Investigating the role of self-peptides in naive CD4+ T cell homeostasis

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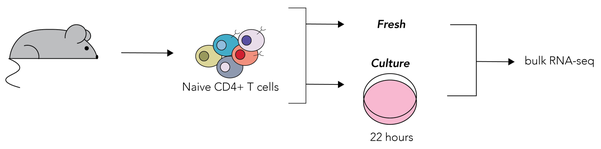
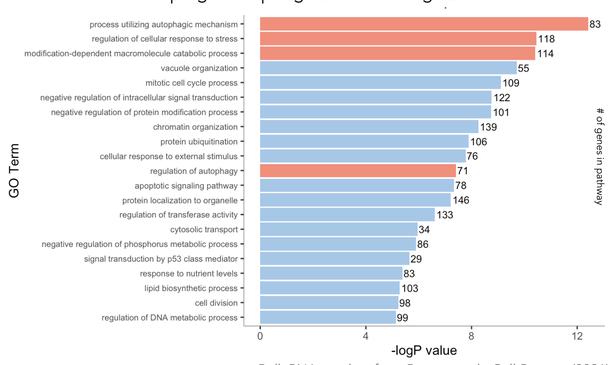
Background

- T cells are **immune cells** that are essential for mounting an adaptive immune response
  - These cells recognize peptides presented on major histocompatibility complex (MHC) molecules through their unique receptors
- T cells that have yet to encounter their cognate antigen are naive
- As naive T cells circulate the periphery, they must maintain sub-threshold interactions with **self-peptides** presented on MHC molecules for their survival
  - "Self" refers to peptides that are endogenous to the host (not foreign or pathogenic)

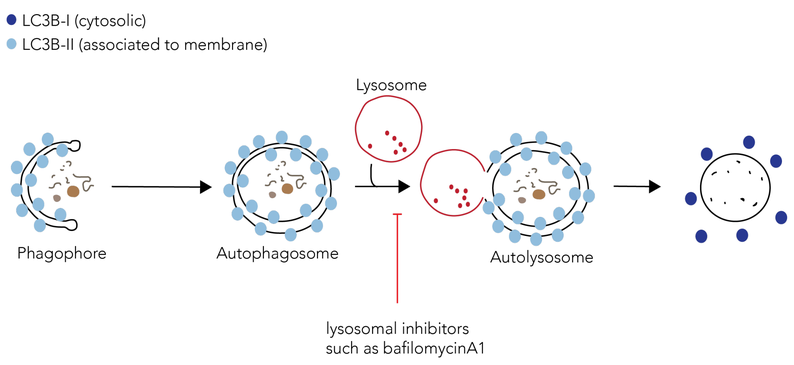


Gene programs up-regulated following 22-hours in culture:



Bulk RNAseq data from Rogers et al., Cell Reports (2021)

- Previous data from the Mandl lab indicates that in vitro self-peptide deprivation leads to an upregulation of catabolic processes in naive CD4+ T cells, such as **autophagy**



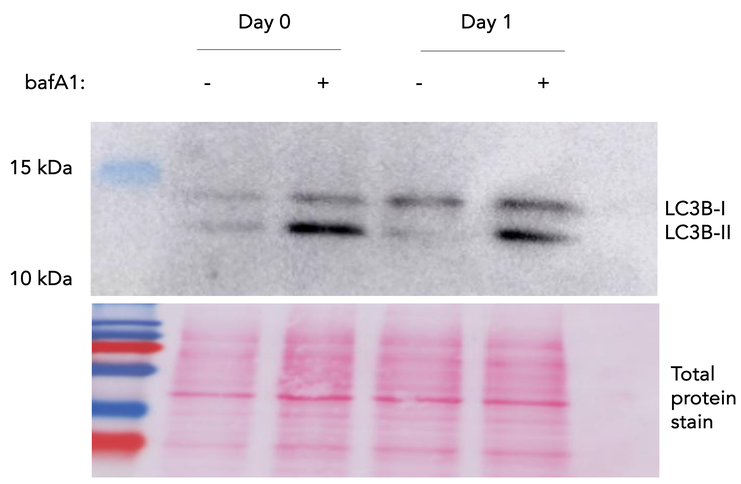
Adapted from Parzych & Klionsky, Antioxid Redox Signal (2014)

- **Autophagy** is a cellular degradation pathway in which organelles and proteins are degraded to be recycled as metabolites for the cell

Is autophagy upregulated at the protein level when naive CD4+ T cells are deprived of self-peptides in vitro?

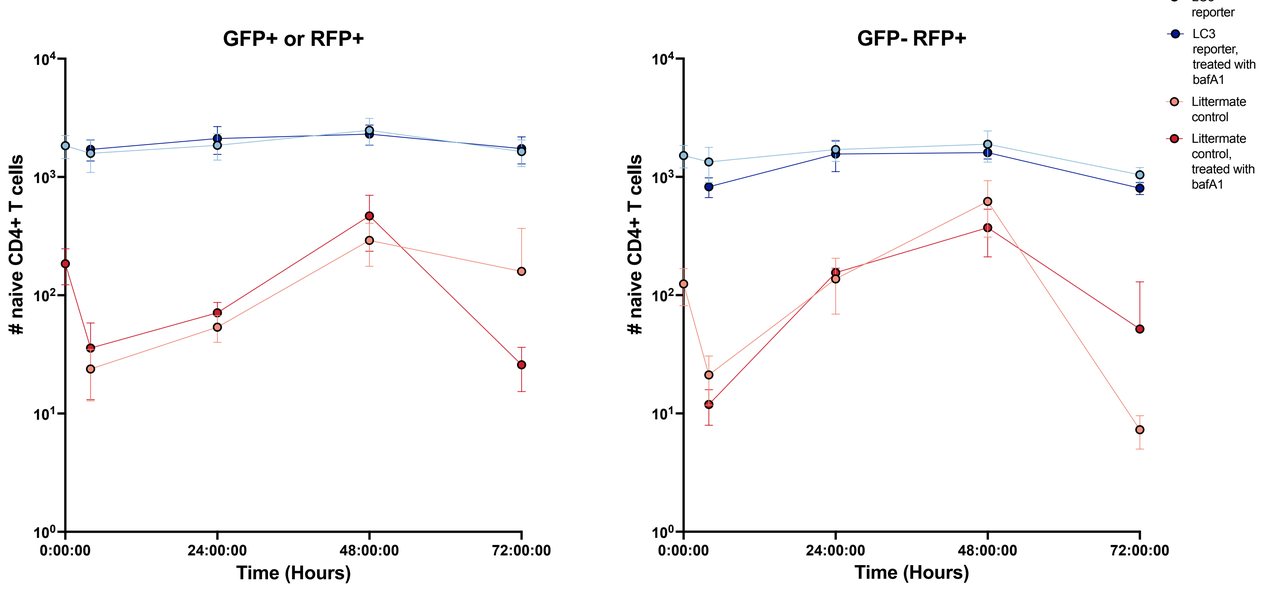
Results

LC3B Western blot indicates that autophagy flux does not significantly change in naive CD4+ T cells within one day of culture:



- Total protein stain is used as a loading control

LC3B reporter has low expression in naive CD4+ T cells:

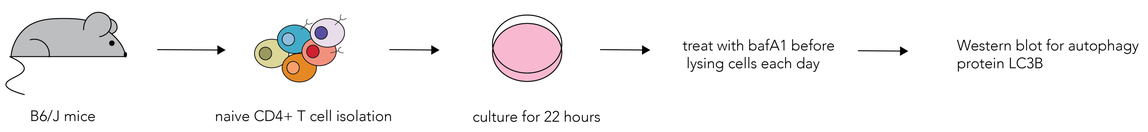


- This suggests the Western blot is the most sensitive assay to assess autophagy overtime

Methods

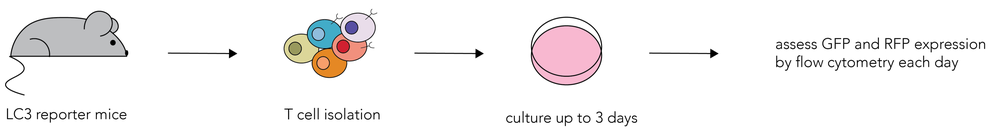
The gold-standard method for measuring autophagy is by quantifying **LC3B**, an essential autophagy protein that is associated with the autophagosome

Measuring LC3B by Western blot:

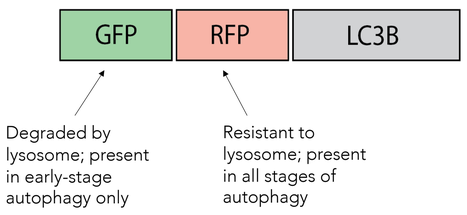


- The drug bafilomycinA1 (bafA1) inhibits lysosomal degradation of autophagosomes, preventing the breakdown of LC3B-II
- In the presence of bafA1, autophagy upregulation is indicated by the ratio of LC3B-II/LC3B-I

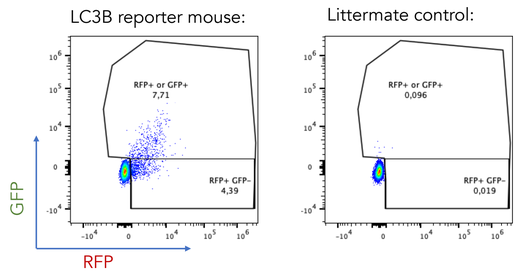
Measuring LC3B with transgenic reporter mice:



- Driven by a CAGG promoter, LC3B is fused to two fluorophores with differing sensitivity to the acidic lysosome
  - This reporter allows for the quantification of autophagy **and** the determination of its stage



Gating example in naive CD4+ T cells (day 3 of culture):



Future Directions

- Assess LC3B protein levels by Western blot up to 3 days in culture
- Identify methods of inducing autophagy in naive CD4+ T cells in vitro to use as a positive control
  - Standard methods of inducing autophagy in cell culture, such as treatment with rapamycin, are ineffective in naive T cells
- Investigate the bulk RNAseq dataset for other gene pathways related to the catabolic signature of naive CD4+ T cells deprived of self-peptides

References & Acknowledgements

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Parzych KR, Klionsky DJ. 2014. An Overview of Autophagy: Morphology, Mechanism, and Regulation. *Antioxid Redox Signal* 20:460–473.

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