Lipid Accumulation Leads to Reactive Macrophage Pathology in settings of GALC dysfunction

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BACKGROUND

Globoid Cell Leukodystrophy (GLD)

- GLD, also known as Krabbe Disease, is a lysosomal storage disease caused by loss-of-function mutations in the lysosomal hydrolase, GALC, which breaks down galactosylceramide (galcer), a major constituent of myelin in the nervous system (Figure 1). GALC dysfunction results in the lysosomal accumulation of lipids like galcer and its toxic metabolite, psychosine. These lipids are thought to result in extensive demyelination and cell death. Brain macrophages also accumulate these lipids and worsen disease pathology. More recently, these cells were found to adopt proinflammatory states (Figure 2, 3). GALC-deficient macrophages worsen disease and adopt proinflammatory states, while GALC-competent cells ameliorate pathology. However, how GALC deficiency leads to abnormal macrophage responses is unknown.

Significance

- The current treatment, hematopoietic stem cell transplant (HSCT) successfully delays disease progression but has limitations such as a narrow treatment window and high mortality. Discovering the mechanisms underlying macrophage dysfunction will be key to the development of safer therapies for GLD. Additionally, our work will uncover links between lysosomes and immune responses that may apply to many other neurodegenerative conditions

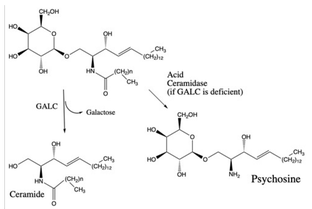

Figure 1: Biochemical Degradation of galcer to psychosine (Herbst et al 2020)

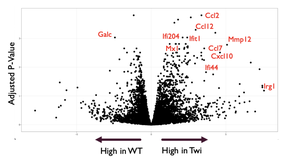

Figure 2: Mutant macrophages highly express proinflammatory signatures (Will Aisenberg, Bennett Lab PostDoc)

QUESTION/HYPOTHESIS

How does defective GALC lead to macrophage dysfunction?

- Lysosomal lipid accumulation is characteristic of GLD. In other settings of lysosomal dysfunction, transcription factor EB (TFEB) is activated in response to lysosomal stress. This lysosomal stress response repairs lysosomes and simultaneously enables proinflammatory immune responses. I hypothesize that lysosomal lipid accumulation activates TFEB in *Galc*^{KO} macrophages but not in WT macrophages.

METHODOLOGY

Cell Culture

- Key to testing this hypothesis is the twitcher (Twi) mouse, a model of GLD that recapitulates human disease (Figure 3). From primary bone marrow, we generated Twi and WT hoxb8-expressing progenitor cell lines. These cell lines can be expanded in culture and differentiated into macrophages upon removal of estrogen and addition of M-CSF1, a macrophage colony-stimulating factor.

Starvation and Lipid Treatment

- Hoxb8-expressing progenitors were differentiated into macrophages for 6 days with 30 ng/ml M-CSF1. These macrophages were then treated with either a starvation buffer called Hanks' Balanced Salt Solution (HBSS) or normal macrophage media (D10) for up to 45 minutes. WT and Twi macrophages were also treated with DMSO vehicle or galactosylceramide in serum-free media for 20 hours.

Immunofluorescence and Image Analysis

- Cells were fixed with 4% PFA for 15 minutes, permeabilized using 0.2% Triton X-100, and blocked. After block, cells were stained with primary antibodies overnight, washed, and stained with secondary antibodies for an hour.
- 40X images were taken using confocal settings on a plate-reading ImageXpress microscope. Images were then analyzed with the image processing software, FIJI.

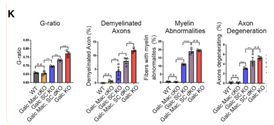

Figure 3: Galc KO brain macrophages worsen disease (Weinstock et al 2020)

RESULTS

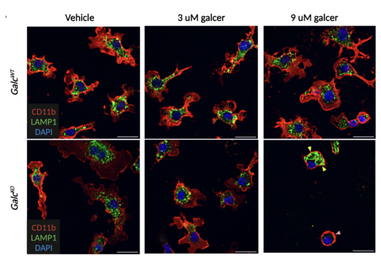

Figure 4: KO, but not WT, macrophages had rounder morphology and larger lysosomes

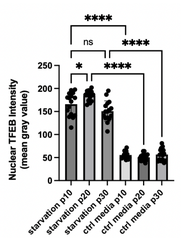

Figure 5: 20 minute permeabilization protocol was best for TFEB staining

RESULTS (CONT'D)

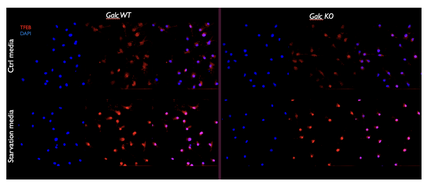

Figure 6: TFEB activation is similar between Galc WT and KO macrophages in response to starvation

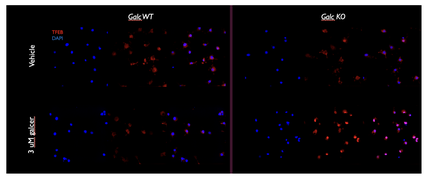

Figure 6: With lipid challenge, TFEB is activated in Galc KO but not WT macrophages

FUTURE DIRECTIONS

Our immediate next steps would be to elucidate how TFEB gets activated in the first place. Secondly, we plan on understanding how macrophage responses are altered by TFEB activation. Thirdly, we want to see if TFEB activation is high in living mice in order to establish if our findings are physiologically relevant

ACKNOWLEDGEMENTS & REFERENCES

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