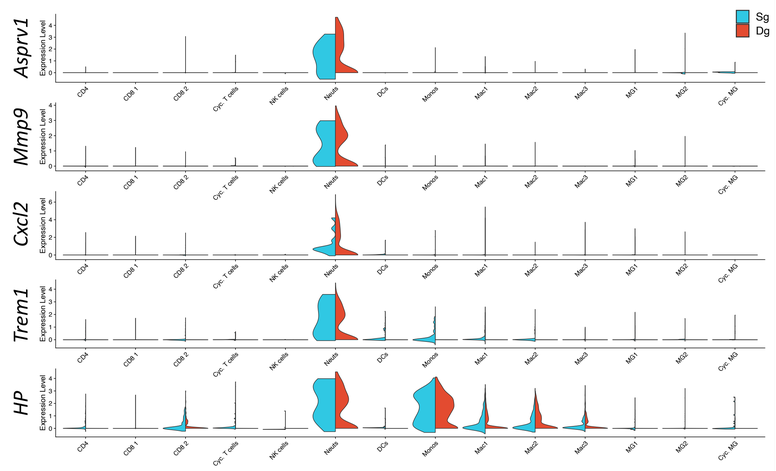


Figure 4: Neutrophil population expressed defense markers. Cluster analysis revealed expression of *Asprv1, Mmp9, Cxcl2, Trem1, and Hp* localized to neutrophil cluster.

Neutrophil Infiltration modulates immune landscape

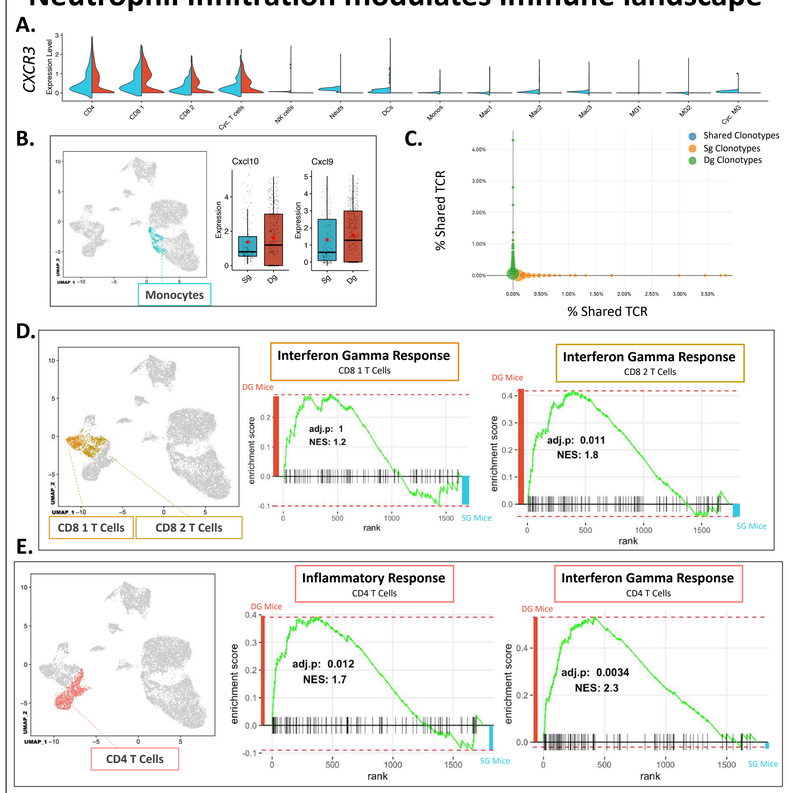


Figure 5: Neutrophil accumulation alters T cell attractant chemokines and response from profiles in T cell subsets. (**A**) Cluster analysis revealed expression of *Cxcr3* localized to T-Cell clusters.(**B**) Expression levels of chemokines *Cxcl10* and *Cxcl9* increases in Dg monocyte subset (**C**) VD(J) sequencing revealed little overlap in TCR clonotype (**D**) Gene set enrichment analysis (GSEA) showed responses to IFN-γ were enriched in both populations of Dg CD8 T cells compared to control Sg mice. (**E**) GSEA plot shows responses to both inflammatory response and IFN-γ response was enriched in the CD4 T cell population of Dg mice compared to control Sg mice.

Conclusion

- Using CXCL1 inducible overexpression we see sustained neutrophil infiltration into the CNS that correlates with increased demyelination
- CNS infiltrating neutrophils express markers associated with neurodegeneration.
- Neutrophil infiltration augments the overall immune response to coronavirus infection of the CNS
- Ongoing studies will further define mechanisms by which neutrophils and microglia regulate demyelination in JHMV-infected mice.

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