Causality of Sex-Driven Dimorphism in a Relapsing-Remitting Murine Disease Model of Multiple Sclerosis

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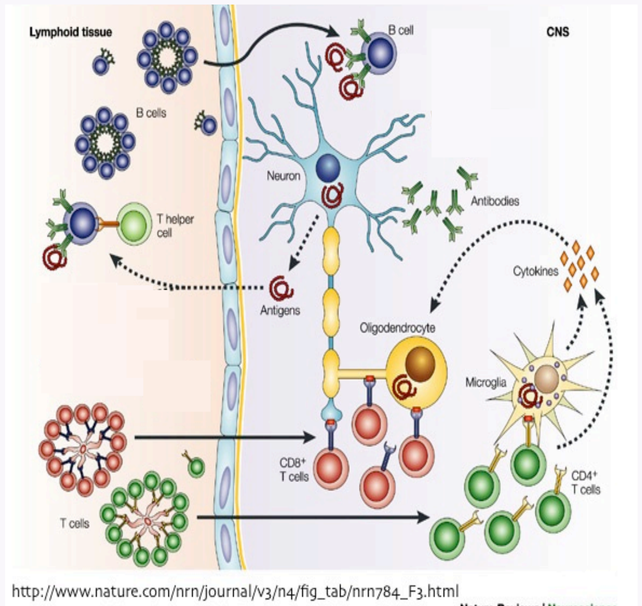
Background

- Multiple sclerosis (MS) is the most common neurodegenerative autoimmune disorder that affects young adults[1]. Autoimmune disorders display sexual dimorphic characteristics in disease susceptibility and progression to females over males. MS is characterized by the destruction of myelin sheaths that cover axons, disrupting neuronal activity and causing neurological deficits[2].
- Relapsing-Remitting Multiple Sclerosis (RRMS) is the most common progression of MS[2]. RRMS disease progression begins with an acute onset of neuroinflammatory events followed by periods of remyelination followed by periods of demyelination[2]. RRMS displays sexual dimorphic characteristics that favor females over males approaching a 4:1 ratio[2].
- Disruption of the blood brain barrier (BBB) allowing for leukocyte entry and chronic inflammation is considered the primary cause of MS pathogenesis[3]. A disease-modifying molecule, sphingosine 1-phosphate receptor 2 (S1PR2), has shown to disrupt BBB integrity[2]. This receptor has an increased expression in the female cerebellum, a disease susceptible region, compared to males[2]. **The underlying cause of the overproduction of S1PR2 has yet to be discovered.**
- Treatment with an S1PR2 antagonist, JTE-013, has previously shown to lower clinical scores of disease severity in a commonly used murine model for MS, experimental autoimmune encephalomyelitis (EAE)[4]. **Phenotypic analysis of inflammatory leukocyte infiltrates found in disease regions of the CNS is needed to further understand the effects of S1PR2 antagonism.**



Objectives

- **Does chromosome compliment define baseline differences of S1PR2 expression between females and males?**
- **What are the phenotypes of inflammatory infiltrating leukocytes into the CNS after treatment with JTE-013, an S1PR2 antagonist?**

1

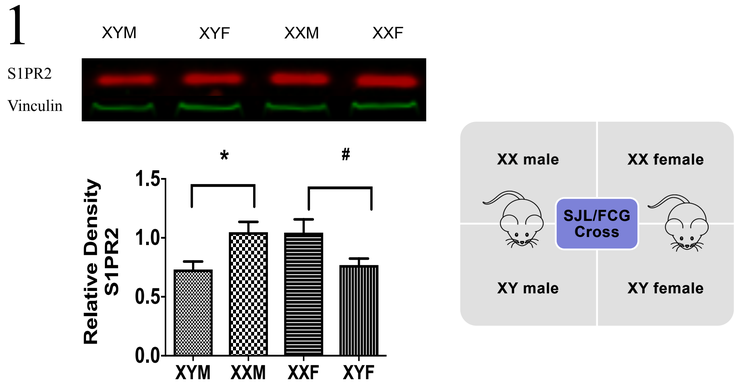
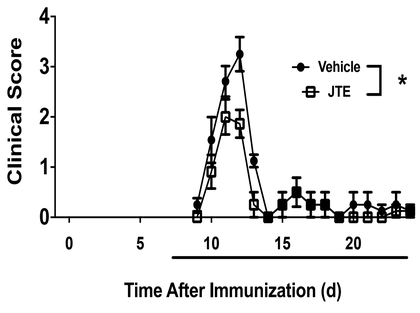


Figure 1: Western blot analysis of S1PR2 levels in the cerebellum of Four Core Genotype (FCG)/SJL mice at 8 weeks old. FCG mice are created by removing the *Sry* gene from the Y chromosome and placing it on an autosomal chromosome. The FCG murine model is the primary approach to study whether sexual dimorphic phenotypes are sourced from XX chromosome compliment vs. XY chromosome compliment.[5] Expression of S1PR2 in XYM is significantly lower than in XXM mice (p=0.029). Expression of S1PR2 in XYF trends towards being significantly lower than in XXF mice (p=0.072).

2



Clinical Scores
1: Limp Tail
2: Disrupted righting reflex
2.5: Knuckling
3: Complete inability to move on hind limb
4: Complete hind limb paralysis
5: Moribund/Death

Figure 2: Mice were immunized with 200 ug/mouse of $PLP_{139-151}$ to induce EAE.
A. Clinical disease scores post immunization. Treatment with JTE-013 or vehicle started at disease onset. Treatment occurred daily.

3

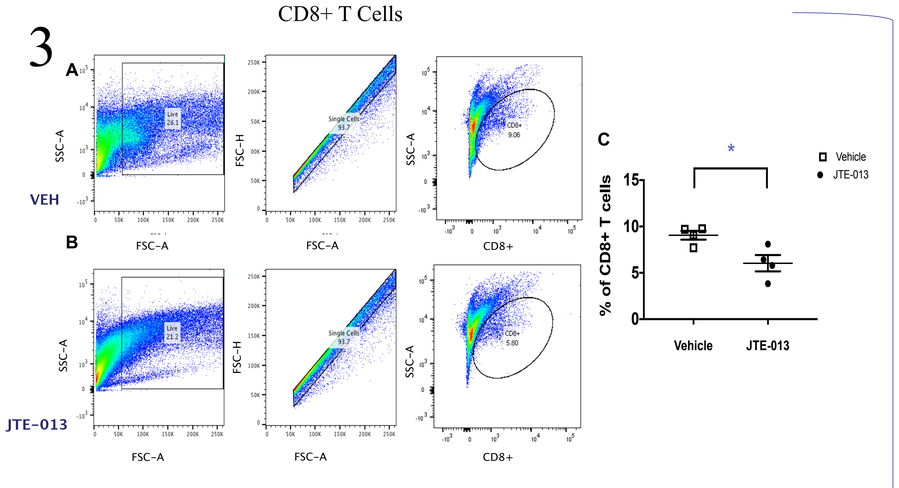


Figure 3: Post EAE, cell isolation and flow cytometric analysis of CD8+ cells in the cerebellum.
A. Representative cell isolation and gating from the vehicle treatment group. **B**. Representative cell isolation and gating from the JTE-013 treatment group. **C.** CD8+ T cells are significantly lower in the the JTE-013 treatment group (p=0.024).

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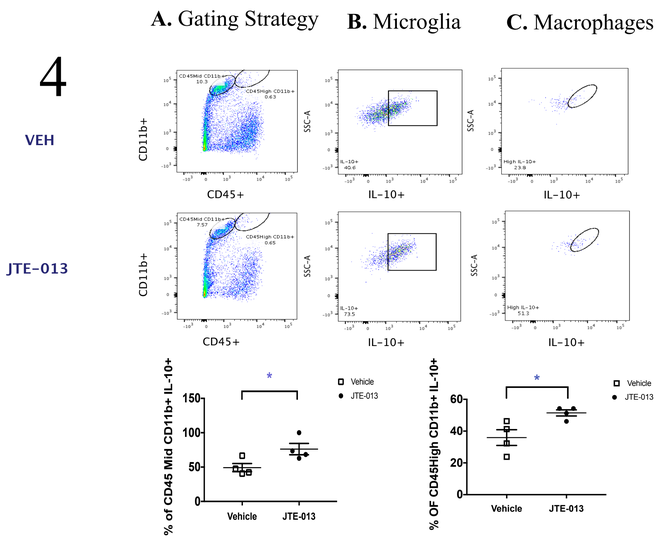

Figure 4: Post EAE, cell isolation and flow cytometric analysis of IL-10 expressing macrophages and microglia in the cerebellum.
A. Representative gating and percentages of CD45High CD11b+ IL-10+ and CD45Mid CD11b+ IL-10 cell types in both vehicle and treatment conditions.
B. Anti-inflammatory, IL-10 expressing, macrophages percentages are significantly lower in the cerebellum of the vehicle treatment group vs. the JTE-013 treatment group. (p=0.0268).
C. Anti-inflammatory microglia expressing IL-10 percentages are significantly lower in the cerebellum of the treatment group vs. the JTE-013 treatment group (p=0.0381)

Conclusions

- **Baseline differences in S1PR2 expression may be due to specific genes found on the X chromosome, or due to the affects of pubescent hormonal fluctuations. The Y chromosome may offer protection for the over expression of S1PR2 in males.**
- **Previous data suggests that treatment with JTE-013 enhances blood brain barrier integrity[2]. Our current data show JTE-013 treatment causes decreased infiltration of CD8+ t cell entry into the central nervous system. Additionally, anti-inflammatory expressing microglia and macrophages were increased with JTE-013 treatment. This suggests S1PR2 antagonism may impact inflammatory events associated with MS disease progression.**

Future Directions

- Western blot of S1PR2 at 2 week time point of FCG mice
- RNA sequencing a. FCG mice b. S1PR2 RNA vs. protein

References

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