Lipid Accumulation in Mononuclear Phagocytes Impairs Autophagy and Contributes to Inflammation after Traumatic Brain Injury

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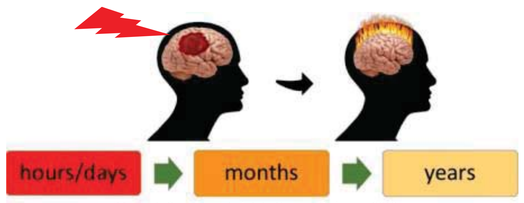
INTRODUCTION



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Prolonged inflammation after TBI

Inflammation resolution does not complete after TBI and leads to chronic neuroinflammation.

Microglia-mediated neuroinflammation is of special focus among factors contributing to second injury after TBI.

Autophagy

Major cellular degradation process by which cells remove toxic macromolecules and damaged organelles.

It is important to maintain normal cellular homeostasis particularly for mononuclear phagocytes including both resident macroglia and infiltrating macrophages after TBI.

Dysregulation of autophagy has been implicated in several neurodegenerative disorders.

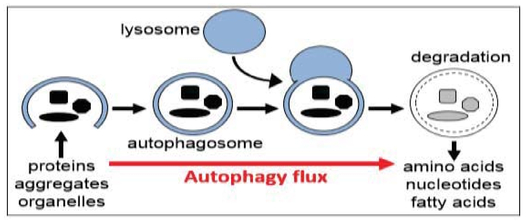

Fig. 1. Schematic representation of the autophagy pathway.

Autophagy impairment in proinflammatory monocytes after TBI

Data from our lab demonstrate that autophagy is inhibited after TBI in parallel with upregulation of proinflammatory markers in mononuclear phagocytes, but the mechanistic explanation behind the autophagy impairment remains unknown.

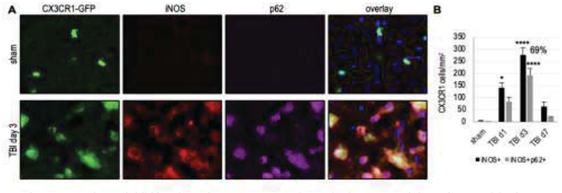

Fig. 2. Autophagy inhibition in active monocytes shown by immunostaining of coronal brain sections of TBI samples

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Lipid accumulation can lead to inhibition of autophagy

It is shown that infiltrating macrophages phagocytose lipids in atherosclerotic plaques and become proinflammatory foam cells.

In multiple sclerosis is also shown that myelin-laden macrophages are reminiscent of foam cells. Cholesterol rich species like myelin are abundant in TBI lesion. We hypothesized that lipid phagocytosis by monocytes in TBI lesion can cause autophagy inhibition.

MATERIALS AND METHODS

Controlled Cortical Impact (CCI)

Moderate TBI was induced in C57BL6/J WT mice using CCI injury model.

After injury animals were sacrificed at different time points and their brains were removed and processed for immuno-fluorescent staining, LC-MS/MS lipidomic analysis, DESI/MSI imaging, and fluorescent activated cell sorting (FACS).

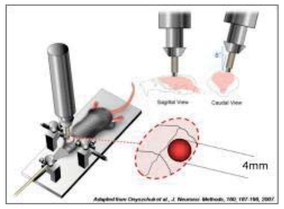

Fig. 2. Schematic representation of controlled cortical impact (CCI) model

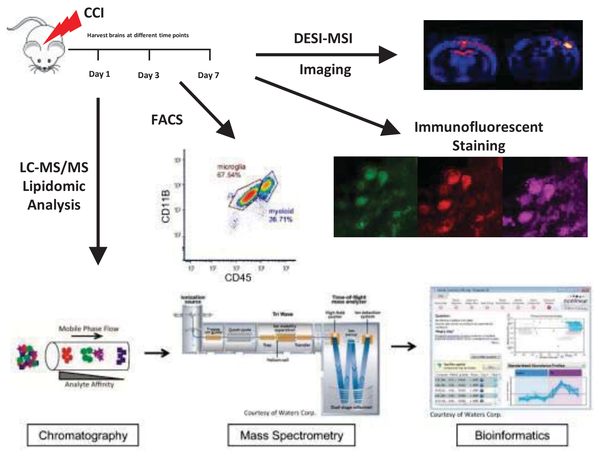

Fig. 3. Schematic representation of our in vivo workflow

RESULTS

Various Neutral Lipids Accumulate in Perilesional Tissue of the TBI Brain

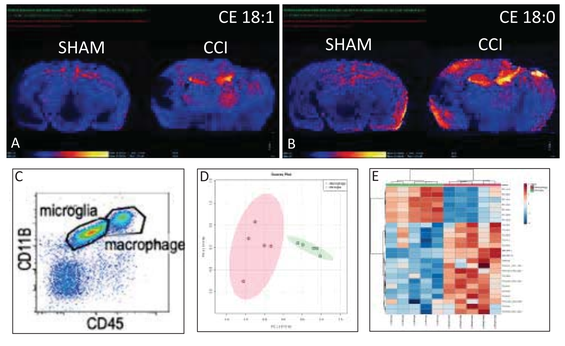

Fig. 4. DESI-MSI and cell-type specific LC-MS/MS data generated from our TBI mouse model. **A** and **B** Desorption electro-spray ionization – mass spectrometry imaging (DESI-MSI) of CCI day 3 brain sections representing two types of cholesteryl esters (esterified with oleic acid and stearic acid respectively). **C**. Fluorescent activated cell sorting (FACS) purified microglia and macrophages from TBI samples further subjected to lipidomic analysis **D.** principal component analysis shows distinguished lipid content in FACS purified microglia versus macrophages **E**. Infiltrating macrophages from TBI day 3 samples show triglycerides and sphingomyelin accumulation whereas in microglia phosphatidyl choline species dominate.

Lipid Droplets accumulate in microglia and macrophages after TBI

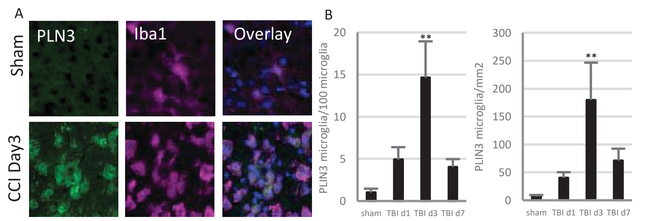

Fig. 5. Expression of lipid droplet marker, Perilipin 3 (PLN3), in ipsilateral cortex of TBI brain. A. Perilipin3 is a ubiquitous lipid droplet coating protein that in this experiment shows elevated accumulation in Iba1 positive cells (monocytes) after CCI that peaks at day 3. Lipid droplet accumulating microglia (LDAM) has been recently identified as a specific phenotype that contribute to more proinflammatory cytokines and ROS production that also show phagocytosis deficiency. **B.** quantification of Iba1 positive cells that also show PLN3 signal. all data are mean ± SEM; n= 3-4/ time point; **p<0.01 vs Sham (One- way ANOVA with Dunnett's post hoc test for multiple comparison)

Neutral lipids accumulate in Autophagy Impaired Monocytes after TBI

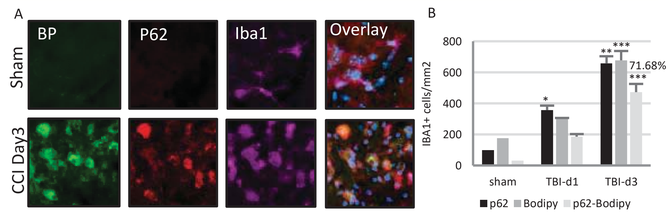

Fig. 6. Colocalization of neutral lipids in monocytes with autophagy impairment. A. BODIPY staining as a well-established neutral lipid dye was used in parallel with P62/SQSTM1 (sequestosome1), the autophagosome cargo receptor that its build up is a marker of autophagy flux inhibition. Both markers demonstrate accumulation after TBI which peaks at day3, also they colocalize in Iba1 positive cells (monocytes) suggesting autophagy impairment in lipid accumulating cells. **B.** Quantification of the number of Iba1 positive cells that show colocalization of BP and P62 signals. Data are mean ± SEM; n= 1-3/ time point; **p<0.01, ***p<0.001 vs Sham (One- way ANOVA with Dunnett's post hoc test for multiple comparison).

Exposure to myelin inhibits autophagy flux in RAW macrophages

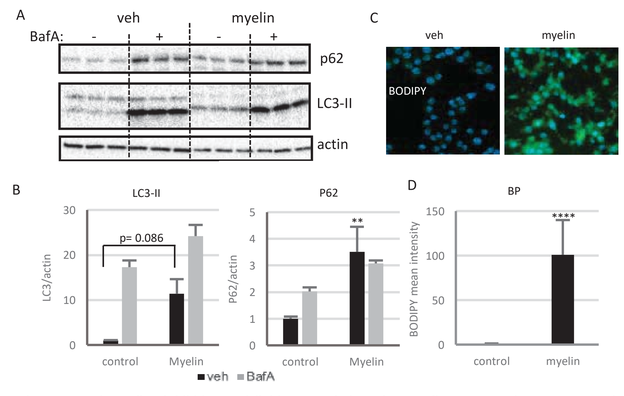

Fig. 7. Autophagy flux inhibition and lipid accumulation after myelin exposure in RAW macrophages. RAW macrophages treated with Myelin and after 48 hours either fixated and stained for neutral lipid dye, BODIPY or lysed in RIPA buffer for western blotting. All data are mean ± SEM. For Western blot (A-B) , n= 3 replicates/group (1 experiment); **p<0.01 vs untreated/no myelin treatment (Two-way ANOVA with Sidak's post hoc test for multiple comparison). For BODIPY staining (C-D), n= 2 replicates/group (1 experiment), ****p<0.0001 vs untreated (Student's t-test).

Exposure to myelin inhibits autophagy flux in Primary macrophages

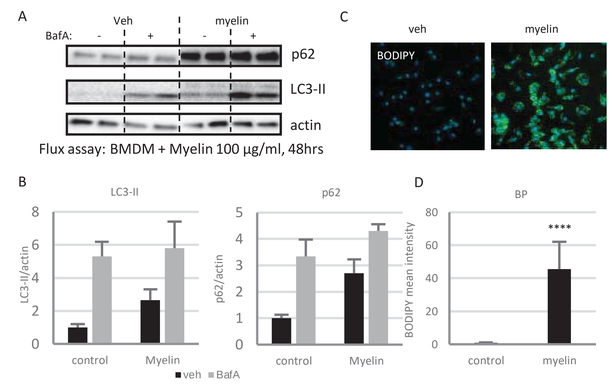

Fig. 8. Autophagy flux inhibition and lipid accumulation after myelin exposure in bone marrow derived macrophages (BMDMs). bone marrow derived macrophages (BMDMs) treated with Myelin and after 48 hour either fixated and stained for neutral lipid dye, BODIPY or lysed in RIPA buffer for western blotting. All data are mean ± SEM; n= 2 replicates/group (1 experiment). For BODIPY staining (C-D) , p****<0.0001 vs untreated (Student's t-test). For Western blot, data was analyzed using Two-way ANOVA with Sidak's post hoc analysis.

Myelin exposure exacerbates proinflammatory response to LPS in macrophages

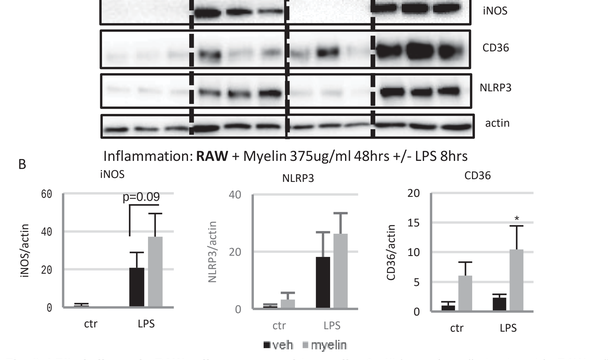

Fig. 9. LPS challenge in RAW cells pre-exposed to myelin. A. 48 hour of myelin treatment in RAW cells followed by 8 hours of LPS treatment shows upregulation of iNOS and NLRP3 inflammasome along with upregulation of the scavenger receptor CD36 resembling the perpetual lipid uptake of proinflammatory foam macrophages. **B**. All data are mean ± SEM; n= 3 replicates/group (1 experiment); *p<0.05 vs untreated/no myelin control (Two-way ANOVA with Tukey's post hoc test for multiple comparison).

Conclusions:

- Various neutral lipid species accumulate in monocytes after TBI.
- Lipid droplet and autophagosome accumulation markers co-localize in monocytes suggesting that autophagy impairment can be the cause of lipid accumulation.
- Exposing both RAW cell line and primary macrophages to Myelin in vitro leads to neutral lipid accumulation, inhibition of autophagy flux, and exacerbation of pro-inflammatory response to LPS.

Future Direction:

- Neutral lipid accumulation in the lysosomes of monocytes needs to be confirmed by FACS and IF staining.
- We propose that either inducing cholesterol efflux or preventing lipid uptake by blocking scavenger receptors like CD36 can prevent autophagy flux inhibition in monocytes.

National Institute of Neurological Disorders and Stroke

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