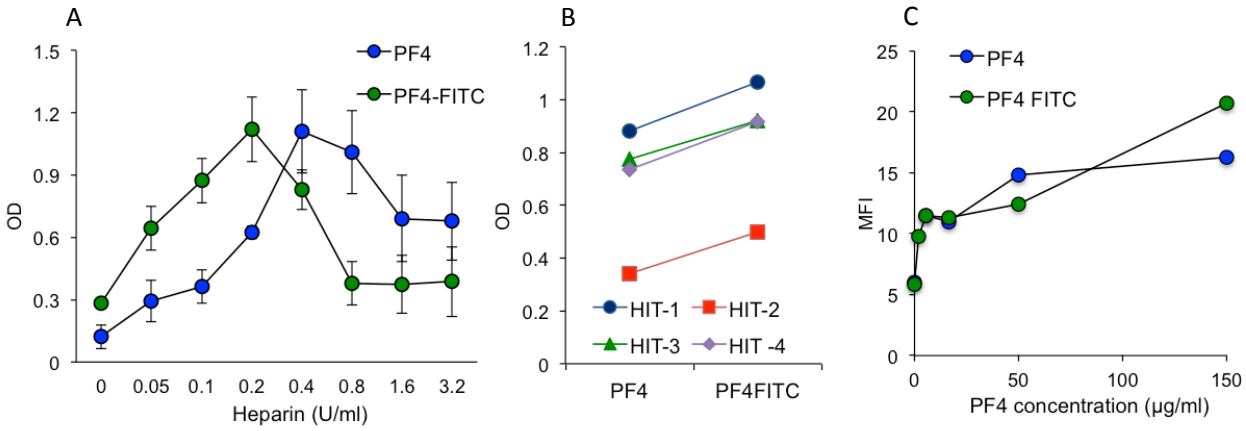
Supplemental Material and Methods

Mouse strains

Transgenic mice expressing platelet-specific hPF4[1] (hPF4+) and/or the R131 isoform of human FcγRIIA[2] (FcγRIIA+) were studied. All transgenic mice were on a Cxcl4$^{-/-}$ background[3]. Genetic alterations were confirmed by the appropriate PCR analyses[1-3]. Mice were studied at 8 to 12 weeks of age. Both male and female mice were studied.

Recombinant proteins and antibodies

Wildtype human (h) PF4 in the plasmid pMT/BiP/V5-His (Invitrogen) was expressed using the Drosophila Expression System (Invitrogen), purified, and characterized as described[4]. Total protein concentrations were determined using the bicinchoninic acid protein assay (Pierce) with the bovine serum albumin (BSA) provided as the standard. hPF4 was conjugated with fluorescein isothiocyanate (FITC) after its heparin-binding sites were protected by binding to heparin-agarose beads (Sigma) as described[5]. FITC-labeled PF4 formed antigenic complexes with heparin and with membrane glycosaminoglycans in the same manner as unlabeled PF4 as tested by ELISA (Figure 1A,B) or flow cytometry (Figure 1C)



Suplemental Methods Figure 1: **FITC labeled PF4 forms PF4/heparin complexes that are recognized by KKO (A,C) or HIT IgG. A.** Binding of KKO to microtiter wells coated with PF4 or PF4-FITC (10 μg/ml) with heparin at the concentrations indicated on the x axis (0-3.2 U/ml). **B.** Binding of HIT Ig (10μg/ml) to microtiter wells coated with PF4 or PF4-FITC plus heparin (0.2U/ml). **C.** Binding of Alexa 647-labeled KKO to platelets incubated with PF4 or PF4-FITC at the concentrations indicated on the x axis (0-150 μg/ml) measured in whole blood.

The HIT-like monoclonal antibody (moAb) KKO the anti-PF4 monomeric moAb RTO and an isotype control moAb TRA (all mouse IgG2bκ), were purified from supernatants of hybridoma cells[6]. Anti-human (h) CD41a moAb conjugated to tandem conjugate system peridinin-chlorophyll-protein (PerCP) and cyanine 5.5 (Cy5.5) (hCD41a-PerCP-Cy5.5), anti-hCD14 conjugated to allophycocyanin (APC) (hCD14-APC), anti-hCD45-PerCP, anti-mouse (m) CD45-PerCP (anti-mCD45-PerCP), anti-mCD11b PE and purified rat anti-mCD16/CD32 (Mouse BD Fc Block™) were from BD Pharmingen. Rat anti-mCD115 PE moAb was from eBioscience. FITC-labeled annexin V was from Abcam. KKO, TRA, affinity purified rabbit polyclonal anti hPF4 (Sigma-Genosys) and Fab fragments from the rat anti-mCD41 antibody (BD Pharmingen) were conjugated with Alexa488 or Alexa647 using Alexa Fluor Protein labeling kits (Thermo Scientific). Biotinylated rat anti-mCD45 antibody was from BioLegend.

References

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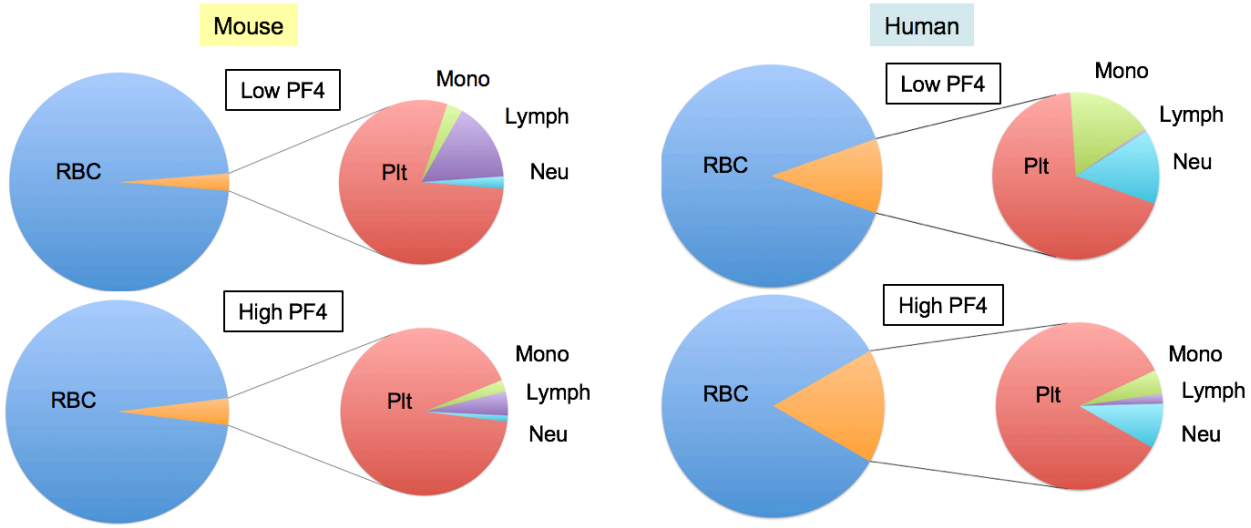
Supplemental Figures



Supplement Figure 1. In vitro distribution of bound PF4 in whole mouse or human blood. Figure is based on the same studies as Figure 1, but corrected for estimated total surface area of individual cell type using the absolute number of analyzed cells in the sample and the following surface area for cell types (mouse/human): platelet = 3.5 μm^2 [1]/8 μm^2 [2]; red blood cell = 96 μm^2 [1]/134 μm^2 [3]; lymphocyte = 124 μm^2 [4]/167 μm^2 [4]; neutrophil = 353 μm^2 [5]/216 μm^2 [6]; and monocyte = 491 μm^2 [7]/243.3 μm^2 [8].

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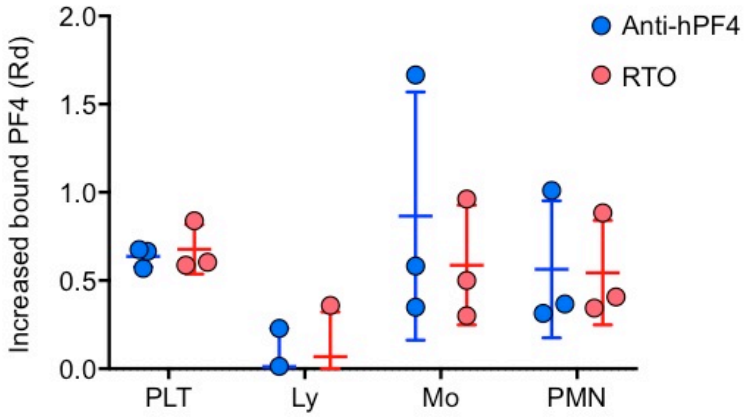

Supplemental Figure 2: Binding of polyclonal anti-hPF4 and monoclonal RTO antibodies to PF4 released from activated platelets and distributed to other cells in whole human blood. TFLLR-NH2 was added to whole human blood. Total cell surface-bound PF4 on platelets (PLT), monocytes (Mono), neutrophils (PMN), and lymphocytes (Lymph) was detected using Alex 488-labeled affinity purified rabbit polyclonal anti-hPF4 antibody or monoclonal antibody RTO. Data are expressed as increase of MFI after stimulation compared to unstimulated samples using distribution resolution metric (R_D). The individual data points and mean ± 1 SD are shown. N = 3 each done in duplicates.