CitH3 Inhibition Attenuates Acute Ischemic Stroke via Targeted Immune Modulation

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ABSTRACT

Ischemic stroke remains a major cause of neurologic disability, with current therapies largely limited to reperfusion and insufficient in addressing secondary brain injury. Neutrophil extracellular traps (NETs) have emerged as a key driver of post-ischemic inflammation, yet their therapeutic targeting remains poorly defined, notably in the context of neural injury. Here, we investigate the role of citrullinated histone H3 (CitH3), a central effector of NET formation, in a mouse model of middle cerebral artery occlusion. We show that intravenous administration of a humanized monoclonal antibody against CitH3 (hCitH3-mAb) leads to a reduction in infarct volume and cerebral edema at 24 h post-stroke. Our data also indicate a shift in immune cell polarization toward an anti-inflammatory, M2-like reparative state as a result of CitH3 inhibition, characterized by reduced expression of iNOS, IL-1β, and TNF-α in tandem with increased CD206 expression. These effects were partially recapitulated in vitro in IFN-γ-stimulated HMC3 cells. Together, these findings highlight the protective function of hCitH3-mAb in the brain, suggesting that targeting CitH3 may be an effective means for the treatment of acute ischemic stroke by modulating immune phenotypes.

INTRODUCTION

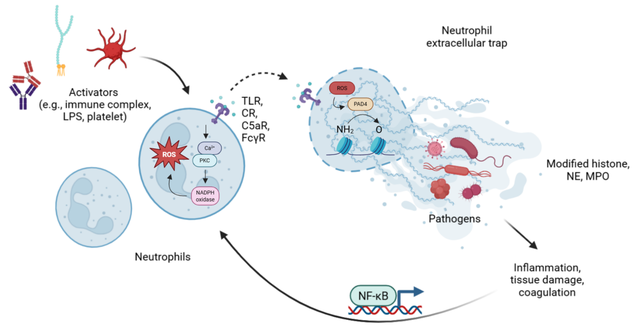

Figure 1. **Positive feed-forward mechanism of NET formation.** Neutrophils undergo lytic NETosis upon activation, causing DNA-protein complexes decorated with citrullinated histones to be extruded from the cell[1]. While NETs function to neutralize pathogens, excessive NETs are engaged in a self-propagating cycle of NET formation via NF-κB signaling to further aggravate inflammation[2].

Hypothesis: hCitH3-mAb improves acute ischemic stroke by shifting immune cell polarization toward repair via the targeting of CitH3-dependent NET formation.

METHODS

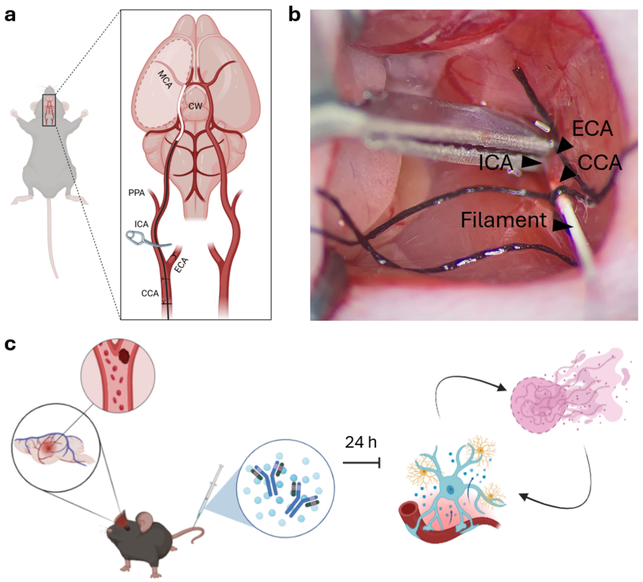

Figure 2. **Experimental paradigm using the MCAO model.** **(a)** Schematic representation and **(b)** dissection of the ventral neck region for transient occlusion of the MCA by a silicone monofilament inserted via CCA arteriotomy. **(c)** C57BL/6J mice were intravenously administered hCitH3-mAb (20 mg/kg) after 1 h occlusion, followed by 24h reperfusion prior to tissue collection.

RESULTS

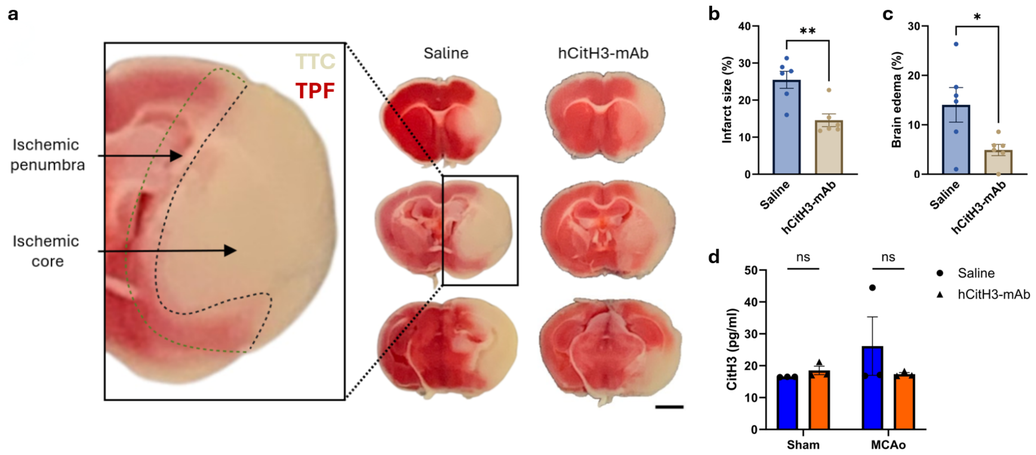

Figure 3. **hCitH3-mAb delivered intravenously limits cerebral infarct growth.** **(a)** Representative TTC-stained brain sections with and without hCitH3-mAb treatment post-stroke (scale bar = 2 mm). **(b-c)** Quantification of infarct and edema volume, compared between groups (n = 6). **(d)** Detection of CitH3 by ELISA across sham- and MCAO-operated mice, with or without hCitH3-mAb treatment (n = 3). Data are shown as mean ± SEM. Statistical significance was determined by either an unpaired t-test or a one-way ANOVA with post-hoc Dunnett's test. ns = not significant, $^{*}p < 0.05$, $^{**}p < 0.01$.

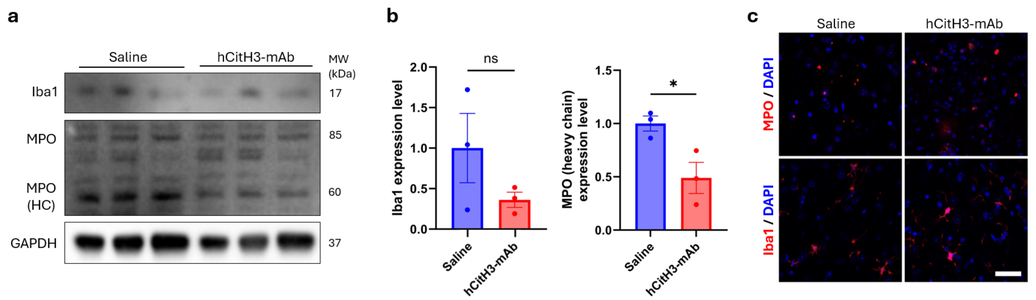

Figure 4. **hCitH3-mAb reduces microglia activation and neutrophil infiltration.** **(a-b)** Western blot analysis quantifying Iba1 and MPO expression after hCitH3-mAb treatment for detection of microglia and neutrophils, respectively, compared against control at 24 h post-stroke and normalized to GAPDH (n = 3). **(c)** IHC characterization of Iba1 and MPO expression across the ischemic core after hCitH3-mAb treatment, compared against control at 24 h post-stroke (n = 1) (scale bar = 25 μm). Data are shown as mean ± SEM. Statistical significance was determined by an unpaired t-test. ns = not significant, $^{*}p < 0.05$

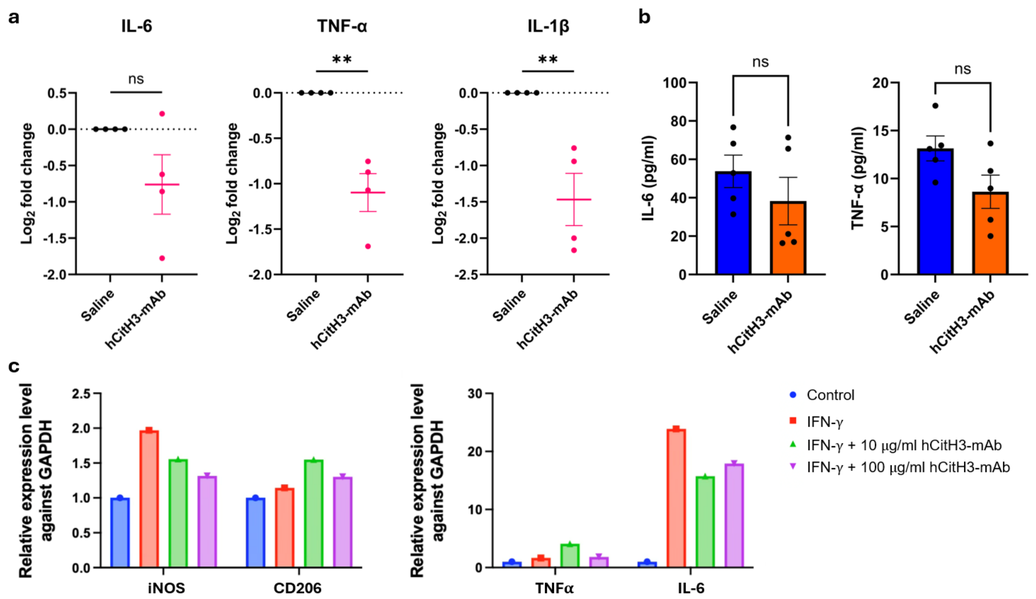

Figure 5. **hCitH3-mAb protects against brain injury by mitigating pro-inflammatory cytokine release.** **(a)** RT-qPCR analysis showing relative gene expression of M1 pro-inflammatory cytokine expression after hCitH3-mAb treatment, compared against control at 24 h post-stroke and normalized to β-actin (n = 4). **(b)** IL6 and TNF-α expression are validated using ELISA and further validated **(c)** in vitro using IFN-γ-stimulated HMC3 cells treated with or without CitH3 antibodies (n = 1). Data are shown as mean ± SEM. Statistical significance was determined by an unpaired t-test. ns = not significant, $^{**}p < 0.01$.

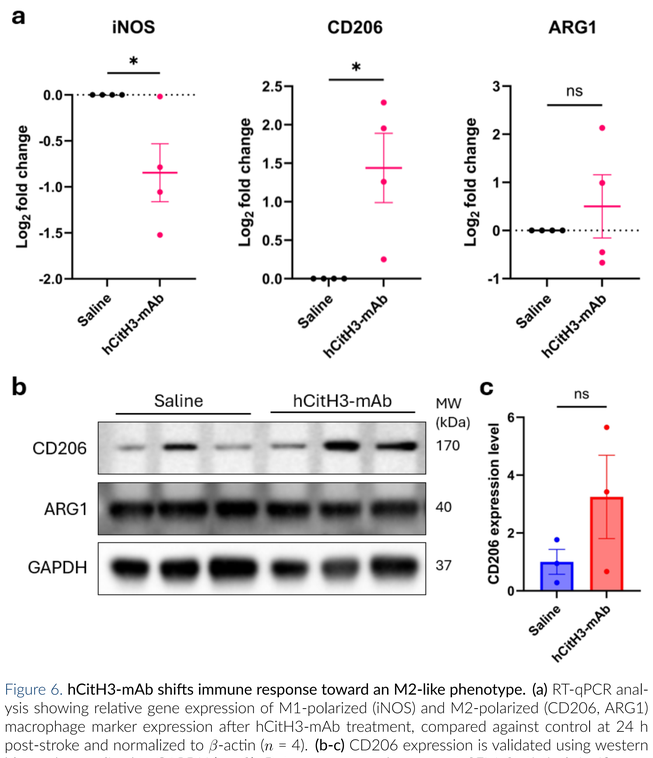

Figure 6. **hCitH3-mAb shifts immune response toward an M2-like phenotype.** **(a)** RT-qPCR analysis showing relative gene expression of M1-polarized (iNOS) and M2-polarized (CD206, ARG1) macrophage marker expression after hCitH3-mAb treatment, compared against control at 24 h post-stroke and normalized to β-actin (n = 4). **(b-c)** CD206 expression is validated using western blot and normalized to GAPDH (n = 3). Data are presented as mean ± SEM. Statistical significance was determined by an unpaired t-test. ns = not significant, $^{*}p < 0.05$.

DISCUSSION

Summary: hCitH3-mAb confers protection to the ischemic brain via attenuation of tissue injury and phenotypic transition of microglia/macrophage toward M2.

Limitations: The model used herein does not fully capture the variability in stroke pathology or reperfusion dynamics observed clinically in human stroke patients.

Future Work: snRNA-seq analysis, longitudinal assessment and optimization

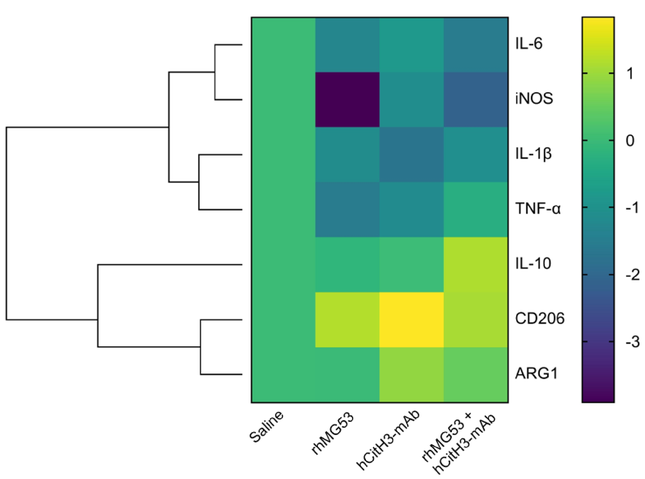

Figure 7. **hCitH3-mAb synergizes with rhMG53 to attenuate pro-inflammation.** Heatmap representation of relative gene expression of M1/M2 markers across different treatment groups (n = 3).

ACKNOWLEDGEMENTS & REFERENCES

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1. Papayannopoulos (2018). *Nat. Rev. Immunol.*, 18(2), 134-147.
2. Zhu et al. (2021). *Exp. Cell Res.*, 405(2), 112707.