Mast Cell-Mediated Mechanisms of Pain in TNBS-Induced Chronic Pancreatitis

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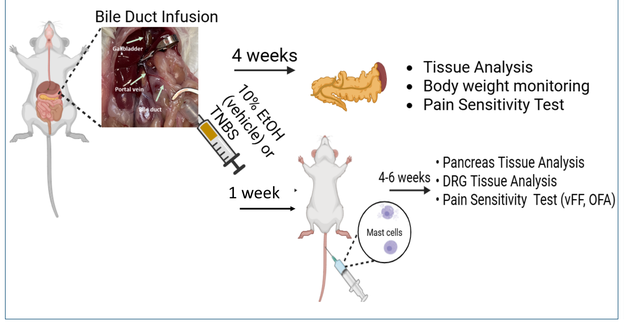
ABSTRACT

Chronic pancreatitis (CP) is a progressive disease characterized by inflammation, fibrosis, and severe pain that greatly reduces quality of life. The mechanisms behind CP-associated pain are still not well understood. In this study, we investigated the cellular and molecular mechanisms contributing to CP pain using a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced CP mouse model. We found increased mast cells in the pancreas and dorsal root ganglia (DRG) in TNBS-CP mice. Mast cell-deficient mice (MCDM) showed significantly reduced CP pain, while reconstituted mast cells could restore pain, demonstrating that mast cells are critical for CP pain development. Further experiments revealed reduced expression of the high mobility group box 1 protein (HMGB1), a damage-associated molecular pattern molecule, in the pancreas and DRG. Compared to healthy mice, DRG from TNBS-induced CP mice increased HMGB1 expression. Pancreatic lysate from CP mice co-cultured with primary DRG neuron cells and human iPSC-derived neuron-like cells, triggered the release of substance P, a mediator of pain. Additionally, elevated serum HMGB1 levels correlated positively with pain levels in CP patients. Our findings highlight mast cells as key mediators of CP pain and implicate HMGB1 in the pancreas-neuron crosstalk underlying persistent CP pain. Unveiling these novel mechanisms holds promise for developing targeted therapies for chronic pain management in CP patients.

Design



RESULTS

Fig.1

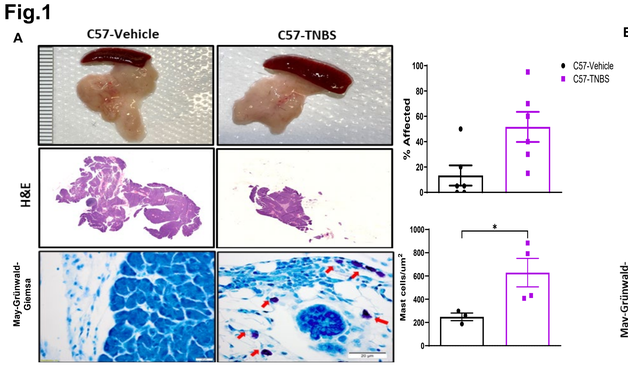


Fig.1A Mast cells increased in the injured pancreas following TNBS treatment. Representative images of the pancreas and spleen from C57 mice receiving vehicle or TNBS. Histological scoring of pancreatic % damage area; Quantification of mast cells using May-Grünwald-Giemsa staining demonstrated mast cell presence in pancreatic tissues.

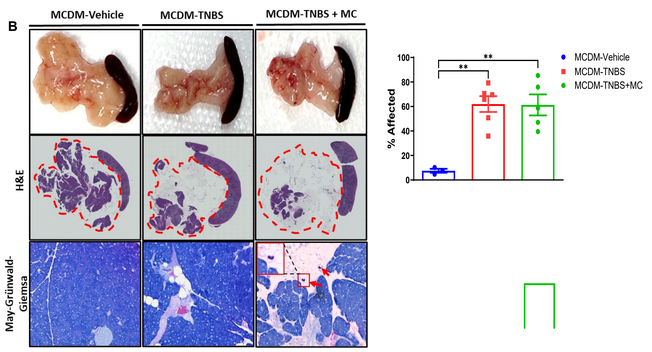


Fig.1B Mast cell reconstitution in MCDM mice does not prevent pancreatic damage during CP induction. Representative images of the pancreas from MCDM mice treated with vehicle (MCDM-Vehicle), TNBS (MCDM-TNBS), or TNBS with mast cell reconstitution (MCDM-TNBS+MC); Histological scoring of pancreatic % affected area; Quantification of mast cells via May-Grünwald-Giemsa staining.

Fig.2

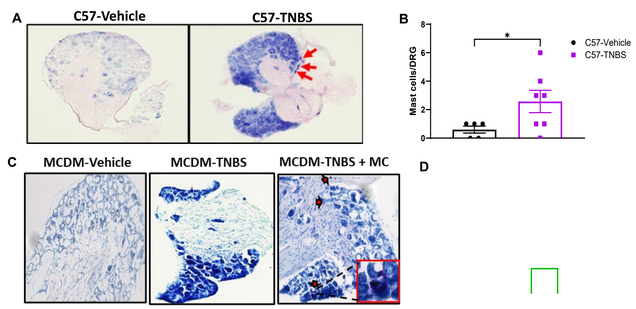


Fig.2 Mast cell accumulation in dorsal root ganglia (DRG). May-Grünwald-Giemsa staining showing increased mast cell numbers in T9-T12 DRGs of TNBS-treated mice compared to vehicle controls (A-B). Mast cell reconstitution in MCDM-TNBS mice significantly increased mast cell density compared to non-reconstituted MCDM-TNBS mice (C-D).

Fig.3

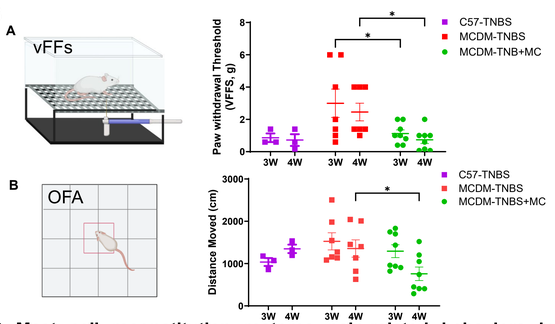


Fig.3 Mast cell reconstitution restores pain-related behaviors in TNBS-induced CP. (A) Mechanical hypersensitivity was measured via von Frey filaments (vFFs) testing on the paw. MCDM exhibits reduced hypersensitivity after TNBS, which is restored to wild-type levels following mast cell reconstitution. (B) Open-field test results showing distance moved. Mast cell-reconstituted MCDM-TNBS mice display reduced movement compared to non-reconstituted MCDM-TNBS mice, consistent with increased pain behaviors.

Fig.4

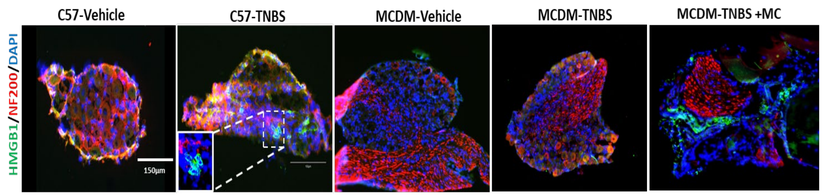


Fig.4 Mast cell reconstitution may restore pain by modulating HMGB1 expression in the DRG. Representative immunofluorescence images of DRG sections showed that HMGB1-positive cells increased in C57-TNBS and mast cell-reconstituted MCDM-TNBS mice.

Fig.5

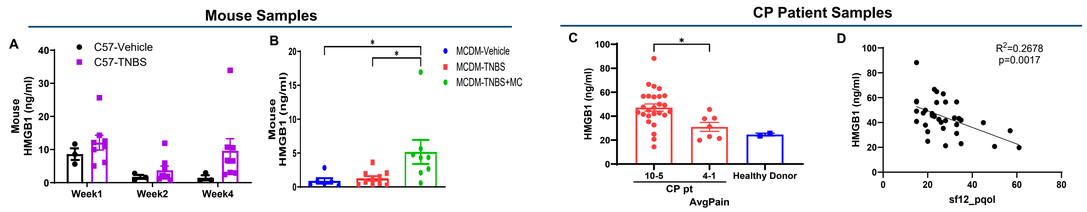


Fig.5 Serum HMGB1 levels are associated with pain. (A) Serum HMGB1 levels in C57 mice at 1, 2, and 4 weeks after TNBS induction of CP. (B) Serum HMGB1 levels in MCDM mice at 4 weeks post-TNBS. (C-D) Serum HMGB1 levels in CP patients. Each dot represents one patient. Patients were stratified by pain scores: 5-10 (n = 26) and 1-4 (n = 7); non-CP (CTR, n = 2).

Fig.6

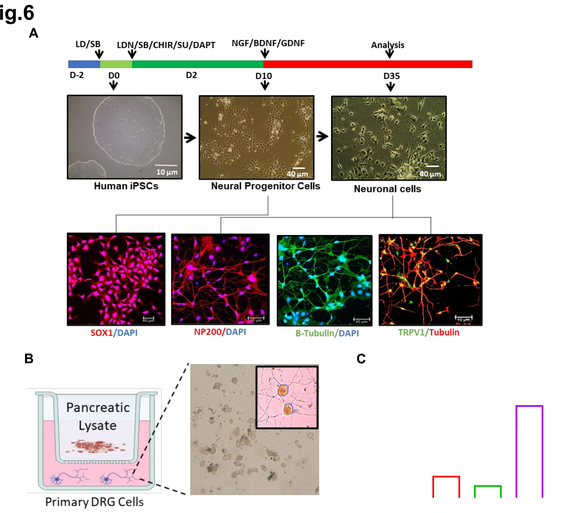


Fig.6 Increased neuronal substance P release from CP pancreatic lysate co-cultured neurons. (A) Differentiation of iPSCs into sensory neuron-like cells. Immunofluorescence staining of iPSC-derived neuronal progenitors for SOX1. Differentiated neurons stained positive for NeuN, β-tubulin, and TRPV1. (B-C) Substance P release from primary DRGs or iPSCs-neurons 24h after exposure to vehicle, and 0.06% of the TNBS or pancreatic lysate from normal or CP mice. (D) Neutralization of HMGB1 with an antibody significantly reduced substance P release from DRGs; HMGB1 protein, anti-HMGB1 antibody, and CP pancreatic lysates were applied in different combinations

CONCLUSIONS

- Mast cells mediate CP pain via HMGB1-dependent pancreas-neuron signaling.
- Targeting HMGB1 could be a promising therapeutic strategy for managing persistent pain in CP patients.

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