FcγRIIB on liver sinusoidal endothelial is essential for antibody-induced GPVI ectodomain shedding in mice

– Supplemental Material –

Running title: FcγRIIB promotes GPVI-shedding

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MATERIALS AND METHODS

Mice

Mice lacking ADAM10 in endothelial cells were generated by intercrossing Adam10^fl/fl mice\textsuperscript{11} using the Tie2-Cre system.

For the generation of bone marrow chimeras, 6 week old WT or FcgR2b^-/- mice\textsuperscript{17} were lethally irradiated with a single dose of 10 Gy, and bone marrow cells from 6-8 week old WT or FcgR2b^-/- mice were injected intravenously into the irradiated mice (4 x 10\textsuperscript{6} cells per mouse). All recipient animals received acidified water containing 2 g/liter neomycin sulfate for 2 weeks after transplantation. Experiments were performed 10-12 weeks after transplantation.

Western blot analysis

Washed platelets (2 x 10\textsuperscript{5}/μL) were solubilized in lysis buffer (TRIS-buffered saline containing 15 mM TRIS, 155 mM NaCl, 1 mM EDTA, 0.005% NaN\textsubscript{3}, protease inhibitor cocktail (Sigma-Aldrich) and 0.5% IGEPAL® CA-630). Samples were separated by SDS-PAGE and transferred onto a polyvinylidene difluoride membrane (Millipore) and probed with the indicated antibodies under non-reducing conditions.
SUPPLEMENTAL FIGURES

Supplemental Figure 1. JAQ1-IgG, but not JAQ1-F(ab')_2 induces transient thrombocytopenia. (A) Platelet counts of WT mice were monitored upon injection of JAQ1-IgG or JAQ1-F(ab')_2 fragments. (B) Presence of GPVI on the platelet surface was determined by flow cytometry using an anti-rat IgG-FITC antibody. (C) The plasma levels of soluble GPVI (sGPVI) were determined using an ELISA system. Data are expressed as mean ± SD (n ≥ 4) and are representative of 3 individual experiments.

Supplemental Figure 2. Kupffer cell depletion using clodronate liposomes has no effect on JAQ1-induced thrombocytopenia and the generation of sGPVI. (A) Sections of snap-frozen liver samples of vehicle and clodronate-treated WT mice were probed with F4/80 (Kupffer cells; green), anti-CD105 antibodies (LSEC; red) and counterstained with DAPI (blue). Scale bar = 50 µm. (B) Presence of GPVI on the platelet surface was determined by flow cytometry using an anti-rat IgG-FITC antibody. (C) The plasma levels of soluble GPVI (sGPVI) were determined using an ELISA system. Data are expressed as mean ± SD (n=4) and are representative of 3 individual experiments.
Supplemental Figure 3. Endothelial FcγRIIB mediates anti-GPVI induced thrombocytopenia and GPVI shedding. (A) Platelet counts of bone marrow chimeric FcγRIIB (FcgR2b−/−) mice were monitored upon injection of the anti-GPVI antibody JAQ1 biotin by flow cytometry. (B) Presence of GPVI on the platelet surface was determined by flow cytometry using an anti-rat IgG-FITC antibody. (C) The plasma levels of soluble GPVI (sGPVI) were determined using an ELISA system. The labels of B and C are depicted in B. Data are expressed as mean ± SD (n=4) and are representative of 2 individual experiments.
Supplemental Figure 4. Endothelial ADAM10 is not required for the generation of sGPVI. (A) Platelet counts of mice with an endothelial-specific ADAM10 deficiency (Adam10<sup>−/−</sup>) were monitored upon injection of the anti-GPVI antibody JAQ1<sub>biotin</sub> by flow cytometry. (B) Presence of GPVI on the platelet surface was determined by flow cytometry using an anti-rat IgG-FITC antibody. (C) The plasma levels of soluble GPVI (sGPVI) were determined using an ELISA system. (A-C) Data are expressed as mean ± SD (n=4) and are representative of 3 individual experiments. (D) Total GPVI levels were determined by Western blotting. GPIIIa served as loading control. The Western blot is representative of 3 individual experiments.