Effects of Systemic Influenza Infection on Remyelination in an Animal Model

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Introduction

- Multiple Sclerosis (MS) is an autoimmune inflammatory disease of the nervous system.
- MS is characterized by episodes of demyelination followed by partial remyelination.
- Cuprizone is known to induce demyelination in specific regions of the C57BL/6 mouse brain, which remyelinate upon termination of cuprizone.
- Upper respiratory infections are known to exacerbate MS symptoms.
- We hypothesize that infection may impede remyelination, possibly due to a direct effect on oligodendrocyte maturation.
- If this is the case, we may find a reduced amount of mature oligodendrocytes and large amounts of oligodendrocyte precursor cells (OPCs) in areas of heavy demyelination in flu mice compared to controls.
- The aim of this study is to observe the impact of peripheral infection on remyelination in the corpus callosum, a known area of heavy demyelination in the cuprizone model.

Oligodendrocyte Maturation

A number of transcription factors are required for the differentiation of OPCs to mature oligodendrocytes. Olig2-knockout mice had no oligodendrocyte lineage cells, showing that initial oligodendrocyte lineage commitment is reliant on the transcription factor Olig2. Other downstream transcription factors are required for the progression into mature oligodendrocytes, all of which are present in OPCs. This suggests that their roles in promoting differentiation at myelin gene promoters must be subject to regulation by additional factors, some of which have been identified as being active in preventing OPCs from undergoing maturation. Importantly, only mature oligodendrocytes myelinate. (Emery, 2010)

Methods

Animals and Cuprizone administration: C57BL/6 mice aged 8 weeks were fed a diet of 0.2% cuprizone for 3 weeks and then placed on normal rodent chow. All mice were inoculated at the 3 day timepoint with either influenza A or saline, then were sacrificed at the 5, 6, and 7 week timepoints for further analysis. All mice were age and gender-matched and sourced from Jackson Laboratories. Weight change was recorded daily.

Immunohistochemistry:

After post-fixation and cryoprotection, brains were sectioned sagittally at 20um on a Cryostat. Tissue was permeabilized and blocked in 0.1-0.3% Triton X in phosphate buffered saline with 5% goat serum and then permeabilized and blocked in 0.1% Triton X in phosphate buffered saline with 5% goat serum. Tissue sections were incubated with antibodies against Olig2 (1:500), Iba1 (1:1000), CC1 (1:200), GFAP (1:500). Appropriate Alexa Fluor-coupled secondary antibodies were used to visualize the antigen–antibody complexes.

Results

1: Infection suppressed specific genes involved in myelination

Figure 1. Schematic of the oligodendrocyte lineage showing some of the intrinsic and extrinsic factors that influence oligodendrocyte differentiation and the myelination of individual axons. (Emery, 2010)

Figure 2: Effects of cuprizone and infection on body weight

Figure 3. The effect of influenza A virus infection on the weight change: Mice were fed a normal chow supplemented with 0.2% cuprizone for five weeks to induce demyelination. During the demyelination process, the gradual weight loss with time duration is observed. After five weeks post-cuprizone diet, the diet was changed into the normal chow for the rodents. The group injected with saline demonstrated weight increasing right after the diet change, while the group inoculated with flu virus showed weight decreasing a week after the diet change.

Figure 4: OPC and Mature oligodendrocytes cell numbers for saline and flu inoculated groups 6 weeks post infection.

Conclusions

- RNA-sequencing on cerebellum and spinal cord tissues of infected mice indicate upper-respiratory infection downregulates genes associated with oligodendrocyte maturation, which is critical to myelin formation and maintenance.
- Bioinformatics analysis indicated the transcription factors Tcf7l2 and Sox10 were deregulated by infection.
- We currently lack the statistical power to determine the effect of influenza infection on mature oligodendrocytes and lesioned corpus callosum during remyelination.

Future Work

In the future we plan to continue the study by delving deeper into the mechanism of myelination. Simply observing the presence of mature oligodendrocytes and OPCs isn’t enough to say whether or not a neuron is fully myelinated. We will immunolabel the myelin sheath itself in order to directly quantify its presence, as well as stain Fox1 and GFAP in order to observe astrocytes and microglia as it is believed that they inhibit remyelination. There are also other basal transcription factors that impact whether OPC differentiate into mature oligodendrocytes that remain to be fully elucidated.

References

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