Surface expression of high mobility group box-1 in lipopolysaccharide-activated canine platelets

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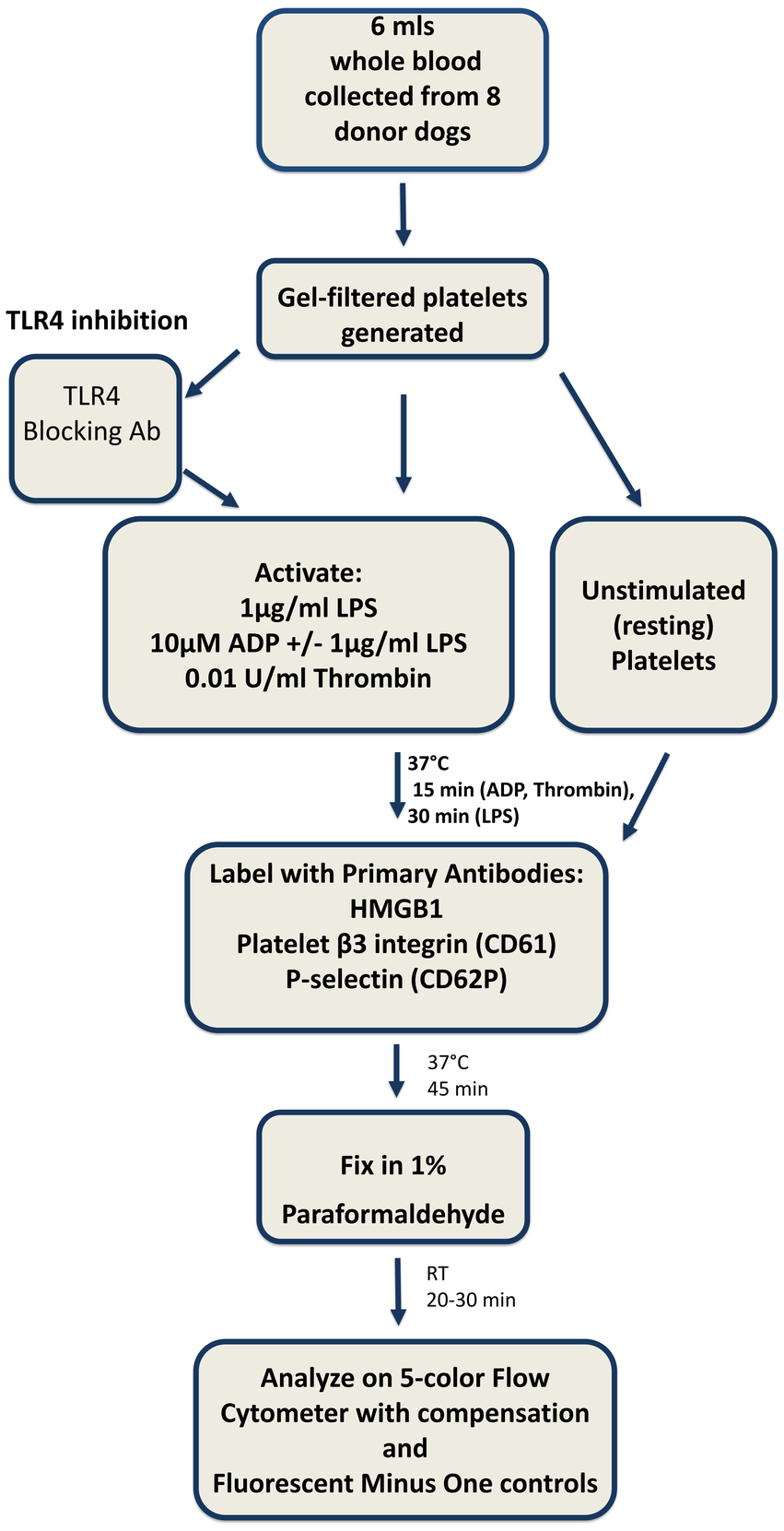
Introduction

- Sepsis is a life-threatening syndrome associated with high mortality in dogs[1,2,3].
- High Mobility Group Box-1 (HMGB1) plays an important role as a damage-associated molecular pattern (DAMP) in innate immunity during sepsis [4,5,6,7].
- Canine platelets function as innate immune cells by expressing functional Toll-like receptor 4 (TLR4), suggesting that canine platelets may respond to DAMPs like HMGB1.[8,9]
- Human and murine platelets have been shown to express and secrete HMGB1 upon activation.[10]
- Surface expression of HMGB1 in canine platelets has not been studied.
- Considering that platelet HMGB1 may contribute to the link between coagulation and inflammation, a better understanding of HMGB1 expression in canine platelets provides potential for future antiplatelet and anti-inflammatory treatments for sepsis.

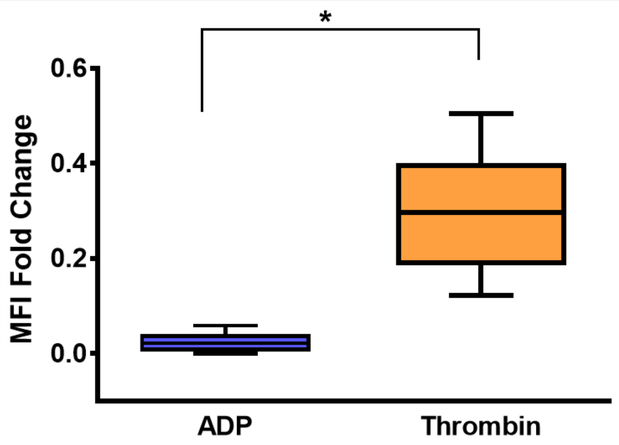
Methods



Hypotheses

- Canine platelets will upregulate surface expression of HMGB1 in response to the agonists, ADP and thrombin, compared to unstimulated (resting) platelets .
- Under LPS stimulation, ADP-primed canine platelets will upregulate surface expression of HMGB1.
- Upregulation of HMGB1 in LPS-activated platelets will be dependent on platelet TLR4.

Results



- **Thrombin upregulates HMGB1 surface expression**
  - Thrombin-activated platelets had significantly increased mean fluorescent intensity (MFI) fold change for HMGB1 compared to ADP-activated platelets (P=0.0004).

Figure 1. HMGB1 MFI fold change in platelets treated with ADP or thrombin. Fold change was calculated by calculating the difference between the logarithmic transformed MFI value for each treatment group and that of resting platelets. N=8

- **LPS in the presence of ADP upregulates HMGB1 surface expression**
  - ADP priming prior to LPS stimulation significantly increased MFI fold change in HMGB1 compared to platelets treated with either ADP or LPS alone (P=0.049, P=0.026, respectively)

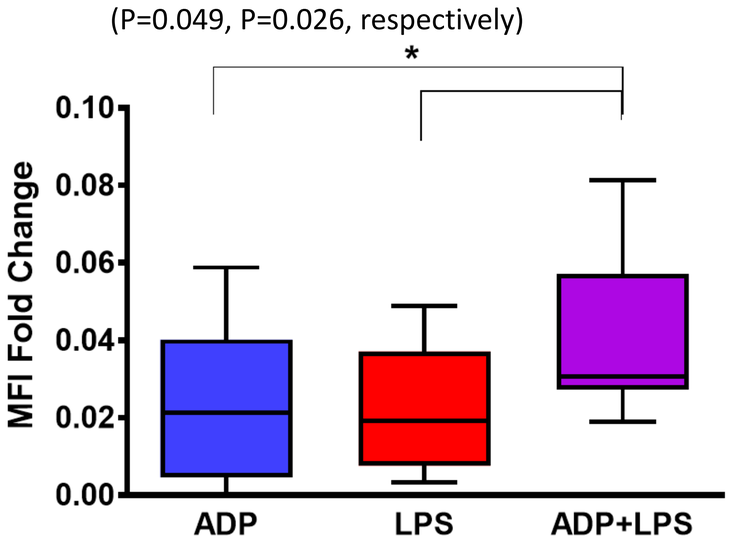


Figure 2. HMGB1 MFI fold change in platelets treated with ADP, LPS or LPS in the presence of ADP. N=8

- **HMGB1 surface expression is associated with α-Granule secretion**
  - Regardless of treatment, CD62P+ platelets expressed significantly higher levels of HMGB1 than CD62P- cells (P<0.05). CD62P or P-selectin is a marker for α-Granule secretion.

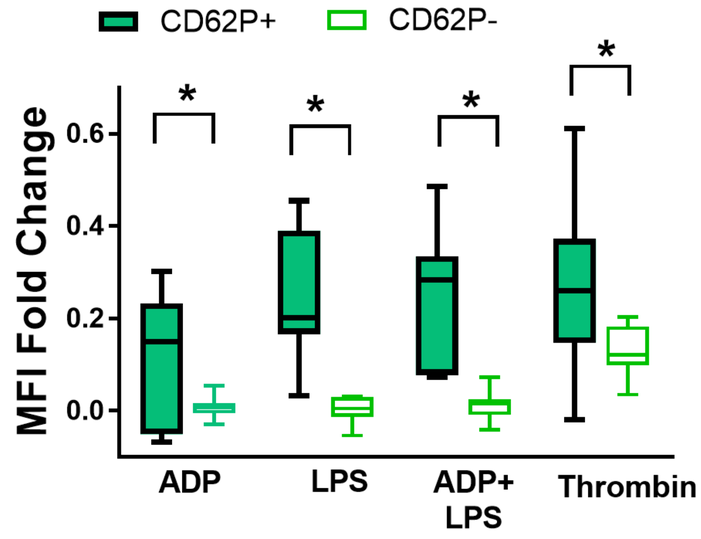
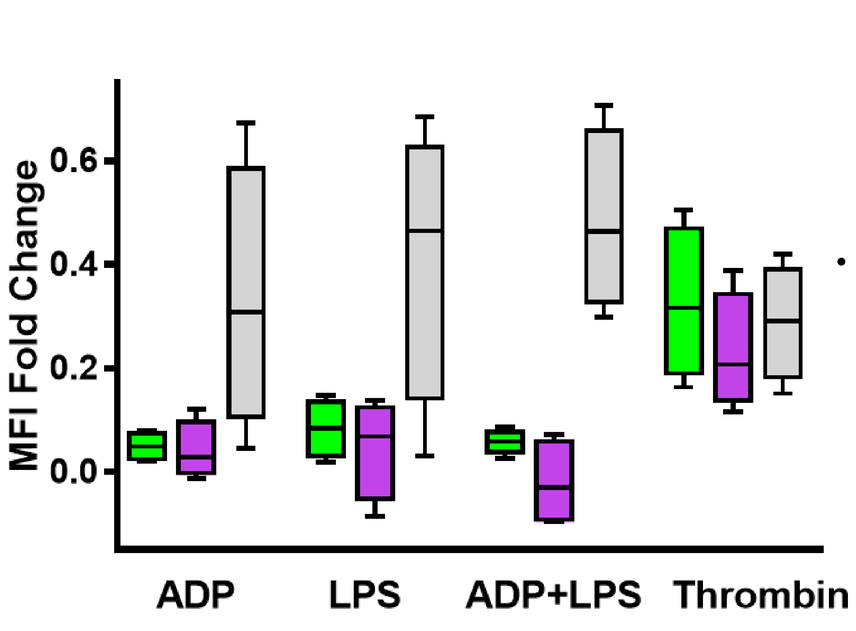


Figure 3. HMGB1 MFI fold change for platelets treated with ADP, LPS, ADP+LPS, or thrombin comparing CD62P+ and CD62P- platelets. N=8

- **Upregulation of HMGB1 surface expression is dependent on platelet TLR4**



  - Inhibition of platelet TLR4 with a function blocking antibody reduced HMGB1 MFI fold change HMGB1 MFI fold change across all treatments.

Figure 5. HMGB1 MFI fold change in platelets treated with the TLR4 functional blocker, CD284, prior to activation with ADP, LPS, ADP+LPS, or thrombin. N = 4

Conclusion

- Canine platelets express HMGB1 on their surface upon activation with thrombin and LPS in the presence of ADP.
- Upregulation of surface HMGB1 occurs primarily in activated platelets that have undergone α-Granule secretion.
- TLR4 is required for upregulation of surface HMGB1 in activated platelets. As a HMGB1 receptor, binding of HMGB1 to platelet TLR4 further amplifies the expression of surface HMGB1 on nearby platelets.
- Platelet HMGB1 or TLR4 presents a novel therapeutic target for hypercoagulable dogs with sepsis.

References:

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