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14. ABSTRACT

We have established base line criteria that will allow us to evaluate the role of platelets during trauma. These studies have started to unravel the relationship between platelets and complement and their contribution to tissue damage. It seems that complement C3 does not deposit on the surface of platelets following ischemia/reperfusion. Yet, we have seen the deposition of both C3 and platelets in various tissues following IRI in a similar time frame. Further studies will evaluate if these factors co-localize in tissue. We have developed B6.lprPF4-/- mice which will allow us to better study the role of PF4 in tissue damage. Preliminary studies indicate a decreased level of platelets in these mice, suggesting a decreased level of disease. Further studies will expand upon these observations better outlining the function of platelets in the injury associated with trauma. Ultimately these studies will allow us to develop specific treatment strategies that limit battlefield tissue injury without affecting haemostatic and coagulation properties of platelets.

15. SUBJECT TERMS
Platelets, Complement, Trauma, Tissue Damage

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Introduction

The primary function of platelets is the maintenance of hemostasis, antimicrobial host defense and tissue repair (Klinger and Jelkmann, 2002). Platelets are the smallest and most abundant blood cell type found in the circulation. Activation of platelets in response to injury initiates an inflammatory response resulting in platelet aggregation, expression of adhesion molecule receptors and co-stimulatory molecules such as P-selectin (CD62P), CD40, and CD154 as well as release of cytokines such as interleukin-1 beta (IL-1β) and transforming growth factor-beta 1 (TGFβ1) (Elzey et al., 2003; Klinger and Jelkmann, 2002; Soslau et al., 1997).

While platelets are traditionally thought to be regulators of hemostasis and coagulation, there is mounting evidence that they may also be important in the development and progression of inflammatory processes (Coppinger et al., 2004; Danese et al., 2003). Recent studies have demonstrated a role for platelets in the development of both innate and adaptive immune responses (Elzey et al., 2003; Shiraki et al., 2004). Platelets have also been shown to participate directly in the immune response through interaction with vascular endothelium, with antigen presenting cells (APC) and with lymphocytes, or through the release of soluble mediators that include pro-inflammatory cytokines, cell adhesion molecules or chemokines (Elzey et al., 2003). Platelets have also been shown to actively participate in ongoing inflammatory responses such as those observed in atherosclerosis, arthritis and inflammatory bowel disease (Danese et al., 2003). Nonetheless, it is not clear by what mechanisms platelets may be involved in the progression of cellular or tissue injury after severe trauma. Activated platelets and platelet-derived microparticles (PMPs) may participate in specific receptor-ligand interactions through co-stimulatory molecules such as CD40-CD154, or adhesion molecules CD62P-CD162 (PSGL-1) interaction (Elzey et al., 2003). Conversely, activated T cells or APC may stimulate platelets through similar receptor-ligand interactions and/or through exposure to cytokines including IL-6, other acute-phase reactants, and pro-coagulant factors such as thrombin (Elzey et al., 2003; Klinger and Jelkmann, 2002; Soslau et al., 1997).

The complement system comprises more than 30 proteins that interact in proteolytic cascades with three initiating arms. The classical, lectin, and alternative pathways are each activated by distinct mechanisms: antibodies initiate the classical pathway, while mannose-binding lectin and bacterial polysaccharides initiate the lectin and alternative pathways, respectively. Each initiating arm produces the enzymatic complexes, C3 and C5 convertases. The cleavage of C3 and C5 advances the cascade in all three pathways, culminating in a common terminal arm, the membrane attack complex (MAC; C5b-9). MAC inserts into the membrane of target cells, forming a pore that result in cell lysis. In addition to cell lysis, the complement cascade also increases phagocytosis, generates inflammatory molecules that recruit inflammatory cells, and it instructs the adaptive immune system to produce appropriate humoral responses (Thurman and Holers, 2006).The complement pathways initiated during trauma have not been well defined.

Activated platelets can exist in either pro-aggregatory or pro-inflammatory states (Kulkarni et al., 2007). Thus, platelets have the capacity to respond to diverse systemic stimuli that include complement fragments, nucleotides, cytokines, integrins, adhesion molecules, and co-stimulatory molecules. Hence, differences in the kinetics and extent of platelet responses depend on the type and concentrations of stimuli encountered. The overall goal of the following series of experiments is to advance our understanding of the beneficial or detrimental role of platelets in trauma patients. We will evaluate the protective and destructive effects of platelets and of their products in our models of ischemia/reperfusion (IR) injury, and hemorrhagic shock (HS). This will allow us to develop specific treatment strategies that limit battlefield tissue injury without affecting haemostatic and coagulation properties of platelets.

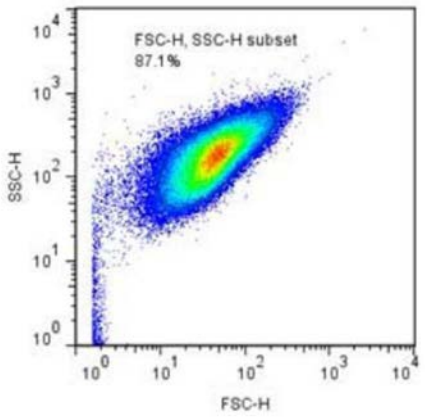
Body

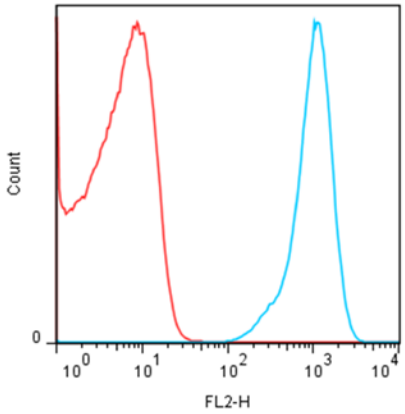
Goal: Determine whether platelets from trauma patients are decorated with complement and whether this results in altered function.

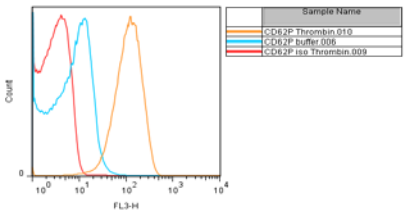
1. Determine whether complement deposits on the surface of platelets from trauma patients.

We optimized the condition to isolate platelets from human whole peripheral blood and stained them for CD41 (population marker). We also optimized the conditions for platelet activation by thrombin and measured CD62P by flow cytometry. Thrombin activated platelets will serve as a positive control for our further experiments to study complement deposition on the surface of platelets.

Fig 1. The gated population represents platelets. Platelets stained with CD41. Activated platelets with thrombin stained with CD62.

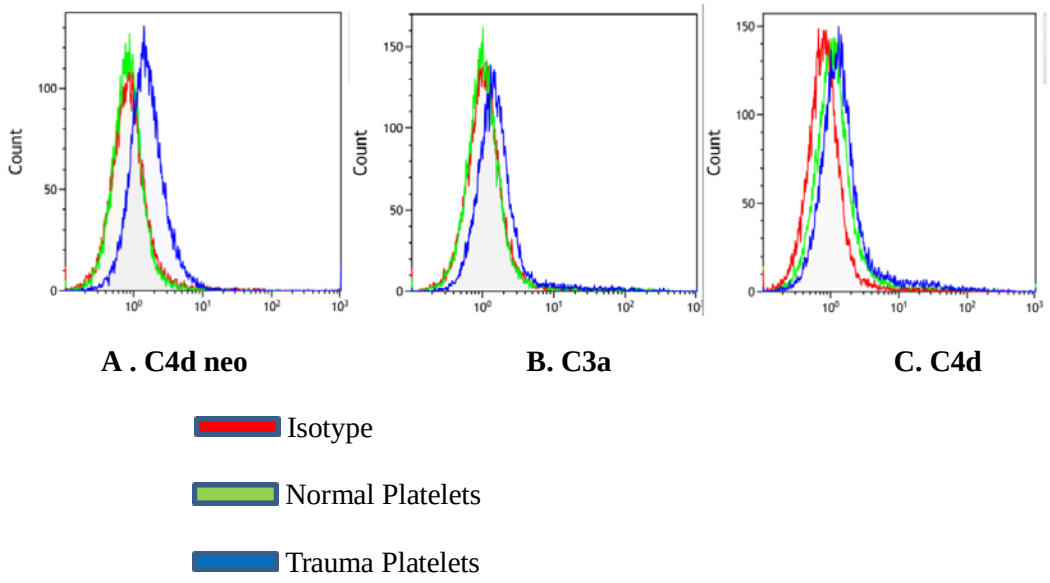






We then isolated platelets from whole peripheral blood, collected from healthy donors as well as patients presented to the emergency room after a localized trauma. Deposition of complement on platelets was evaluated by flow cytometry using monoclonal antibodies to C4d, C4d neo, C3a, C3d, C1q. As shown in a representative sample, trauma platelets have more C4d neo, C4d ,and C3a deposition compared to healthy platelets.

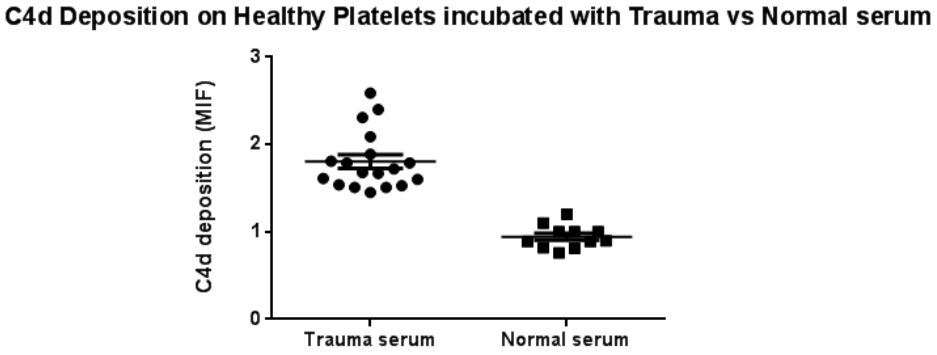
Figure 2. Complement deposition on trauma vs healthy platelets.

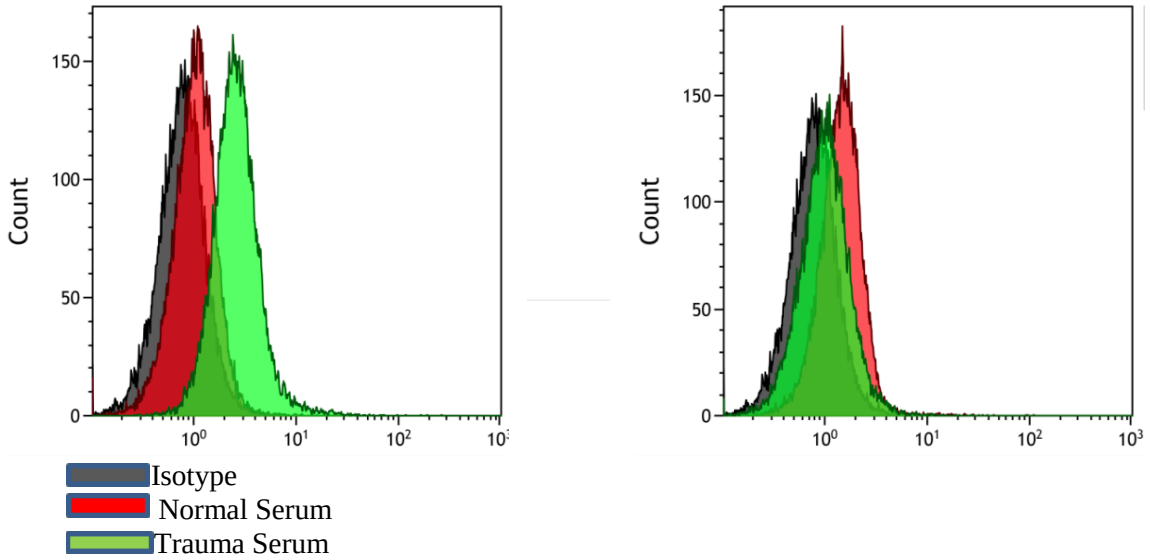


2. Determine whether serum from trauma patients can decorate healthy platelets with complement.

Platelets were isolated from whole peripheral blood and collected from healthy donors. Isolated platelets were incubated with serum, obtained from healthy donors as well as patients presented to emergency room after a localized trauma. The platelets were then washed and incubated with monoclonal antibody to C4d, C3d,and C1q. Deposition of complement on platelets was evaluated by flow cytometry. Figure 3, **Panel A** illustrated cumulative data of C4d deposition on the healthy Platelets incubated with trauma serum(n=20) and normal serum(n=11). **Panel B** is a representative data illustrating deposition of C4d , and C3a on platelets from healthy donors after incubation with either normal or trauma serum . As shown in a representative sample, trauma serum promotes C4d and C3a deposition on healthy platelets.

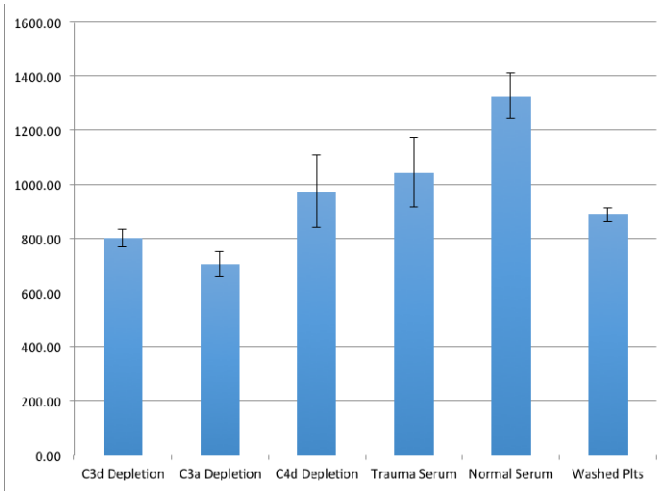
Figure 3. Complement deposition on healthy platelets after incubation with normal vs trauma serum.



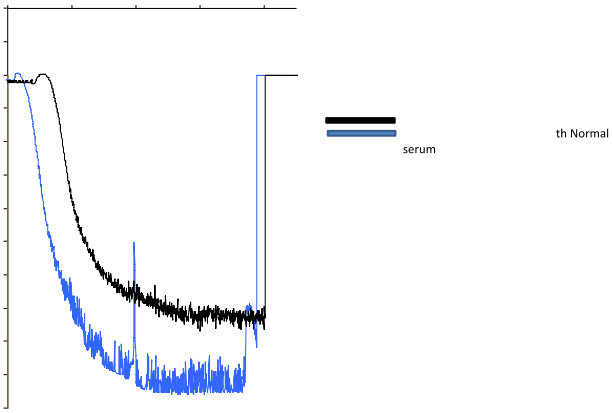


3. Complement deposition and Calcium flux.

Platelets were isolated from whole peripheral blood, collected from healthy donors. Isolated platelets incubated with serum, obtained from healthy donors as well as patients presented to emergency room after a localized trauma like described previously. Platelets were incubated with normal and trauma serums as described previously. However this time we also incubated the healthy platelets with trauma serum, which were depleted from C4d, C3d, and C3a and compare them with non-depleted trauma sera. As it shown in the graph complement depletion decrease calcium flux, more prominently C3a depletion.



4. Determine whether trauma serum alters ability of healthy platelet aggregation
Platelets incubated with trauma serum showed reduced aggregation compared to normal serum. Isolated Platelets were immediately incubated with serum (either normal or trauma serum) for 1 hr at room temperature. The platelets were then washed and their aggregation capacity was measured by aggregometer in response to 0.1 U thrombin.



Key Research Accomplishments

Trauma platelets have more C4d neo, C4d, and C3a deposition compared to healthy platelets.

Incubation of healthy platelets with trauma serum decreases their ability to aggregate.

Reportable Outcomes

Nothing to report

Conclusions

We have established base line criteria that will allow us to evaluate the role of platelets during trauma. These studies have started to unravel the relationship between platelets and complement and their contribution to tissue damage. It seems that complement C3 does not deposit on the surface of platelets following ischemia/reperfusion. Yet, we have seen the deposition of both C3 and platelets in various tissues following IRI in a similar time frame. Further studies will expand upon these observations better outlining the function of platelets in the injury associated with trauma.

Ultimately these studies will allow us to develop specific treatment strategies that limit battlefield tissue injury without affecting haemostatic and coagulation properties of platelets.

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