Novel mechanisms of thrombopoietin generation: The essential role of Kupffer cells

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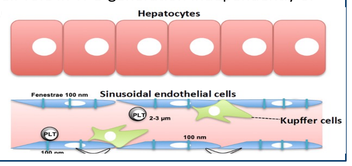
INTRODUCTION

- Thrombopoietin (TPO) is the physiological regulator of megakaryocyte differentiation and therefore platelet production.[1,2]
- Our lab has shown that platelet glycoprotein (GP) Ibα is required for hepatocellular TPO generation (Blood, 2018).[6]
- However, based on liver architecture, platelets (2-3 µm) are separated from hepatocytes by fenestrated (~100nm) endothelium, challenging the notion that platelets directly interact with hepatocytes for TPO generation.
- Kupffer cells reside along and within the sinusoidal endothelium, and have recently been implicated in senescent platelet clearance. However, their role in TPO generation independently or in concert with hepatocyte luminal protrusions has nev

Figure 1. Schematic representation that platelets circulation are physically separated from hepatocytes fenestrated endothelium.[7]

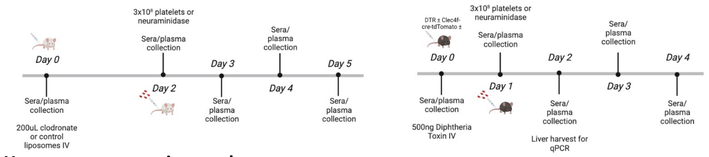


HYPOTHESIS

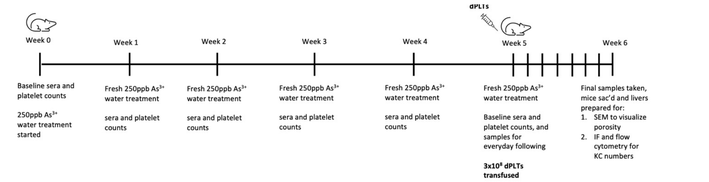
Kupffer cells are required for platelet-mediated hepatic TPO generation through direct contact with hepatocytes or by indirect cytokine release.

METHODS

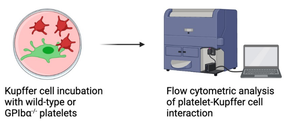
Kupffer cell depletion models



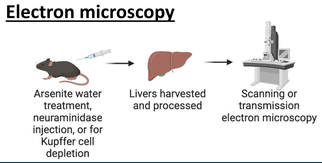
Hepatocyte protrusion study



Phagocytosis assay



Electron microscopy



CONCLUSIONS

Kupffer cells immobilize senescent platelets prior to their clearance for direct hepatocyte protrusion contact and subsequent TPO generation. These data demonstrate for the first time Kupffer cells as a critical intermediary TPO generation. Our data elucidates regulatory mechanisms of TPO generation, highlighting the link between platelet clearance and thrombopoiesis, as well as providing novel insights into liver function. These findings will allow for refined TPO agonist therapies for thrombocytopenia patients, and is crucial for hemostasis and maintenance of HSC niche.

REFERENCES

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RESULTS

Figure 2. Macrophage depletion attenuates platelet-mediated TPO levels

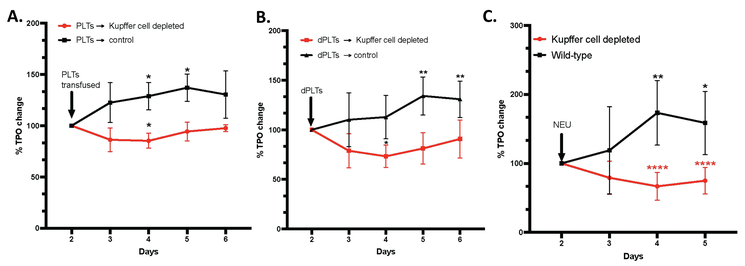

Figure 2: A. Serum TPO levels normalized to wild-type platelet transfusion ($3x10^8$) in clodronate liposome treated mice (n=5-7). **B.** Serum TPO levels normalized to desialylated platelet transfusion ($3x10^8$) in clodronate liposome treated mice (n=9-12). **C.** Plasma TPO levels normalized to 50mU neuraminidase injection in clodronate treated mice (n=9-12). One-way ANOVA with multiple comparisons to determine statistical significance. *, P < 0.05; **, P < 0.01; ***, P < 0.001, n=5.

Figure 3. Kupffer cells are required for hepatocellular TPO generation

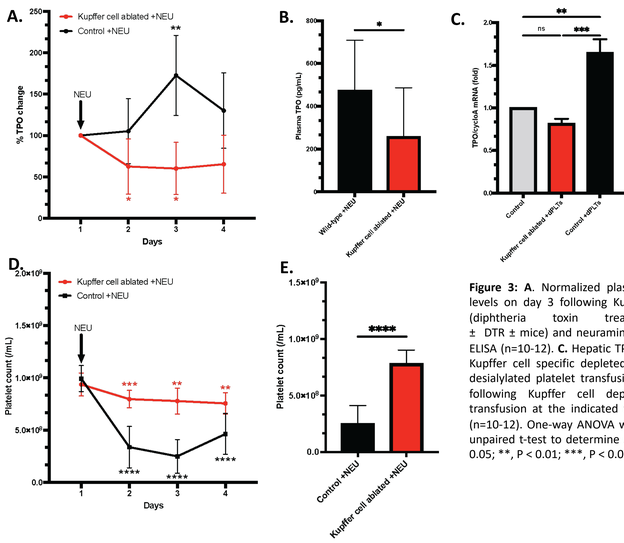

Figure 3: A. Normalized plasma TPO levels and **B.** raw levels on day 3 following Kupffer cell specific depletion (diphtheria toxin treated Clec4f-cre-tdTomato ± DTR ± mice) and neuraminidase injection measured by ELISA (n=10-12). **C.** Hepatic TPO expression via RT-qPCR of Kupffer cell specific depleted mice 24 hours post $3x10^8$ desialylated platelet transfusion (n=5). **D.** Platelet counts following Kupffer cell depletion and neuraminidase transfusion at the indicated timepoints, and **E.** at day 3 (n=10-12). One-way ANOVA with multiple comparisons or unpaired t-test to determine statistical significance. *, P < 0.05; **, P < 0.01; ***, P < 0.001, n=5.

Figure 4. Platelet-mediated TPO generation is interdependent on GPIbα and Kupffer cells

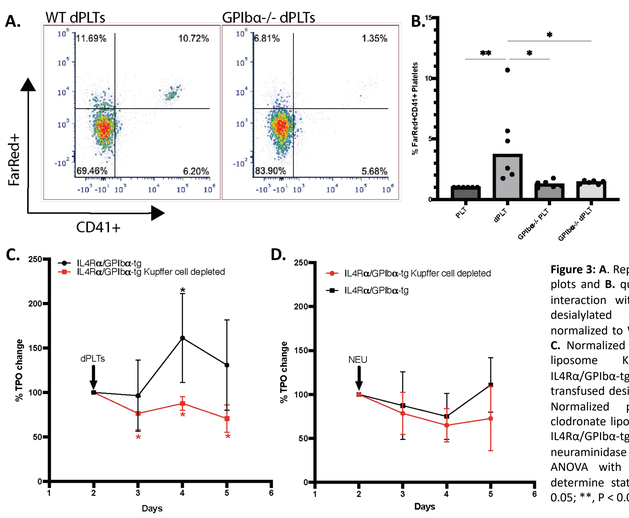

Figure 3: A. Representative flow cytometry plots and **B.** quantification of Kupffer cell interaction with WT or IL4Rα/GPIbα-tg desialylated platelets. Quantification normalized to WT non-desialylated. (n=6). **C.** Normalized plasma TPO in clodronate liposome Kupffer cell depleted IL4Rα/GPIbα-tg mice intravenously transfused desialylated platelets (n=7). **D.** Normalized plasma TPO levels in clodronate liposome Kupffer cell depleted IL4Rα/GPIbα-tg mice injected 50mU neuraminidase (n=10-11). One-way ANOVA with multiple comparisons to determine statistical significance. *, P < 0.05; **, P < 0.01; ***, P < 0.001.

Figure 5. Kupffer cells orchestrate TPO generation in concert with hepatocyte luminal sinusoid protrusions

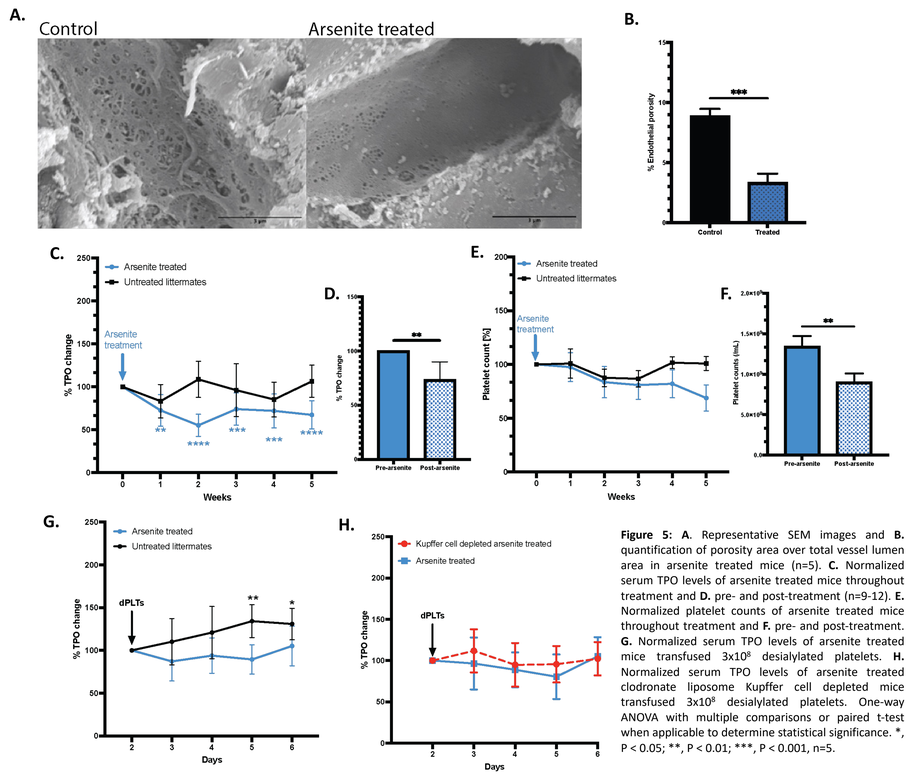

Figure 5: A. Representative SEM images and **B.** quantification of porosity area over total vessel lumen area in arsenite treated mice (n=5). **C.** Normalized serum TPO levels of arsenite treated mice throughout treatment and **D.** pre- and post-treatment (n=9-12). **E.** Normalized platelet counts of arsenite treated mice throughout treatment and **F.** pre- and post-treatment. **G.** Normalized serum TPO levels of arsenite treated mice transfused $3x10^8$ desialylated platelets. **H.** Normalized serum TPO levels of arsenite treated clodronate liposome Kupffer cell depleted mice transfused $3x10^8$ desialylated platelets. One-way ANOVA with multiple comparisons or paired t-test when applicable to determine statistical significance. *, P < 0.05; **, P < 0.01; ***, P < 0.001, n=5.

Figure 6. Kupffer cells adherent platelets directly contact hepatocytes for TPO generation

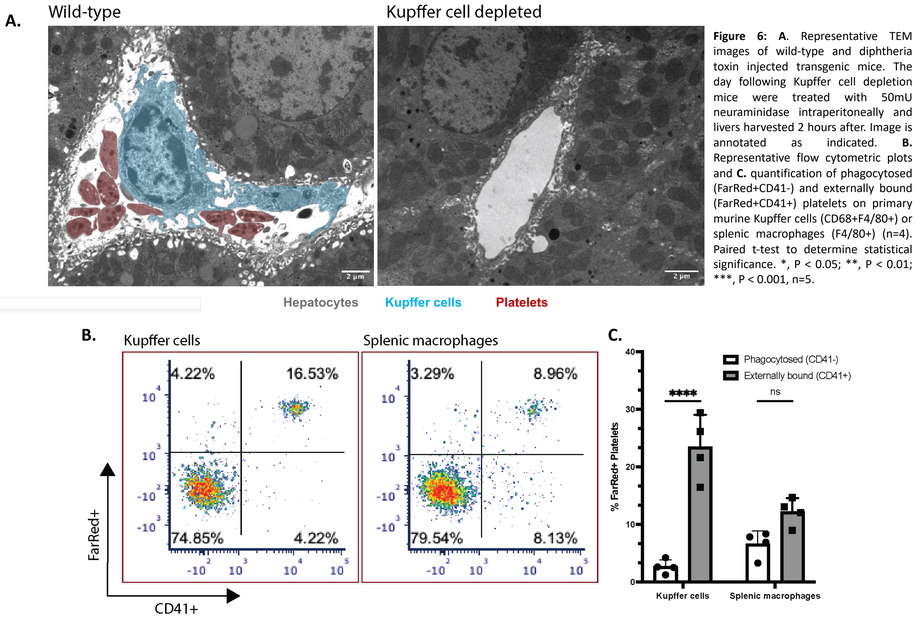

Figure 6: A. Representative TEM images of wild-type and diphtheria toxin injected transgenic mice. The day following Kupffer cell depletion mice were treated with 50mU neuraminidase intraperitoneally and livers harvested 2 hours after. Image is annotated as indicated. **B.** Representative flow cytometric plots and **C.** quantification of phagocytosed (FarRed+CD41-) and externally bound (FarRed+CD41+) platelets on primary murine Kupffer cells (CD68+F4/80+) or splenic macrophages (F4/80+) (n=4). Paired t-test to determine statistical significance. *, P < 0.05; **, P < 0.01; ***, P < 0.001, n=5.