The Role of BATF2 in Experimental Autoimmune Encephalomyelitis and Multiple Sclerosis

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Background

Multiple Sclerosis (MS)

- An inflammatory, demyelinating disease primarily affecting the central nervous system.
- Demyelination leads to lesion formation.
- Astrocytes play critical role.
- Symptoms include limb weakness, decreased mobility, pain, and impaired motor dexterity.

Experimental Autoimmune Encephalomyelitis (EAE)

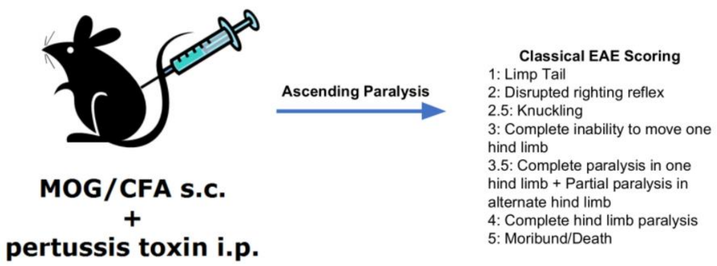

Figure 1. Explanation of EAE. The mice are injected with MOG/CFA and pertussis toxin to induce and immunize against EAE. During the disease course ascending paralysis occurs. Each day of the disease course each mouse is scored on a scale of 1 to 5; 1 being limp tail and 5 being death.

BATF2

- A basic leucine zipper transcription factor belonging to the activator protein 1 family.
- Functional role in innate immune response by controlled expression of immune regulatory genes and in the development and differentiation of dendritic cells.
- Shown to be downstream of interferon-γ, regulating expression of inflammatory genes such as CCL5 and TNF in macrophages.

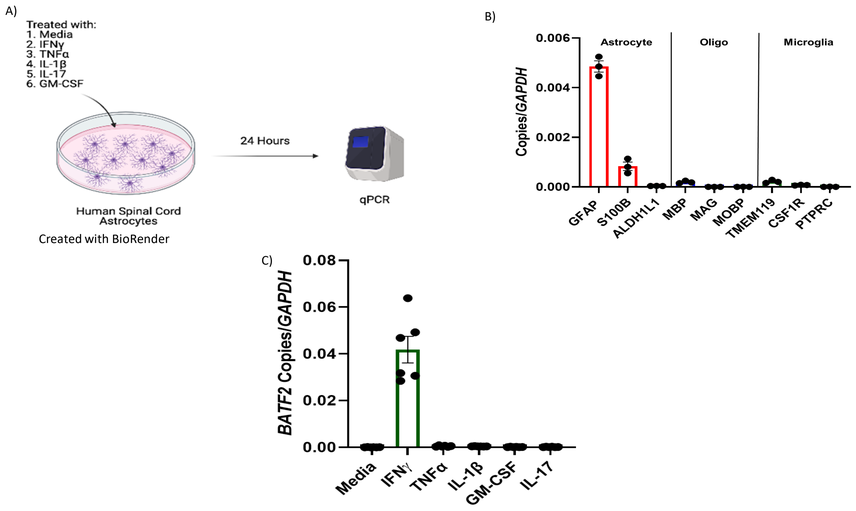

Figure 2. qPCR validation of RNA sequencing data. A) Schematic representing the process of treating human spinal cord astrocytes with astrocytes followed by mRNA alalysis by quantitative (q)PCR. B) Validation that the cells used were astrocytes. C) BAFT2 mRNA expression was analyzed by qPCR after human spinal cord astrocytes were treated with normal media, interferon-γ, tumor necrosis factor alpha (TNF-α), interleukin 1 beta (IL-1β), granulocytes-macrophage colony-stimulating factor (GM-CSF), or interleukin (IL-17).

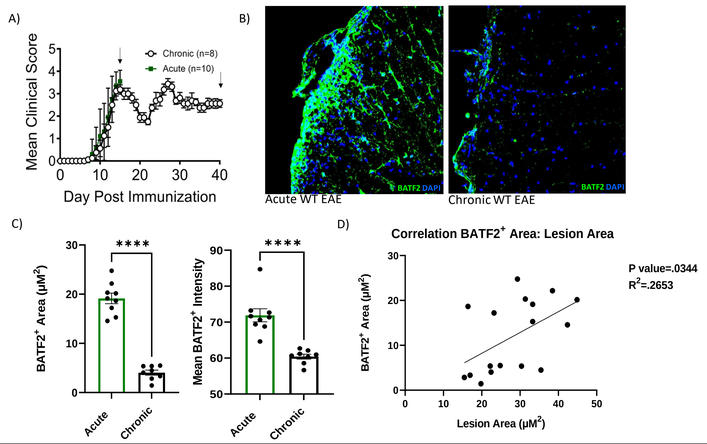

Figure 3. Classification of BATF2 found during acute and chronic EAE in wild-type (WT) mice. A) A graphical representation of the clinical scoring of mice during EAE disease course. The arrows indicate the timepoints that spinal cord tissue was collected. Acute WT tissue was collected on day 15 and chronic tissue was collected day 40 post-EAE induction. B) IHC staining of acute and chronic WT EAE tissue. BATF2 expression (green), and nuclei (blue) are labeled. C) The area and intensity of the BATF2 stain in both acute and chronic WT EAE were quantified. D) Correlation graph of BATF2 with lesion size.

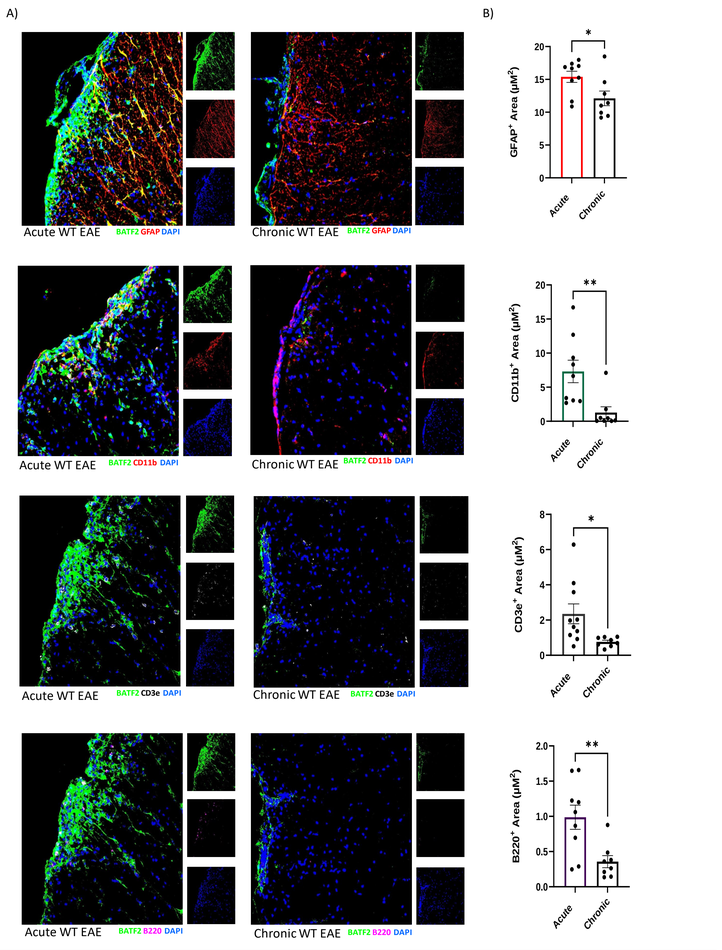

Figure 4: Characterization of cell types in acute and chronic EAE lesions. A) representative IHC images for GFAP (red), CD11b (red), CD3e (white), B220 (purple), and BATF2 (green) labeling. GFAP is staining for astrocytes, CD11b for myeloid lineage cells (i.e., macrophages, microglia, neutrophils), CD3e for T cells and B220 for B cells. B) Cell type-specific labeling was quantified.

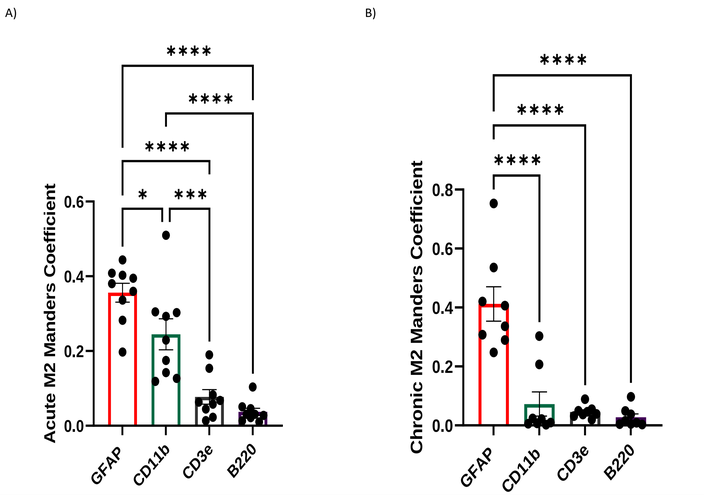

Figure 5. The M2 Mander's Coefficient for Acute and Chronic EAE tissue labeling. A and B) The M2 Mander's coefficient measuring the probability that each cell type is also expressing BATF2 in A) acute and B) chronic EAE lesions. *P < 0.05, **P < 0.01, ***P<0.001, and ****P<0.0001.

Summary

- BATF2 has a greater area and intensity in acute vs. chronic EAE.
- BATF2 expression positively correlates with lesion size.
- Astrocytes are the main cell type followed by CD11b that expresses BATF2 in acute EAE.
- Astrocytes are the main cell type to express BATF2 in chronic EAE.

Conclusions

The upregulation of BATF2 under inflammatory conditions may present a new therapeutic target for MS.

Future Directions

- Finish cell characterization by doing a stain on acute and chronic EAE for TMEM 119, a marker for microglia.
- Perform Western blots on cytosolic and nuclear BATF2 to determine if BATF2 is translocated from the nucleus.
- Perform qPCR on human and mouse astrocyte cells treated with IFN-gamma, HMGB1 and IFN-gamma/HMGB1 media to determine transcription levels.
- Induce EAE in WT and BATF2 knockout mice to identify changes in disease course and lesion formation in the absence of BATF2.

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