Key mechanisms of anti-HPA-1a mediated platelet destruction in FNAIT

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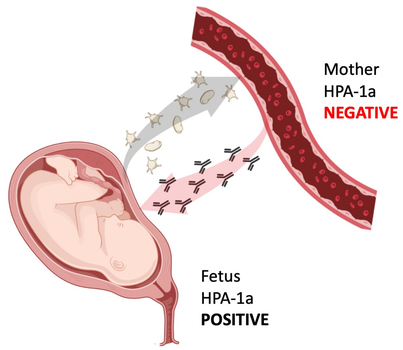
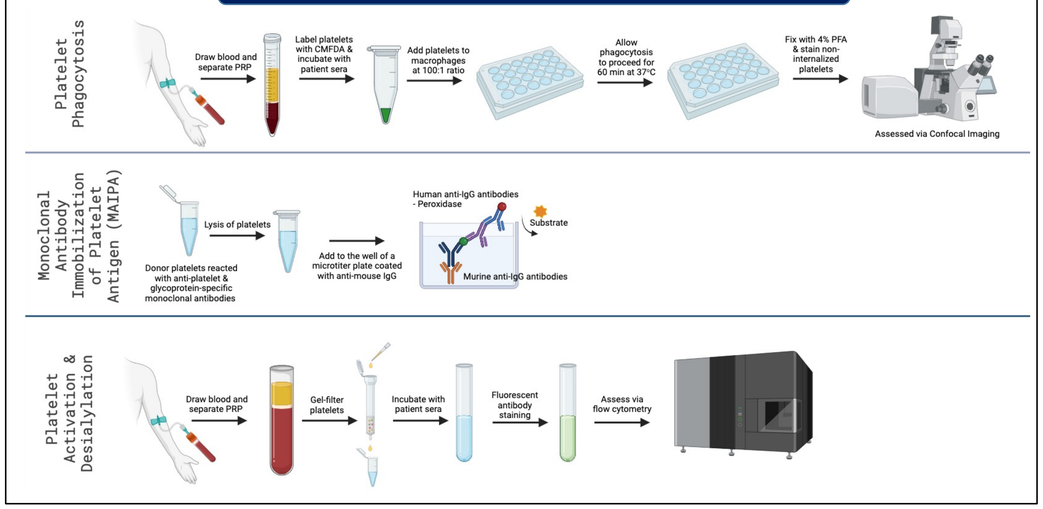
INTRODUCTION

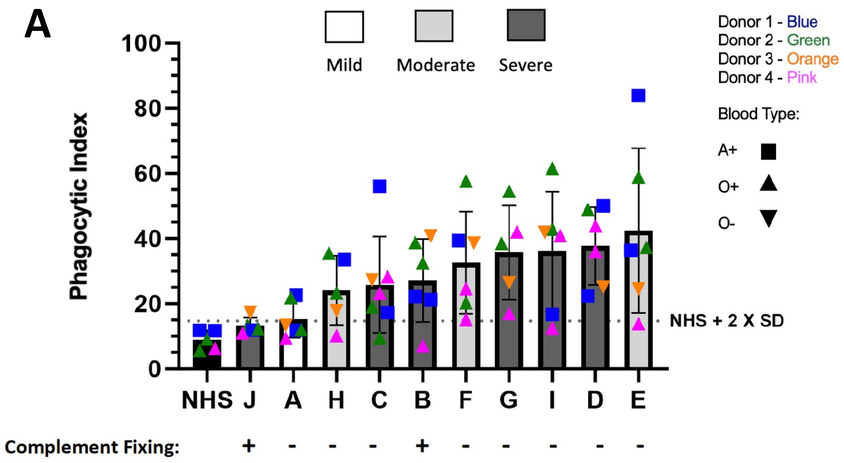
- → Fetal and neonatal alloimmune thrombocytopenia (FNAIT) is a pregnancy complication that occurs when the mother produces antibodies against fetal platelets.[1-3]
- → Maternal-fetal incompatibility for HPA-1a antigen is the most common cause.[2]
- → The pathophysiology involved in fetal platelet destruction remains largely unclear.
- → The objective of this study was to assess the ability of sera from 10 FNAIT patients (anti-HPA-1a positive) to mediate Fc gamma receptor-dependent platelet phagocytosis, complement fixation, platelet activation and desialylation to determine which of these mechanisms could explain thrombocytopenia in FNAIT.



METHODS



RESULTS



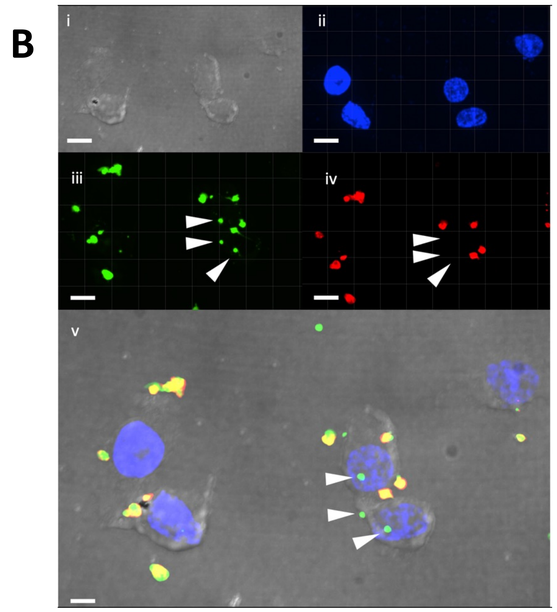


Figure 1. 90% of FNAIT patient sera mediated macrophage phagocytosis of opsonized third-party healthy donor platelets. (A) The ability of FNAIT patient sera or normal human serum (NHS) to trigger THP-1-CD16A macrophage phagocytosis of platelets was evaluated (n=5-6). Four healthy platelet donors were included and represented by blue, green, orange, and pink data points. These donors had three different blood types, as indicated by the symbols: ■, ▲ and ▼. The cut-off to determine if phagocytosis was "positive" for triggering phagocytosis is the PI value higher than 2x the standard deviation (SD) of the mean of the NHS. Phagocytic index: number of phagocytosed platelets per 100 counted macrophages. Thrombocytopenic severity is indicated by the colour of the bar based on the fetal platelet count. The + or – below the x-axis display the sera's ability to fix complement. **(B)** Fluorescent images were combined with differential interference contrast (DIC) images. Platelets were labeled with the cytoplasmic dye 5-chloromethylfluorescein diacetate (CMFDA) (green). Non-phagocytosed platelets were identified using an AlexaFluor 647-conjugated anti-CD42a antibody (red). Macrophages were stained with DAPI (blue). Arrows: phagocytosed platelets. Scale bar = 10 µm

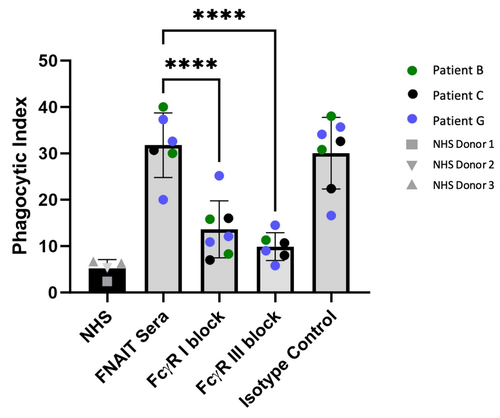


Figure 2. THP-1-CD16A macrophages phagocytose anti-HPA-1a alloantibody-opsonized platelets predominantly through FcγR I and FcγR III. Healthy platelets from third-party donors were opsonized with three different (B,C,G) severe thrombocytopenic FNAIT patient sera (n=4-7 experiments depending on sera availability). NHS was used with platelets as negative controls. FcγRs were blocked using deglycosylated antibodies to FcγRI (clone 10.1) or FcγRIII (3G8), and isotype control (mouse IgG1). Significance: one-way analysis of variance (ANOVA) with multiple comparisons against all means with a Tukey post-hoc test. P value: ****P<0.0001. Data error: mean ± standard deviation.

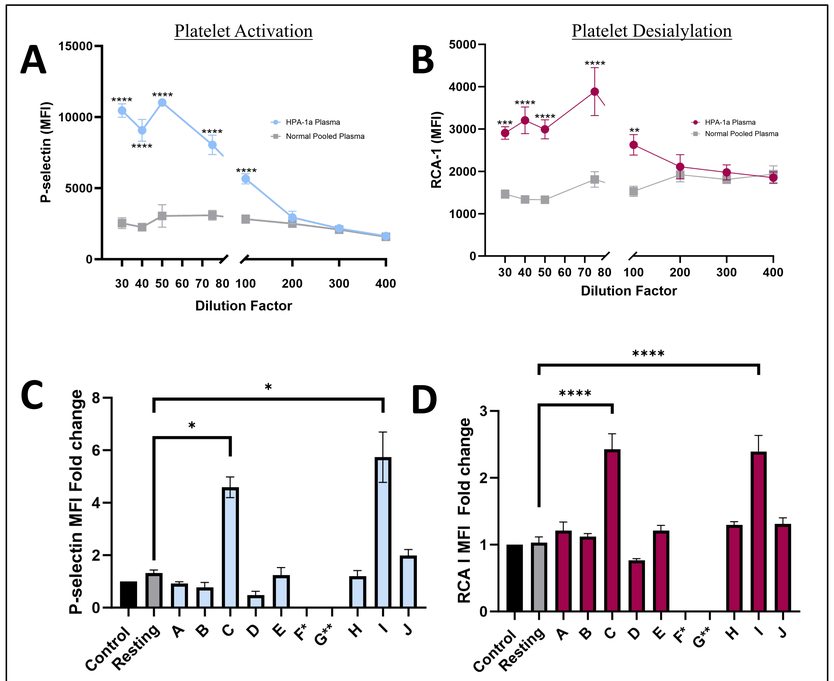


Figure 3. Anti-HPA-1a IgG induces evidence of platelet activation and desialylation in vitro. (A) Surface P-selectin expression **(B)** and RCA-1 binding to gel-filtered human platelets either treated with normal pooled plasma or pooled anti-HPA-1a plasma and assessed by flow cytometry. **(C)** Surface P-selectin expression and **(D)** RCA-1 binding to gel-filtered platelets treated with normal human sera (control), and patient sera (1/100 dilution) measured by flow cytometry. Data analysis was performed using FlowJo v10. Median fluorescence intensity (MFI) fold change was calculated from healthy control serum in each individual assay (n=5). The data are represented as the mean (from duplicate measurements) anti-HPA-1a antibody activation of platelets measured with the error bars depicting the standard deviation of the group. P values: **: P <0.01, ***: P <0.001, ****: P <0.0001.

SUMMARY AND FUTURE DIRECTIONS

In conclusion, this study provide insights into the complex interplay of mechanisms contributing to thrombocytopenia in FNAIT. Platelet phagocytosis and/or complement fixation are present in patients with moderate or severe thrombocytopenia, while platelet desialylation and activation may contribute to specific cases. The involvement of FcγRI and III suggests a potential therapeutic approach.

Future directions for this project include translating into an in vivo model to investigate the potential ameliorative effects of Fc receptor blockade.

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