**SUMMARY**

In idiopathic thrombocytopenic purpura (ITP), autoantibodies bind to platelets which are then phagocytosed by monocytes/macrophages and removed by the reticuloendothelial system. PRTX-100 (Staphylococcal protein A) is being investigated for the treatment of ITP. We developed an in vitro flow cytometric assay to assess the effect of PRTX-100 on phagocytosis of opsonized platelets. Human monocytes were treated with PRTX-100 at concentrations of 250, 25, 2.5, 0.25 and 0.025 ng/ml and incubated with allogeneic human platelets that were opsonized with WE32 IgG and labeled with a PE-labeled anti-CD61 antibody. The assay was used to assess for surface bound platelets versus ingested platelets. Phagocytosis was determined by flow cytometric analysis. Percent phagocytosis was calculated as the fraction of ingested platelets (PvCP-CD61−) from the total PvCP population (PvCP-CD61− + PvCP-CD61+) within the gated monocyte population. PRTX-100 was found to inhibit the phagocytosis of opsonized platelets in a dose-dependent fashion. Phagocytosis was dependent on opsonization with WE32, indicating a role of Fc receptors on the effector monocytes. Since prevention of platelet phagocytosis is an important treatment goal in ITP, PRTX-100 is currently under investigation as a possible therapy for patients with ITP.

**METHODS**

**Platelet Phagocytosis assay**

- Culture monocytes for 6 days
- 48 hour PRTX-100 treatment
- 1 hour culture with WE32 opsonized/PvCP labeled platelets
- Measure phagocytosis by flow cytometry

**RESULTS**

Human monocytes gated for analysis verified by CD14 expression

Phagocytosis of platelets is dependent on WE32 opsonization

- **Opsonized platelets 37°C**: 37% Phagocytosis
- **Non-opsonized platelets 37°C**: 4% Phagocytosis
- **Opsonized platelets 4°C**: 1% Phagocytosis

**CONCLUSIONS**

PRTX-100 inhibits the phagocytosis of WE32 opsonized platelets by human monocytes. Phagocytosis of WE32 opsonized platelets in the absence of PRTX-100 was 60% while that of non-opsonized platelets was 10%. These data demonstrate that the observed phagocytosis is antibody-dependent and presumably involves Fc receptors on the monocytes.

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**DISCLOSURE**

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