A comparison of brain MRI features and disability between two strains of interferon-γ receptor deficient mice in a virus induced model of MS

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BACKGROUND

➢***What is MS?***

Multiple Sclerosis (MS) is a potentially disabling neurological disease characterized by demyelinated lesions [1] and in some cases atrophy of the brain and spinal cord [2]. It affects at least 350,000 people in America alone [1]. MS is widely considered as an autoimmune disease [1].

➢***Why can't we study the pathomechanisms in humans and what can we do instead?***

Studying the active pathogenic mechanisms involved in MS would involve biopsying the brain, a procedure which is typically not needed for diagnosing this disease. Experimental circumstances can't be readily modified in humans. Therefore, a variety of mouse models have been developed to increase our understanding of the mechanisms leading to CNS tissue injury in MS, with the ultimate goal of developing more effective preventive and restorative therapeutic modalities. These models include autoimmune-based models, such as Experimental Autoimmune Encephalitis; virally-induced models such as Theiler's Murine Encephalitis Virus (TMEV), and toxin induced models [3].

➢***What's the TMEV model?***

In the TMEV models, mice are intracrainially injected with a picornavirus common to mice; normally, it causes a gastrointenstinal flu-like illness. After intracerebral inoculation, a biphasic disease develops: every mouse develops the initial meningo-encephalitis stage, and in susceptible strains, this stage is followed by a demyelinating disease similar to what is seen in progressive forms of MS [4].

➢***What impact does the strain of mice have on TMEV infection?***

Various mice strains have been used as models, with differing types of disease seen in different strains. Two oft-studied strains are B6 and 129; classically, these two strains are resistant to TMEV induced demyelination. However, interferon-gamma receptor deficiency allows the virus to perpetuate, inducing demyelination in the brain and spinal cord in an accelerated fashion compared to the classic model in SJL/J mice [4].

➢***What's the purpose of this study?***

This study aims to compare two rarely used interferon-gamma receptor deficient strains, the Ifngr1^{tm1Agt}/J (G129 mice) and B6.129S7-*Ifngr1*^{tm1Agt}/J (B6G mice). G129 mice have previously been studied; their disease is characterized by lesion formation in the brainstem and periventricular area [4]. Disability is apparent by six weeks after infection, and survival beyond eight weeks is rare. B6G mice are not well-studied and little is known about the characteristics of their disease.

METHODS

Mice: infected intracerebrally with DAV VIII TMEV virus; 7 each B6G and G129 infected, 3 control each

Outcome measures:

1. disability – rotarod once weekly;
2. MRI based outcome measures on biweekly in vivo MRI-s;
   - A. T2 hyperintense lesion load by volumetric MRI;
   - B. Analysis of T1 hypointense lesion formation : what proportion of T2 hyperintense lesions is detectable as T1 hypointensity?
   - C. Analysis of gadolinium enhancement in newly forming lesions: Is it always present, pattern of enhancement (ring/rim, arc, homogeneous, nodular)

Statistical analysis: Sigma Stats 11.2 will be used for intra- and inter-group analysis

MRI acquisition methods

- Brueker Avance 300 MHz (7 Tesla) NMR spectrometer (Brueker Biospin, Ettlingen, Germany) equipped with imaging gradients and small animal imaging probes
- Scan time points: before infection, biweekly after infection, until death or eight weeks
- Continuous inhalational anesthesia with 2.5% V/V% isoflurane
- Thermocouple controlled regulation of scanner bore temperature to prevent hypothermia
- Monitoring of respiratory rate using SA Instruments 1025 monitoring and gating system for small animal MRI imaging (SAII, Inc, Stony Brook, NY)

Acquisition Parameters

- 3D T2 weighted imaging: RARE (FSE) sequence, TR:1500 ms, TE: 45 ms, RARE factor: 16, FOV: 4 x 4 x 2 cm, matrix: 192 x 192 x 96, NEX: 1
- Pre-and post gadolinium multislice T1 weighted acquisition: Inversion Recovery RARE sequence, TR:3200 ms, TE: 7.5ms, TI: 1300 ms; RARE factor: 4, FOV: 4 x 4 cm, matrix: 192 x 192, Number of slices: 18, slice thickness: 0.5mm, interslice distance: 0.5mm, NEX: 2
- Gadolinium dose: weight based administration of 0.2 mmol/kg gadolinium-DTPA diluted to 0.2 ml with normal saline injected intraperitoneally. Scanning delay: 5 minutes

Image analysis

- Analyze 10.0 (Biomedical Imaging Resource, Mayo Clinic) was used for slice extraction and visualization from 3D datasets
- Image analysis using the ROI tool, semiautomated thresholding and seed growing based segmentation and volumetry of lesions and ventricles

Rotarod

- Weekly rotarod measures were used to quantify disability. Disability is defined as 2 standard deviations from baseline.
- Mice ambulate on a constantly accelerating rotating rod, total time spent on the rod is measured.
- 3 trials at each time point, the better performance is recorded as final performance.
- Rotation starts at 10 rpm, which increased by 0.4 rpm every 2 seconds until the animals fall. Measures included passive time—i.e., total time stayed on the wheel, including when mice weren't actively running. Passive rotation was set to 25 seconds.

PRELIMINARY RESULTS

ROTAROD

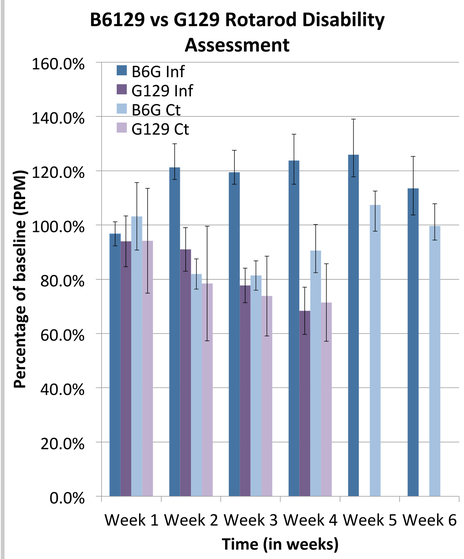


Figure 1: Rotarod data of four different groups: B6G infected, B6G controls, G129 infecteds, and G129 controls. B6G infected show statically significant differences between B6G controls at Week 2 (p=. 040), Week 3 (p=.023) and a trend at Week 4 (p= . 095). Error bars display SE.

VOLUMETRIC ANALYSIS

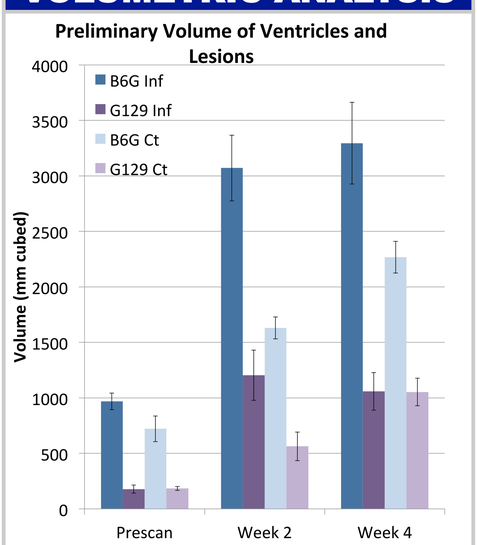
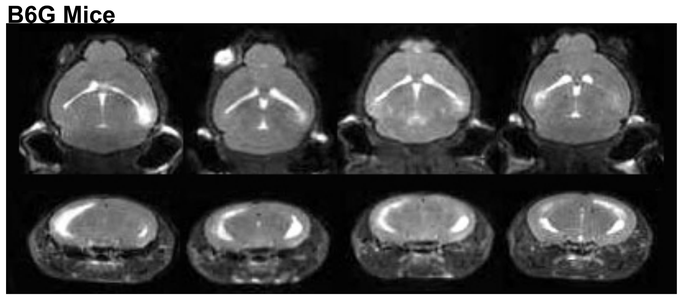


Figure 2: This figure compares lesion and ventricle data data of four different groups: B6G infected, B6G controls, G129 infecteds, and G129 controls on T2 weighted scans. There are no statistically significant differences between G129 and B6G mice, but B6G infecteds at week two gained significantly more lesion/ventricle volume than at baseline (p=.0017).

B6G Mice



G129 Mice

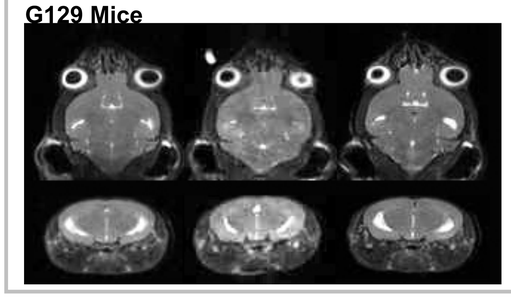


Figure 3: Axial and coronal cuts of a B6G brains (above) and G129 brains (below) at week 2 post-infection on a T2-weighted RARE scan. The B6G mouse brain shows a large area of confluent signal abnormality adjacent to the lateral ventricles; the G129 brain shows minimal changes compared to B6G. In G129 mice, the abnormality is located slightly dorsal compared to abnormality in B6G mice.

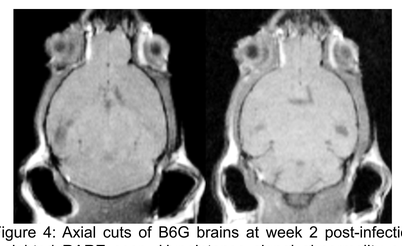


Figure 4: Axial cuts of B6G brains at week 2 post-infection on a T1 weighted RARE scan. Hypointense signal abnormality can be seen next to the ventricles, confirming the abnormality in Figure 3.

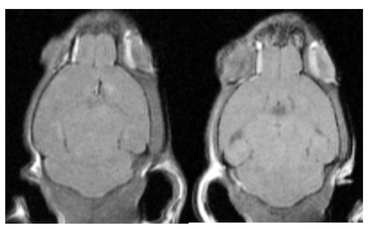


Figure 5: Axial T1 weighted RARE scans of G129 brains at week 2 post-infection.

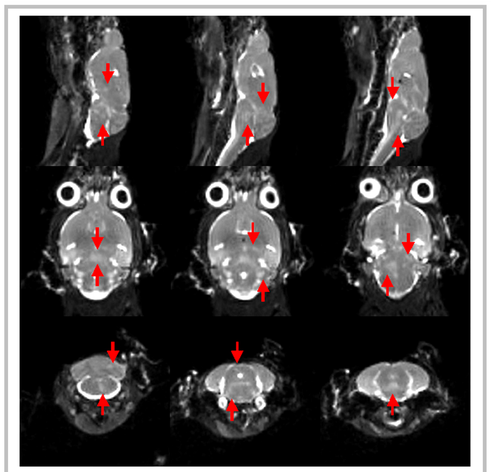


Figure 6: Arrows point to T2 hyperintense lesions. Sagittal (top row), axial (middle row) and coronal (bottom row) T2 weighted MRI scans at week 8 in a G129 mouse using data from a previously published experiment. Our mouse cohort is not yet at this time point.

PRELIMINARY RESULTS

B6G infected mice show statistically significant differences between B6G controls on rotarod; B6G infecteds scored significantly higher at week 2 and week 3 (p=.040, p=.023) and the trend continues on Week 4 (p=.095) (Figure 1).

Rotarod measures in G129 mice do not yet show statistically significant differences (Figure 1).

Volumetric analysis showed no statically significance difference in lesion load and ventricle size in G129 mice. However, in B6129, there was a significant difference (p=. 0017) (Figure 2).

T2 hyperintense lesions are seen in both B6G mice and G129 mice (Figure 3). Lesions in B6G mice are located slightly dorsal compared to G129 mice (Figure 3). T1 hypointense lesions are seen mainly in B6G mice (Figure 4).

Based on our previously published data, G129 mice are expected to develop substantial lesion load by week 8 (Figure 6).

CONCLUSIONS

- B6G mice develop previously unreported unusually large T2 hyperintense and T1 hypointense confluent lesions in the early stages of TMEV infection
- Despite the large lesions, disability as captured by rotarod does not decline in B6G mice at the early time points: B6G mice show an increased activity level from week 2 to week 6. Possible causes:
  - Anxiety or agitation
  - Rotarod is not adequate in capturing the disability they develop. The affected areas appear to involve the hippocampus, so behavioral measures addressing memory and cognitive problems may be more appropriate.
  - Circadian rhythm issues: Mice are nocturnal. The rotarod test are performed during the day, which is normally their sleep time—infected B6G mice might have circadian rhythm abnormalities and may be more awake during the day, as observed by our team during the rotarod and other studies.
- B6G mice may be a good model for "T1 black hole" formation in MS.
- These results are preliminary; this study is ongoing.

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