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 demyelinating 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 biopsy of 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 methods, 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 intracranially injected with a picornavirus common to mice; normally, it causes a gastrointestinal flu-like illness. After intracerebral inoculation, a biphasic disease develops: every mouse develops the initial meningoencephalitis 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 mouse 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 SJU mice [4].

What’s the purpose of this study?

This study aims to compare two rarely used interferon-gamma receptor deficient strains, the Ifngr1⁻/⁻ (B6G mice) and B6-129Stg⁻/⁻ (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 controls each

Outcome measures:
1. Disability – rotarod once weekly:
2. MRI based outcome measures on biweekly in vivo MRI-s:

   - A. T2 hypointense lesion load by volumetric MRI:
   - B. Analysis of T1 hypointense lesion formation:
   - C. Analysis of gadolinium enhancement in newly forming lesions:

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

MRI acquisition methods:

   - Bruker Avance 300 MHz (7 Tesla) NMR spectrometer (Bruker 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 (SAIL, 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, interleave 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.

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.

REFERENCES


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