ITP Antibodies Mediate Complement Activation and Platelet Desialylation

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Background

Immune thrombocytopenia (ITP) is an acquired autoimmune disorder characterized by low platelet count (<100 x 10^9) due to increased platelet destruction and decreased platelet production (**Figure 1**). ITP can be a primary condition or develop secondarily. Symptoms of ITP include bruising (purpura), petechiae (**Figure 2**), and an overall increase in bleeding. However, only about 5% of patients present with severe bleeding. Current treatment, if indicated, involves steroids, IVIG, and anti-D. About 10% of ITP patients present with chronic refractory ITP. Plasmapheresis has been used in these patients who have failed conventional treatments, but there is an ongoing need for more patient-focused therapies. Multiple mechanisms in which ITP facilitates platelet destruction have been discovered. Past studies have shown that ITP antibodies lead to an increase in complement activation and that ITP antibodies bind to desialylated platelets, marking them for apoptosis (**Figure 3**).

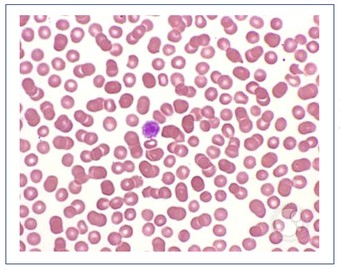


Figure 1: Blood smear indicating decreased platelet counts found in ITP

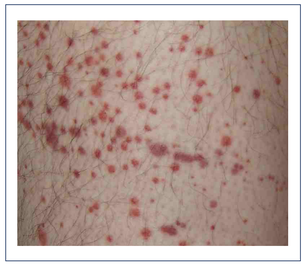


Figure 2: Petechiae found in ITP patients

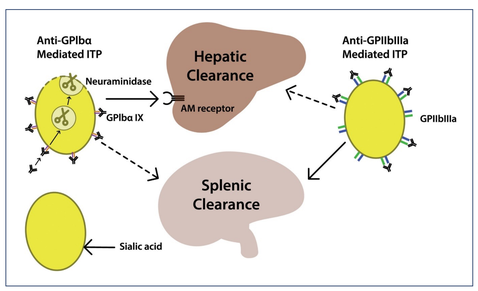


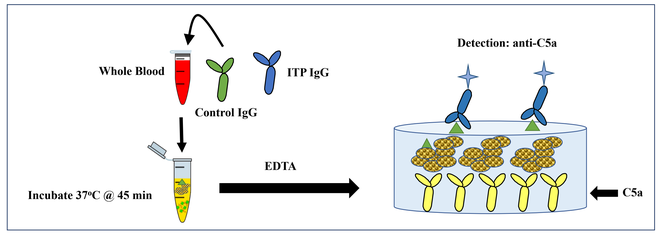
Figure 3: Method of action of Platelet Desialylation in ITP

Objectives

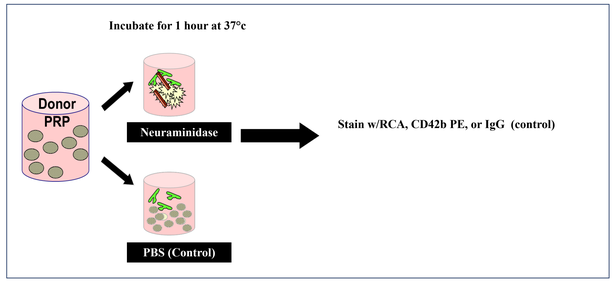
We hypothesize that desialyated platelets show increased binding of ITP antibodies. Increased ITP platelet binding contributes to complement activation.

Methods

Complement activation Whole blood samples were incubated with control IgG or ITP antibodies from the blood of 3 patients for 45 minutes at 37°c. Samples were centrifuged, and the collected supernatant was either frozen at -80°c or used immediately. Complement activation was then measured using a C5a ELISA.



Platelet desialylation Whole blood was treated with ACD (1:9) and was centrifuged at 180g for 20 minutes to isolate platelet rich plasma (PRP). PRP was treated with PGE1 and centrifuged at 1800 rpm for 10min. Neuraminidase (Sigma 11585886001) was added to the PRP and incubated for 1 hour at 37°c. RCA FITC (Vector FL-1081-5) was added to samples and allowed to incubate at RT in the dark for 15 minutes. Samples were fixated with 1% paraformaldehyde for 30 minutes to 24 hours before proceeding with flow cytometry



Results

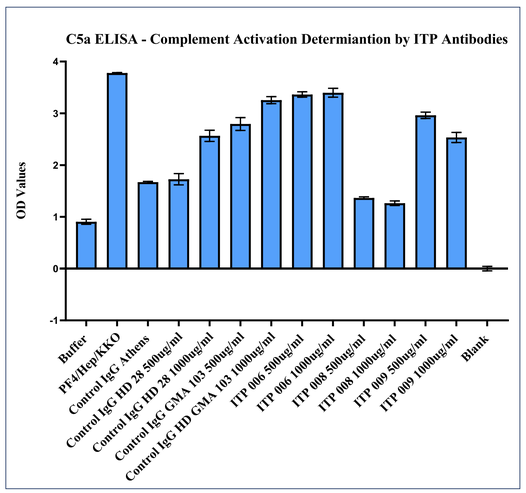
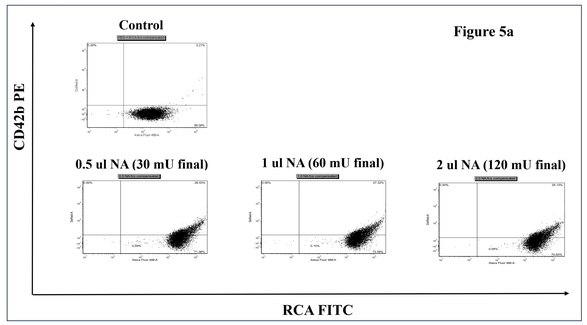


Figure 4: Complement activation in control patients (IgG) vs. ITP patients measured using a C5a assay. PF4/Hep/KKO and Control IgG Athens were used as positive controls. The blank and buffer samples were used as negative controls.

Results C5a ELISA showed no difference in complement activation between samples incubated with IgG and ITP antibodies.

Rationale: Most platelets in whole blood have intact sialic residues on platelet glycoproteins. We undertook studies to see if ITP antibodies activate platelets in whole blood to undergo premature desialylation. To desialyate platelets, we incubated platelet rich plasma with neuraminidase, an enzyme that removes terminal sialic residues from glycoproteins. To examine desialylation, we used a labeled RCA (RCA FITC) and incubated platelets.



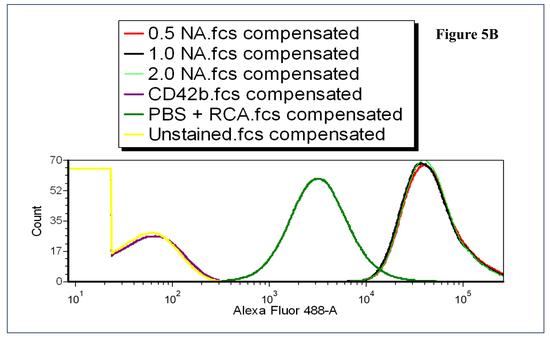


Figure 5a-b: Platelet rich plasma (PRP) flow cytometry to determine the optimal concentration of neuraminidase (NA) needed for platelet desialylation. PRP cells were incubated with NA at 30, 60, and 120 mU and tagged with RCA FITC.

Results Pending results. However, for future platelet desialylation experiments, RCA FITC (1:500) and Neuraminidase (1 ul/60 mU) will be used to stain platelets.

Conclusion

These results suggest that ITP antibodies do not activate complement or more sensitive assays are needed to detect complement activation by ITP antibodies. If our desialylation studies indicate that ITP antibodies show improved binding to desialyated antibodies, we will then examine complement activation using desialyated platelets.

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