Impairment of autophagy in microglia/macrophages exacerbates neuroinflammation after spinal cord injury in mice

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Background

Autophagy is a catabolic process that degrades cytoplasmic constituents and organelles in the lysosome, serving an important role in cellular homeostasis and protection against neurodegeneration. We previously reported that defects in autophagy contribute to neuronal damage and are part of the secondary injury mechanism in traumatic spinal cord injury (SCI) [1, 2]. Recent data implicate autophagy in regulation of immune and inflammatory responses, with high levels of autophagy flux associated with anti-inflammatory, and low levels with pro-inflammatory phenotypes [3, 4]. In the present study, we examined the effects of genetically or pharmacologically manipulating autophagy on posttraumatic neuroinflammation and motor function after SCI in mice. Beclin-1 (BECN1) is a core subunit of the phosphatidylinositol-3 kinase (PI3K) complex necessary for autophagosome formation and is required for the fusion of lysosomes to autophagosomes.

Methods

Young adult male C57BL/6, Cx3cr1-GFP, autophagy hypomorph *Becn1*$^{+/-}$ mice, and their wildtype (WT) littermates were subjected to moderate thoracic spinal cord contusion. Neuroinflammation and autophagy flux in the injured spinal cord was assessed using flow cytometry, immunohistochemistry, Western blot (WB), NanoString and qPCR. Neurological function was evaluated with the Basso Mouse Scale (BMS) and horizontal ladder test. Lesion volume and spared white matter was evaluated by unbiased stereology. To stimulate autophagy, trehalose was introduced into their drinking water immediately after injury and continued through 6 weeks after SCI. In acute time period studies, trehalose or vehicle control water was administered to each mouse via oral gavage until euthanized at 3d SCI.

Results

- SCI causes a temporal dysregulation of autophagic function in microglia and infiltrating myeloid cells.
- In *Becn1*$^{+/-}$ mice, neuroinflammation and proinflammatory cytokines are further increased after SCI, resulting in worse functional outcome.
- Trehalose treatment after SCI resulted in better recovery of locomotor function.
- These findings highlight the importance of autophagy in resident immune cells of the CNS and further elucidates its role in secondary injury after SCI.

Conclusion

Taken together, our data indicates that inhibition of autophagy in microglia/macrophages potentiates proinflammatory activation that is associated with poorer functional outcome following traumatic SCI. These findings highlight the importance of autophagy in resident immune cells of the CNS and further elucidates its role in secondary injury after SCI.

References

1. Liu S et al. Disrupted autophagy after spinal cord injury is associated with ER stress and neuronal cell death. Cell Death Dis. 2015; 6: e1582.
2. Li Y et al. cPLA2 activation contributes to lysosomal defects leading to impairment of autophagy after spinal cord injury. Cell Death Dis. 2019; 10: 531.
4. Wu J et al. Autophagy in Neurotrauma: Good, Bad, or Dysregulated. Cells. 2019; 8.

Autophagy is inhibited in activated microglia and infiltrating myeloid cells after SCI

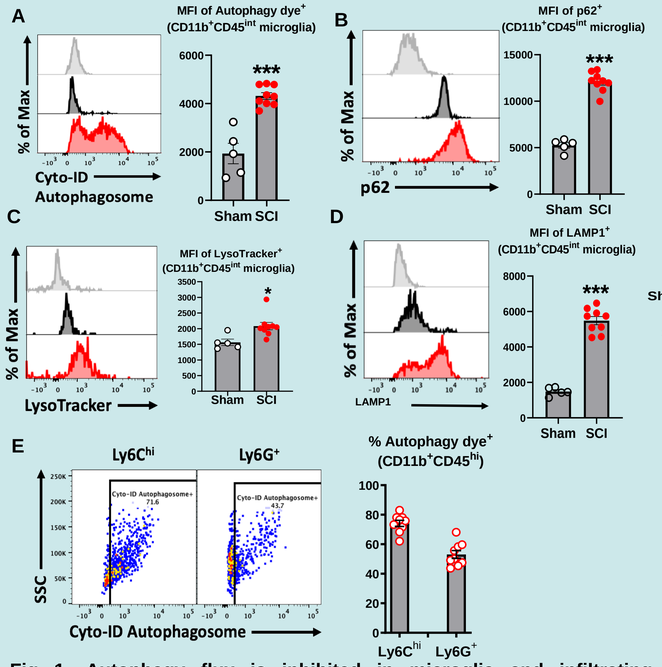

Fig 1. Autophagy flux is inhibited in microglia and infiltrating monocytes in acute phase SCI. (A-C) Representative histograms and mean fluorescent intensity (MFI) quantification of Cyto-ID Autophagy Detection Kit (**A**), p62/SQSTM1 (**B**), lysosomal activity (**C**) and LAMP1 (**D**) in CD11b+CD45int microglia. **(E)** Representative dot plots and quantitative data of Cyto-ID for infiltrating CD11b+CD45hi monocytes.

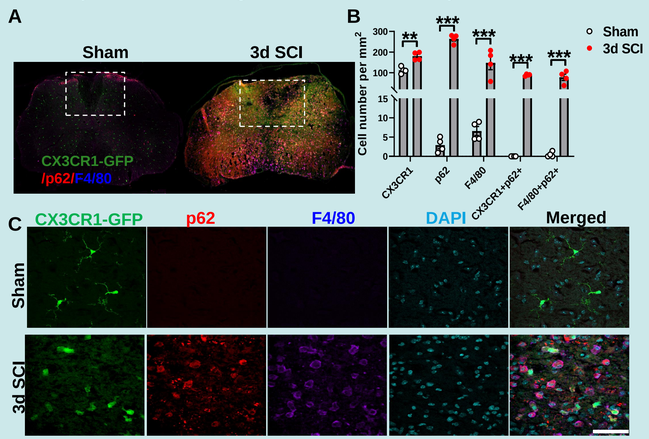

Fig 2. Autophagosomes acutely accumulate in activated microglia and infiltrating macrophages at 3 days after SCI. (A) Representative images of GFP+(green)/p62+(red)/F4/80+ (blue) cells at 0.3 mm rostral to the epicenter. Insets display the dorsal white matter (DWM) for quantification. **(B-C)** Cell counts and representative images of p62/F4/80 in the DWM of CX3CR1-GFP mice.

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Autophagy deficiency leads to transcriptome change in NanoString neuroinflammation panel

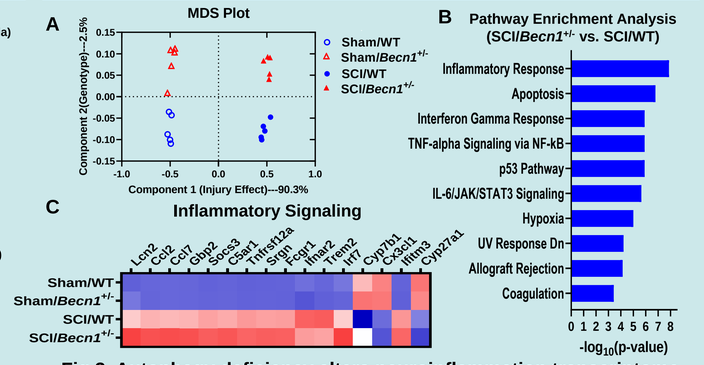

Fig 3. Autophagy deficiency alters neuroinflammation transcriptome within the spinal cord after acute injury. (A) Multi-dimensional scaling (MDS) was performed using all normalized gene counts from the NanoString neuroinflammation panel. **(B)** Pathway enrichment analysis of genes modified by SCI in *Becn1*$^{+/-}$ vs. WT mice. **(C)** Heatmap of DE genes in the inflammatory signaling pathway.

Autophagy deficiency exacerbates pro-inflammatory response after SCI

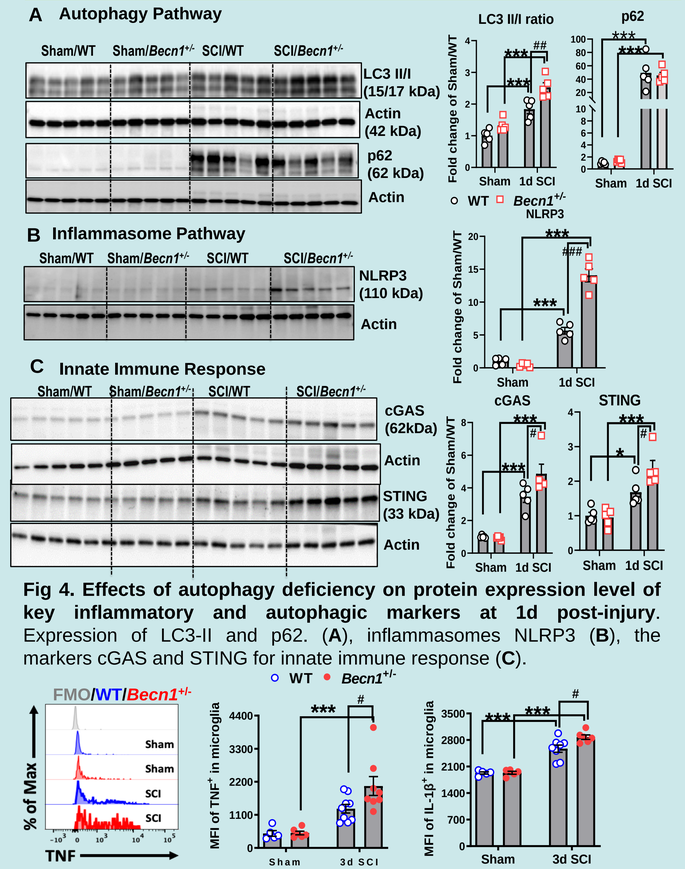

Fig 4. Effects of autophagy deficiency on protein expression level of key inflammatory and autophagic markers at 1d post-injury. Expression of LC3-II and p62. (**A**), inflammasomes NLRP3 (**B**), the markers cGAS and STING for innate immune response (**C**).

Fig 5. Autophagy deficit increased microglial production of proinflammatory cytokines. Representative histograms and mean fluorescent intensity (MFI) quantification of TNF and IL-1beta in microglia

Autophagy deficiency adversely affect functional and tissue recovery after SCI

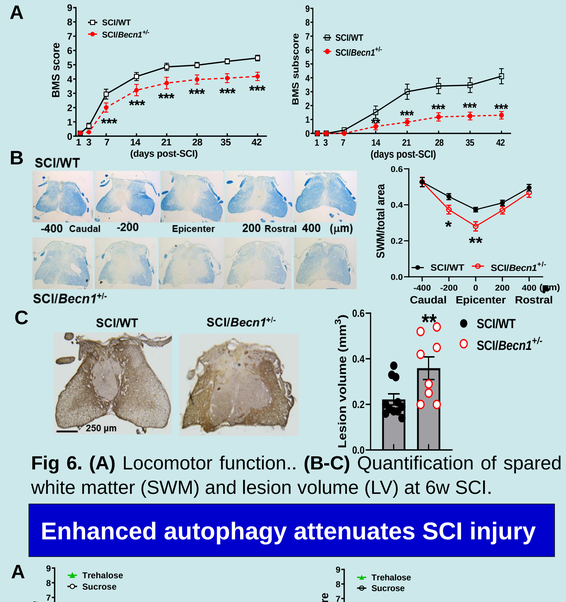

Fig 6. (A) Locomotor function.. **(B-C)** Quantification of spared white matter (SWM) and lesion volume (LV) at 6w SCI.

Enhanced autophagy attenuates SCI injury

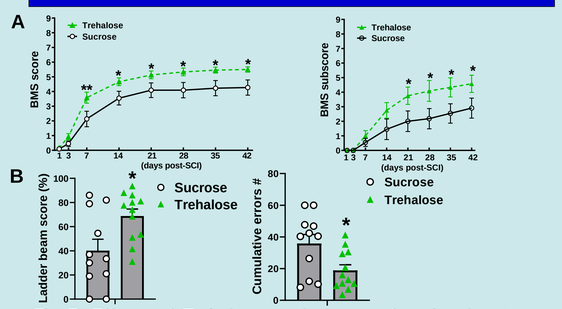

Fig 7. Effects of Trehalose on long-term functional outcome following SCI. (A) BMS and subscores. **(B)** Ladder beam scores and number of cumulative errors in horizontal ladder test at 6w SCI.

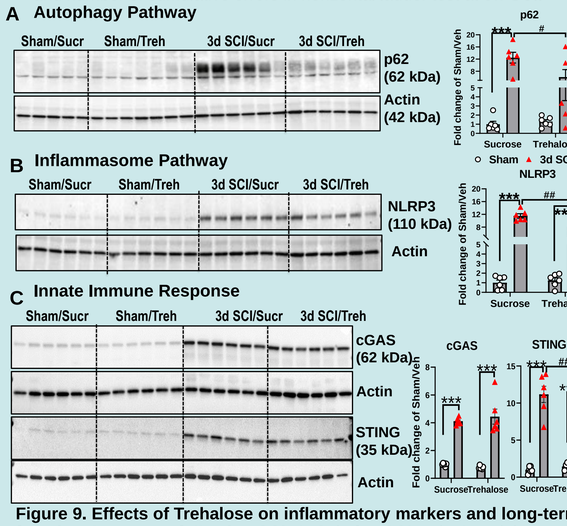

Figure 9. Effects of Trehalose on inflammatory markers and long-term functional outcome following SCI. At 3 d after SCI, spinal cord tissue surrounding injury site were dissected for examination of the autophagic and inflammatory markers. Western blot analysis of protein expression for p62 (A), NLRP3 (B), cGAS and STING (C),