Multiple monocyte subtypes contribute to thrombus resolution and vein wall fibrosis in a murine model of DVT

Bilal Khan Mohammed MD[1,2], Kaijie Zhang PhD[1], Haiying Sun[2], Ankit Bharat MD[2], Bowen Wang PhD[1], Stephen F Chiu MD[1,2]

Division of Cardiac Surgery [1], Division of Thoracic Surgery/Canning Thoracic Institute [2],

Feinberg School of Medicine, Northwestern University, Chicago, IL, 60611

Background

Deep vein thrombosis (DVT) and pulmonary embolism (PE) are major contributors to cardiovascular-related illness and death. Together, they are referred to as venous thromboembolism (VTE). While acute treatments such as anticoagulation can reduce immediate risk, some patients develop long-term complications, including chronic thromboembolic pulmonary hypertension (CTEPH)— a progressive condition caused by unresolved thrombi and associated vascular remodeling.

The immune system, especially monocytes, plays a key role in both the formation and resolution of thrombi. Monocytes are typically divided into two main subtypes:

- Classical monocytes (CM), which are inflammatory and rapidly recruited to sites of injury. These cells rely on C-C Chemokine Receptor Type 2 (CCR2), a chemokine receptor that enables their mobilization from the bone marrow into the bloodstream.
- Non-classical monocytes (NCM), which patrol the endothelium and are involved in tissue repair and remodeling. Their development depends on the transcription factor Nuclear Receptor Subfamily 4 Group A Member 1 (NR4A1).

Although both subtypes are known to participate in vascular injury responses, their specific roles in thrombus resolution and post-thrombotic fibrotic remodeling are not well understood.

To address this, we used a partial inferior vena cava (IVC) ligation model, which mimics the formation of thrombus and resolution leaving fibrotic clot observed similarly in human CTEPH **(Fig. 1).**

We studied NR4A1 knockout (NR4A1KO) mice lacking NCMs, and CCR2 knockout (CCR2KO) mice lacking CMs, to determine how each monocyte subtype contributes to thrombus growth, resolution, and vein wall fibrosis over time.

Fig.1

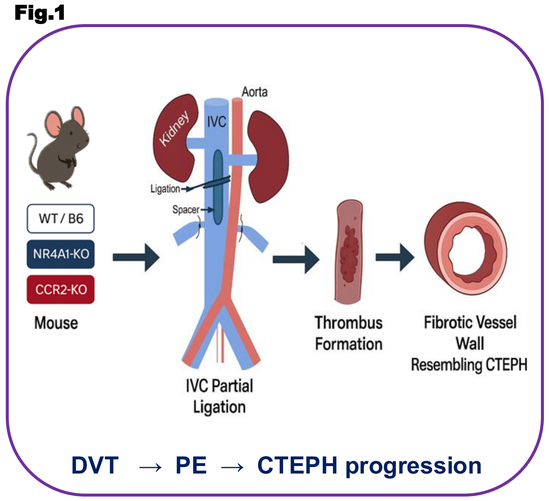
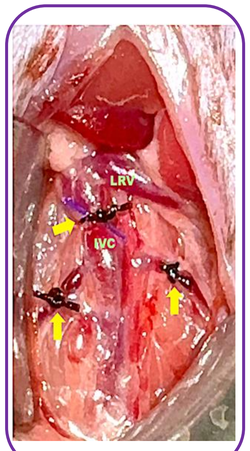


Fig.2



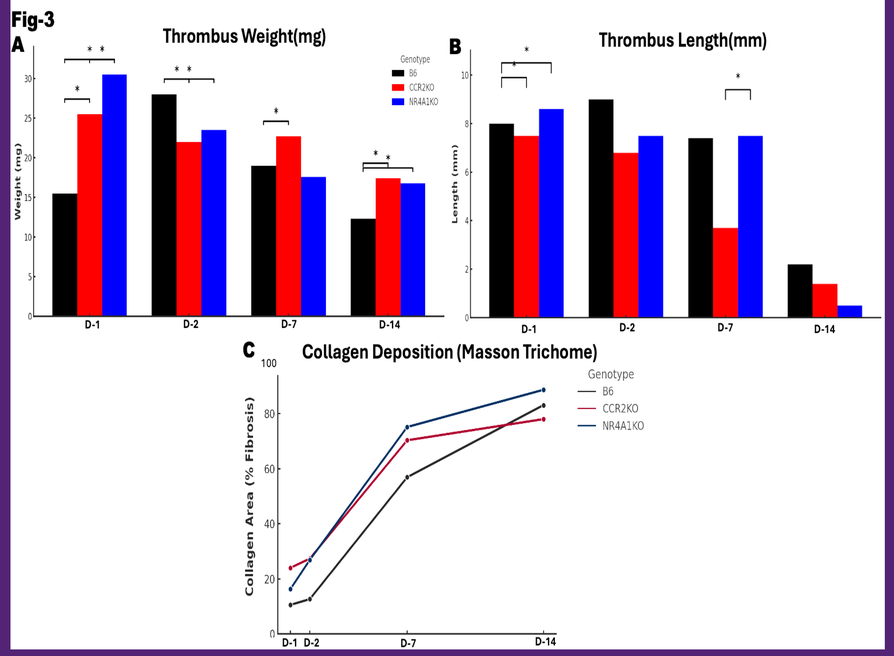
Methods

- Partial inferior vena cava (IVC) ligation with the use of spacer was performed in C57BL/6 (B6), *C-C Chemokine receptor2 knockout* (CCR2KO), and *Nuclear Receptor Subfamily 4 Group A Member 1 knockout* (NR4A1KO) mice. In this experiment we also with side and back branches ligated to ensure consistent flow restriction **(Fig. 2).** Thrombi were collected on Days 1, 2, 7, and 14 after surgery.
- Thrombus weight (mg) and length (mm) were measured, and a new measurement—thrombus linear density (mg/mm)—was used to assess how compact the clot was and how it changed over time **(Fig. 3).** Photos of the thrombus were taken to compare gross phenotypes across C57BL/6 (B6), CCR2KO and NR4A1KO genotypes **(Fig. 4).**
- For fibrosis analysis, 50 µm sections of the thrombus-containing vein were prepared by the Nu Pathology Core. Masson's trichrome staining was also performed by the core **(Fig. 5)**, and collagen area was measured as a percentage of the total vein wall to evaluate fibrosis (% fibrosis) **(Fig. 3C).**

Results

- Both CCR2-KO and NR4A1-KO mice showed accelerated and robust thrombus formation at Day 1 post-ligation compared to B6 controls. Thrombus weights were significantly higher in KO mice on Day 1 (CCR2KO vs B6, $p = 0.02$; NR4A1KO vs B6, $p = 0.005$; Fig 3A), and this difference persisted through Day 14 ($p < 0.05$).By Day 14, B6 mice exhibited a ~60% reduction in thrombus weight from peak levels, while NR4A1-KO and CCR2-KO mice demonstrated only ~47% and ~33% reductions, respectively ($p < 0.05$), indicating impaired thrombus resolution.
- Thrombus length **(Fig.3B)** decreased more rapidly in KO mice, suggesting compaction rather than resolution. NR4A1-KO mice showed significantly shorter thrombi at early and late timepoints compared to B6 ($p < 0.05$).

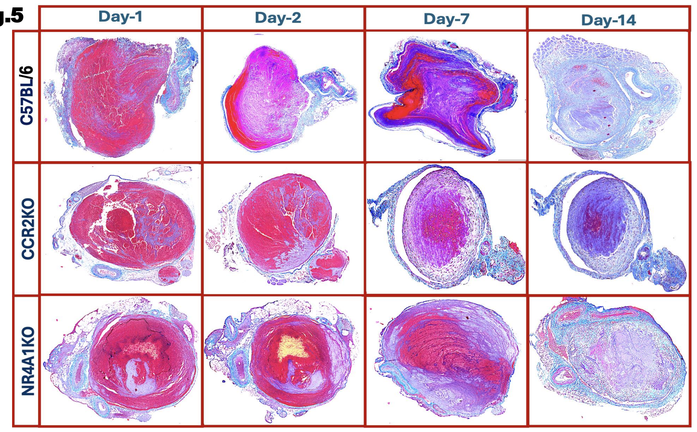
Fig-3



Results

Fig.4

Fig.5



- Histological analysis confirmed progressive collagen accumulation in NR4A1-KO mice, with a 1.8-fold increase in fibrosis at Day 14 relative to B6 controls ($p < 0.001$) suggesting NCMs play a sustained anti-fibrotic role during late thrombus remodeling. **(Fig. 3C).**
- Two-way ANOVA demonstrated significant effects of genotype ($p = 0.028$), timepoint ($p < 0.0001$), and their interaction ($p < 0.0001$), validating genotype-specific and time-dependent differences in thrombus behavior.

Conclusion

- Both non-classical and classical monocyte subtypes appear to play dynamic roles in thrombus formation and resolution of acute thrombus.
- Genetic deletion of NR4A1 which depletes non-classical monocytes, leads to increased thrombus density and appears to aggravate the fibrotic response to thrombus, implying that nonclassical monocytes may play an anti-fibrotic role in the pathogenesis of post-thrombotic vein wall thrombosis and CTEPH.
- CCR2 deletion, which disrupts classical monocyte recruitment, delays thrombus clearance.
- Targeting specific monocyte subset pathways may represent a novel therapeutic strategy to prevent post-thrombotic fibrosis and CTEPH progression.