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POSTER ABSTRACTS

301.VASCULATURE, ENDOTHELIUM, THROMBOSIS AND PLATELETS: BASIC AND TRANSLATIONAL

Platelet Factor 4 Enhances Antimicrobial Function of the Endothelium and Improves Outcome in a Murine Model of Sepsis

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Introduction: In this study, we investigated mechanism(s) by which the platelet-specific chemokine platelet factor 4 (PF4) interacts with bacteria to enhance host-defense function and improve outcome in sepsis. Sepsis, a life-threatening dysregulated response to infection, is the leading cause of mortality worldwide for which there are no targeted treatments. Upon sensing pathogens, platelets release high concentrations of PF4, a positively charged chemokine with high affinity for polyanions including polymers in the bacterial cell wall, the endothelial glycocalyx and cell-free DNA. We previously found that PF4 improves outcomes in the murine lipopolysaccharide endotoxemia and enhances *in vitro* bacterial capture by neutrophil extracellular traps. We now show that PF4-coating of bacteria reduces coagulopathy, limits bacterial dissemination, and improves survival in a murine model of polymicrobial sepsis, in part through the novel mechanism of enhancing the antimicrobial function of endothelial cells (ECs).

Methods: Effects of PF4 on bacterial internalization by ECs was assessed by incubating heat-inactivated *Staphylococcus (S) aureus* conjugated to pHrodo, a dye that only fluoresces following cellular internalization, with PF4 (0-100mg/mL) and then expose those complexes to human umbilical vein endothelial cells (HUVECs) ± tumor necrosis factor a (TNFa) to simulate inflammation. Internalized *S aureus* fluorescent signal was visualized 16hr post-exposure. HUVECs were stained for von Willebrand factor (VWF) release to indicate bacteria-induced endothelial activation. Effects of PF4 on EC killing of bacteria was studied by exposing HUVECs to live *Escherichia (E) coli* pre-treated with PF4 (0-25mg/mL) for 1hr. ECs were washed to remove excess unbound bacteria, and cell lysates were plated on agar plates for 24hr to count colony forming units (CFUs).
To investigate the effects of PF4-bacteria interactions on sepsis survival outcomes, contents from the cecum (cecal slurry, CS) of C57BL6 donor mice (100mg/mL) were pre-treated with PF4 (100mg) for 20min and injected intravenously (IV) into wildtype (WT) or *PF4* $^{-/-}$ recipient mice to induce immediate bacteremia. Animals were evaluated for sepsis severity for up to 72hr, and survival analysis was performed using the Mantel-Cox test. A separate cohort of WT mice was given IV CS (400mg/mL) pre-incubated with PF4 (8mg). Blood was drawn at 3 to 24hr post-CS infusion and subjected to complete blood count. Thrombin anti-thrombin (TAT) and VWF levels were measured by ELISAs in plasma as indicators of intravascular coagulopathy and endothelial activation. To assess bacterial load, blood and liver and spleen homogenates were plated on agar plates to count for CFUs.

Results: PF4 coating of bacteria enhanced bacterial uptake by HUVECs, peaking at 20-50 μg/mL of PF4. TNFa-stimulated HUVECs more readily internalized PF4-coated bacteria at 1-20 μg/mL of PF4. Although PF4 promoted *S aureus* uptake, it limited bacteria-induced VWF secretion by HUVECs. PF4 also enhanced EC killing of *E coli* upon bacterial internalization. In the CS model of murine polymicrobial sepsis, PF4 prevented CS-induced mortality in both WT and *PF4* $^{-/-}$ mice (**Fig. 1**). PF4-pretreatment of CS also reduced leukopenia and thrombocytopenia 3-6hr post-CS challenge in WT mice. Mice given PF4-treated CS exhibited lower TAT and VWF levels as compared to untreated-CS animals. Lastly, PF4 reduced bacterial load in blood, liver, and spleen 24hr post-CS inoculation (**Fig. 2**).

Conclusion: PF4 enhances bacterial uptake and killing by the endothelium *in vitro*, promotes bacterial clearance while preventing bacteria-induced coagulopathy and endothelial dysfunction leading to improved survival in the murine CS model of polymicrobial sepsis. These studies suggest that PF4 binding to bacteria may be an important host defense mechanism. Further studies are underway to define the contribution of EC antimicrobial activity to the observed outcomes. The ability of PF4-based therapeutics to enhance clearance of microbes merits further translational studies in sepsis.

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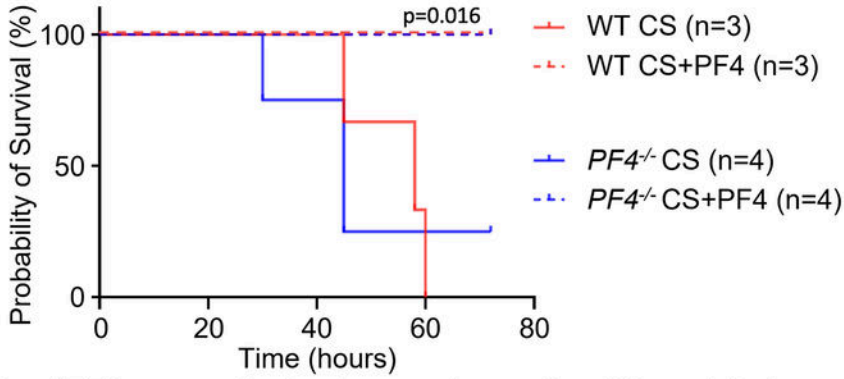

Figure 1. Effects of PF4 on survival outcomes in murine CS model of sepsis. WT or *PF4*$^{-/-}$ mice on C57BL6 background were injected with 20mg CS that was pre-treated with 5% dextrose or PF4 (100µg). Survival was assessed up to 72hr post-CS infusion. Survival analysis was performed using a log-rank (Mantel-Cox) test, and differences in survival were found to be significant (*P*=0.016).

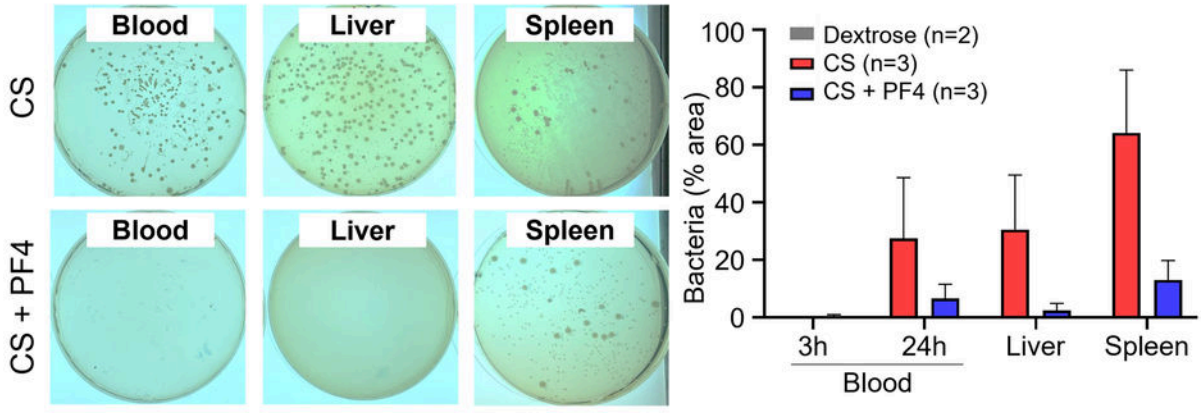

Figure 2. Effects of PF4 on bacterial load in polymicrobial sepsis. C57BL6 mice were injected with dextrose alone (n=2, gray bars), or 80mg CS that was pre-treated with 5% dextrose (n=3, red bars) or PF4 (8µg; n=3, blue bars). Blood was drawn into EDTA from the retro-orbital sinus at 3, 6 and 24hr. Animals were euthanized at 24hr, and liver and spleen were homogenized. Blood samples and organ homogenates were plated on agar plates and incubated for 24hr at 37°C. Representative agar plates for CS vs. CS+PF4 are shown (left), and bacterial growth was quantified using Fiji (right). Data were presented as mean ± SEM.

Figure 1

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