Immune thrombocytopenia patient sera mediates phagocytosis of healthy donor platelets by macrophages

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INTRODUCTION

Immune thrombocytopenia (ITP) is an autoimmune bleeding disorder characterized by low peripheral blood platelet count[1]. Previous studies have shown the presence of autoantibodies on the surface of platelets in 50-60% of patients, but only 10-20% of patients have detectable autoantibodies in sera[3]. There is a discordance between detecting autoantibodies in patient sera and the ability of sera to trigger thrombocytopenia in naïve recipients[4]. These autoantibodies are presumed to mediate platelet clearance through splenic macrophages dependent on FcγRs[5]. This project explores the ability of unselected ITP patient sera to trigger phagocytosis in vitro.

METHODS

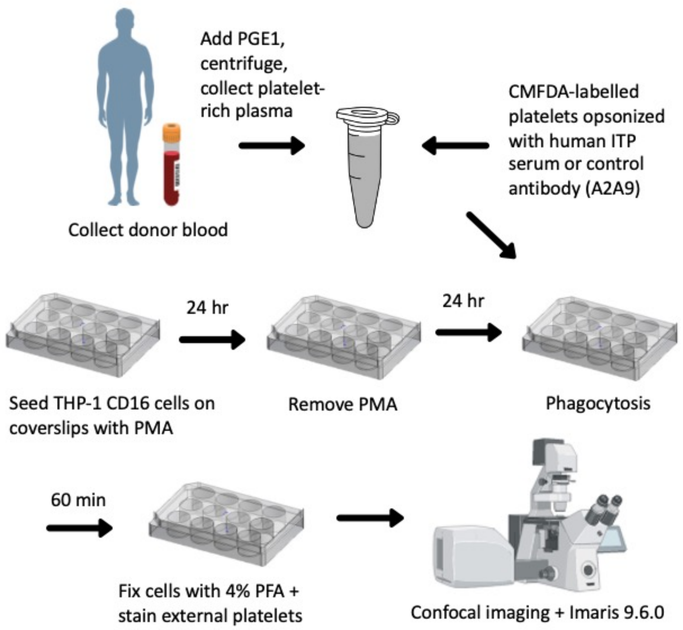
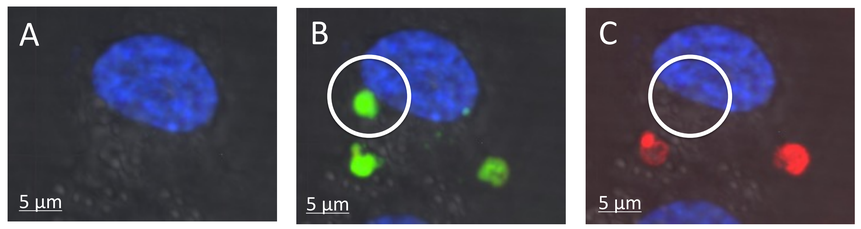


Figure 1. In vitro phagocytosis of ITP serum-opsonized platelets by THP-1 CD16 macrophages. Adult patients with primary ITP were recruited with platelet count <100 x 10^9/L. ITP sera was used to opsonize healthy donor platelets. Platelets were fluorescently labelled and incubated with THP-1 macrophages expressing all human activating Fc receptors. THP-1 CD16 macrophages have been transfected with FcγRIII (CD16). The ability of macrophages to uptake platelets was quantified in the presence and absence of FcγR-blocking antibodies by confocal microscopy.

RESULTS



Differential Interference Contrast + DAPI | Platelets (CMFDA) | External platelets (anti-CD42a AF647)

Figure 2. Phagocytosis of ITP serum-opsonized platelet by THP-1 CD16 macrophages. (A) Fluorescent images were combined with differential interference contrast (DIC) images. (B) Platelets were labeled with the cytoplasmic dye 5-chloromethylfluorescein diacetate (CMFDA) (green). (C) Non-phagocytosed (external) platelets were identified after phagocytosis using an AlexaFluor 647-conjugated anti-CD42a antibody (red). Images were taken using a Quorum multi-modal imaging system with 63x objective oil immersion. Images were Z-stacked, and 3D reconstructed for analysis using Imaris v9.6. Circle: one phagocytosed platelet.

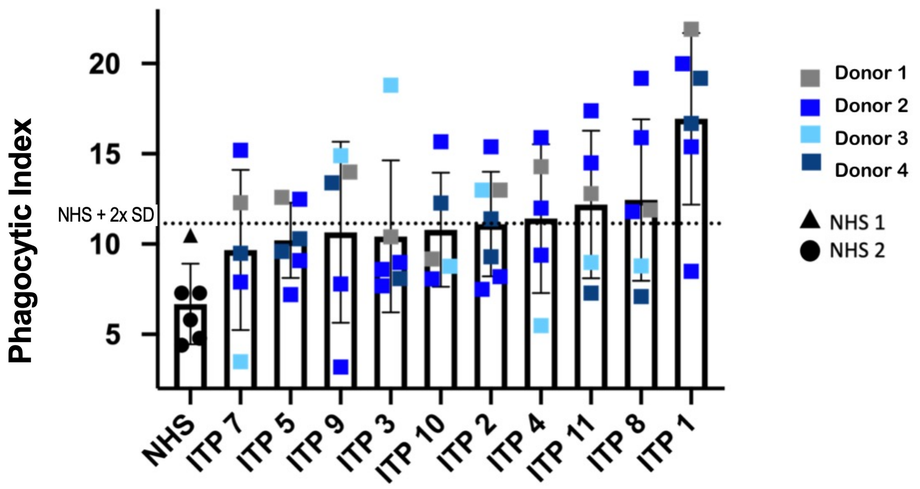
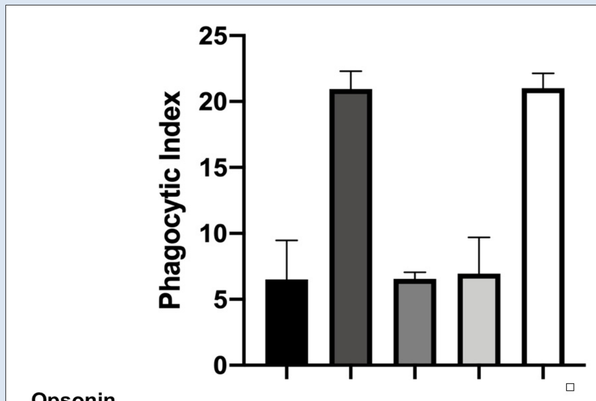


Figure 3. ITP serum opsonized platelets (increasing order). Healthy platelets from four donors were opsonized with one of ten different ITP sera (n=5-6 experiments each). Two normal human sera (NHS) specimens were used to opsonize platelets as controls (n=5 experiments). The baseline set to detect the ability of sera to trigger phagocytosis was NHS plus 2 times its standard deviation. 40% of the sera was positive for phagocytosis. The phagocytic index was calculated as the number of phagocytosed platelets per 100 macrophages.



Opsonin					
NHS	+	-	-	-	-
ITP Sera	-	+	+	+	+
Blocking Antibodies					
FcγRI	-	-	-	+	-
FcγRIII	-	-	+	-	-
Isotype Control	-	-	-	-	+

Figure 4. FcγR-Blocking with THP-1 CD16 macrophages. FcγRs were blocked using deglycosylated antibodies to FcγRI (clone 10.1), or FcγRIII (3G8). Healthy donor platelets were opsonized with the ITP sera (ITP 1) demonstrating high phagocytosis (n=2 experiments). Isotype control: 10 ug/mL deglycosylated mouse IgG1.

CONCLUSIONS

These findings may suggest the ability to trigger phagocytosis could be greater than the proportion of patients that have autoantibodies present in sera. This work also highlights the role of FcγRI and FcγRIII in clearance of ITP sera-opsonized platelets with one patient studied thus far. Further studies will analyze patient sera for autoantibodies and FcγR involvement.

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