Anti-GPIbα antibodies in FNAIT associated with recurrent miscarriages

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Introduction

- ***Recurrent miscarriage (RM)*** *is clinically* defined as three or more consecutive pregnancy losses before week 20 of gestation and is common, affecting approximately 1-2% of couples.[1,2]
- Many factors can contribute to RM; however, an exact cause for RM cannot be identified in 50% of cases.[1]
- **Fetal and neonatal alloimmune thrombocytopenia (FNAIT)** is one suspected cause of miscarriage.
- FNAIT affects ~1/1000 neonates and results from maternal antibody generation against fetal platelets bearing certain paternally-inherited platelet surface receptor polymorphisms.[3]
- We previously described in a murine model of FNAIT that anti-GPIbα antibodies led to increased platelet activation and thrombosis in the placenta, resulting in miscarriage rather than the bleeding diatheses and thrombocytopenia common in anti-β3 FNAIT.[4]
- FNAIT is not tested for in cases of RM, and no studies have been done on large cohorts of women experiencing unexplained RM to test for this disease.

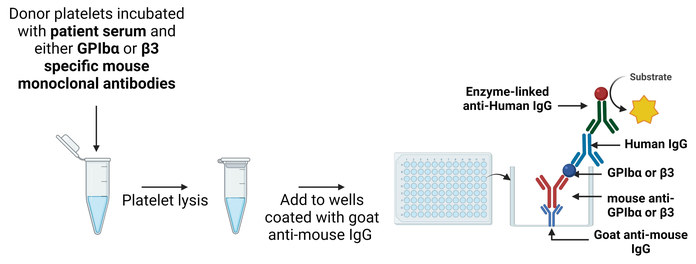
In this report the presence of anti-GPIbα antibodies in patients with RM of an unknown etiology, suggesting the need to investigate FNAIT as a potential cause of RM in humans.

Hypothesis

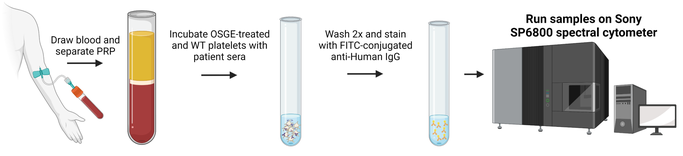
Anti-GPIbα alloantibodies induce a severe phenotype of FNAIT which may cause recurrent miscarriages in humans

Methods

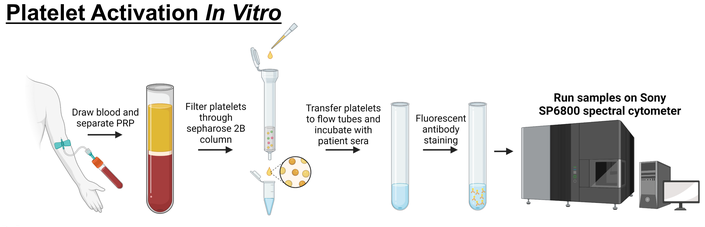
Monoclonal Antibody Immobilization of Platelet Antigen (MAIPA)[5]



Serum IgG Binding to Donor Platelets *In Vitro*



Platelet Activation *In Vitro*



References

1. Stirrat GM. Recurrent miscarriage I: definition and epidemiology. The Lancet. 1990 Sep 15;336(8716):673–5.
2. Rai R, Regan L. Recurrent miscarriage. The Lancet. 2006 Aug 12;368(9535):601–11.
3. Curtis BR. Recent progress in understanding the pathogenesis of fetal and neonatal alloimmune thrombocytopenia. Br J Haematol. 2015;171(5):671–82.
4. Li C, Piran S, Chen P, Lang S, Zarpellon A, Jin JW, et al. The maternal immune response to fetal platelet GPIbα causes frequent miscarriage in mice that can be prevented by intravenous IgG and anti-FcRn therapies. J Clin Invest. 2011 Nov;121(11):4537–47.
5. Kiefel V, Santoso S, Weisheit M, Mueller-Eckhardt C. Monoclonal antibody--specific immobilization of platelet antigens (MAIPA): a new tool for the identification of platelet-reactive antibodies. Blood. 1987 Dec 1;70(6):1722–6.

Results

Detection of anti-GPIbα IgG in sera of two RM patients with MAIPA

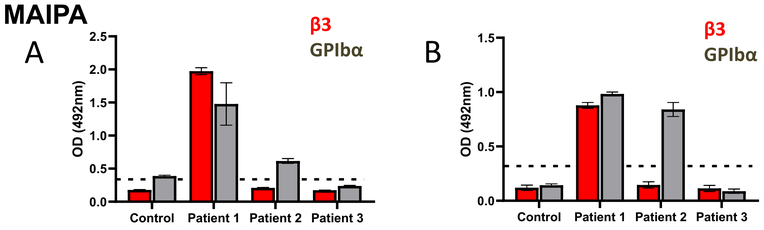


Figure 1. (A,B) Two independent MAIPA assays found IgG specific to GPIbα in serum of RM patient 1 and patient 2. IgG specific for β3 was also detected in sera of patient 1.

Significant IgG binding to donor platelets treated with RM patient serum *in vitro*

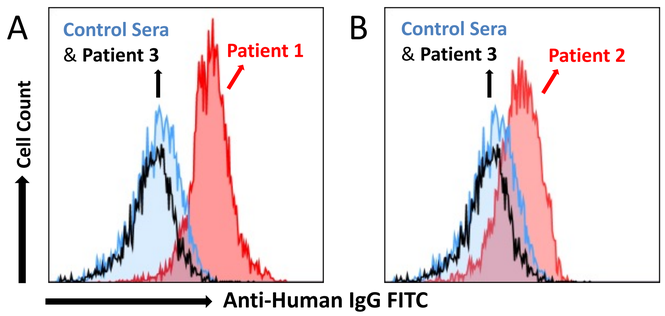


Figure 2. Donor platelets treated with sera from **Patient 1 (A)** and **Patient 2 (B)** show high levels of IgG binding compared to healthy control (blue).

IgG binding to donor platelets is attenuated when GPIbα is cleaved on OSGE-treated platelets

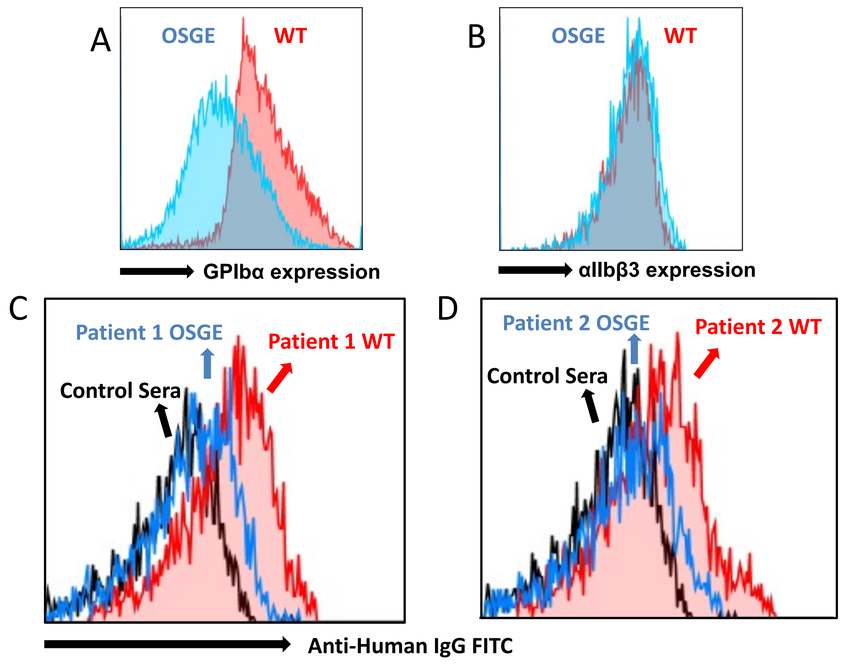


Figure 3. (A) GPIbα expression is significantly reduced on **O-sialoglycoprotein endopeptidase** (OSGE)-treated platelets compared to **wild-type** (WT). (B) αIIbβ3 expression is unchanged with OSGE treatment. IgG binding to donor platelets treated with patient 1 (C) and patient 2 (D) sera was attenuated OSGE-treated platelets compared to WT.

Sera from RM patients positive for anti-GPIbα IgG induce platelet activation and desialylation *in vitro*

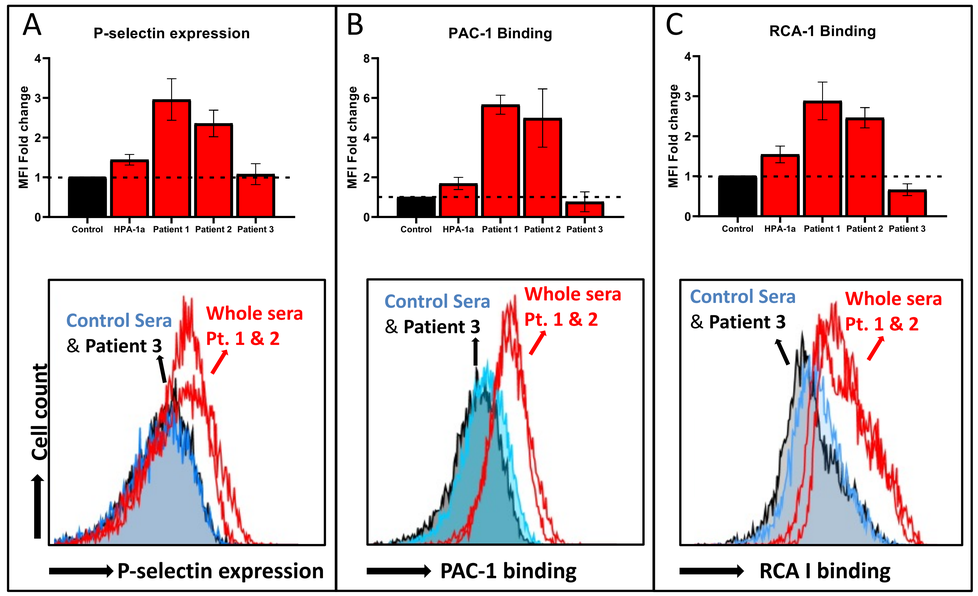


Figure 4. (A) Surface P-selectin expression, (B) PAC-1 binding, (C) and RCA-1 binding to gel-filtered human platelets treated with control and patient sera (1/100 dilution) measured by flow cytometry with representative histograms. MFI fold change was calculated from healthy control serum treatment in each individual assay. Error bars expressed as mean with SEM (n=3, different donor platelets).

IgG purified from RM patient serum induces platelet activation and desialylation *in vitro*

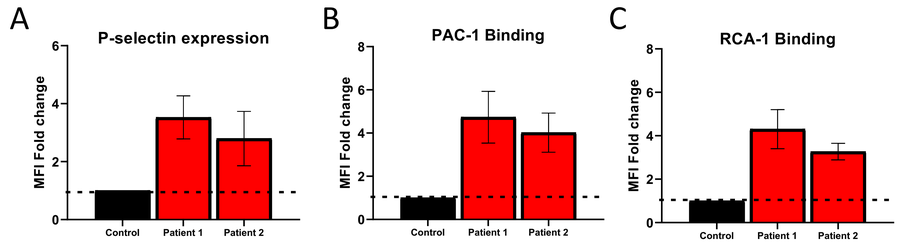


Figure 5. (A) Surface P-selectin expression, (B) PAC-1 binding, (C) and RCA-1 binding to gel-filtered human platelets treated with IgG (7ug) purified from patient serum measured by flow cytometry. MFI fold change was calculated from IgG control treatment in each individual assay. Error bars expressed as mean with SEM (n=3, different donor platelets).

Summary and Future Directions

- These findings provide the first evidence that maternal anti-GPIbα antibodies in FNAIT may cause miscarriage in humans.
- Additional testing is required on these patients to confirm diagnosis of FNAIT diagnosis (maternal/paternal genotyping for common HPAs in FNAIT and MAIPA using maternal/paternal platelets).
- Elucidate mechanism of miscarriage caused by anti-GPIbα in these patients.
- Study large cohorts of women with RM to determine prevalence of FNAIT in these patients.