A novel approach for generating monoclonal HPA-1a antibodies from single HPA-1a specific B-cells

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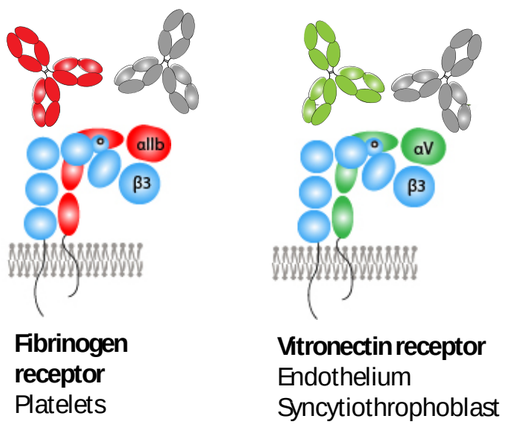
Introduction

- **Fetal and neonatal alloimmune thrombocytopenia (FNAIT)** is a rare but potentially serious bleeding disorder in pregnancy caused by maternal alloantibodies targeting paternally-inherited antigens on platelets of the fetus/newborn
- The majority of FNAIT cases is caused by antibodies targeting the **human platelet antigen (HPA)-1a**, located on the β3-integrin that is expressed on platelets, endothelial cells and placental syncytiotrophoblasts
- The factors contributing to severe bleeding are largely **unknown**
- However, recent observations have highlighted that **afucosylation** of HPA-1a-antibodies correlates with disease severity.
- In addition, some antibodies only recognize **aIIbb3** (unique to platelets, in red) whereas others only recognize **aVb3** (expressed on syncytiotrophoblasts and endothelial cells, in green) of which the latter type has been associated with enhanced bleeding.
- **Cloning of HPA-1a-antibodies** could provide valuable insights into anti-HPA-1a IgG biology and contribution to FNAIT disease severity.

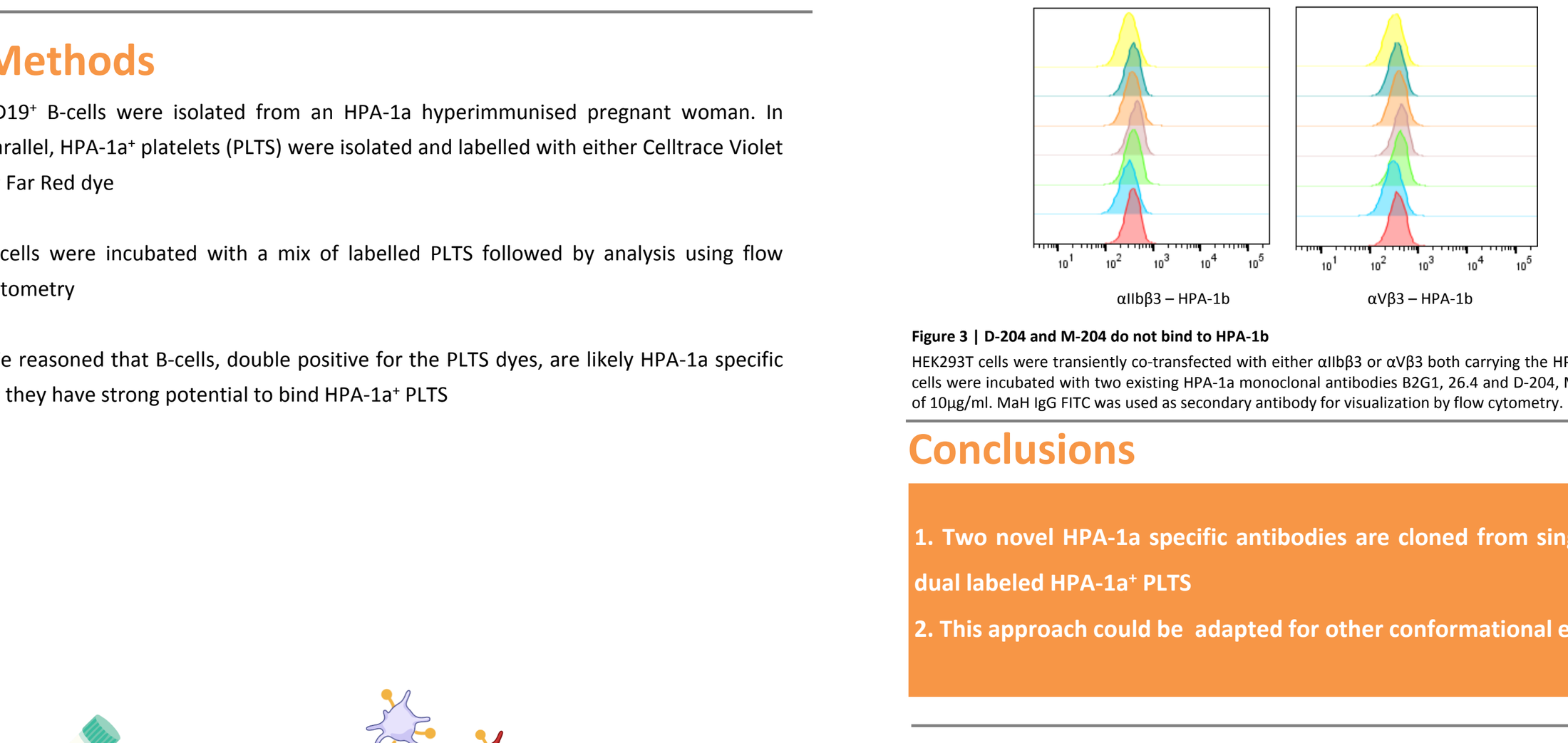


Research aim

Obtaining insights into anti-HPA-1a IgG biology and contribution to FNAIT severity via generation of monoclonal HPA-1a antibodies

Methods

- CD19⁺ B-cells were isolated from an HPA-1a hyperimmunised pregnant woman. In parallel, HPA-1a⁺ platelets (PLTS) were isolated and labelled with either Celltrace Violet or Far Red dye
- B-cells were incubated with a mix of labelled PLTS followed by analysis using flow cytometry
- We reasoned that B-cells, double positive for the PLTS dyes, are likely HPA-1a specific as they have strong potential to bind HPA-1a⁺ PLTS



Results

Single cell sorting of platelet reactive memory B-cells and antibody cloning

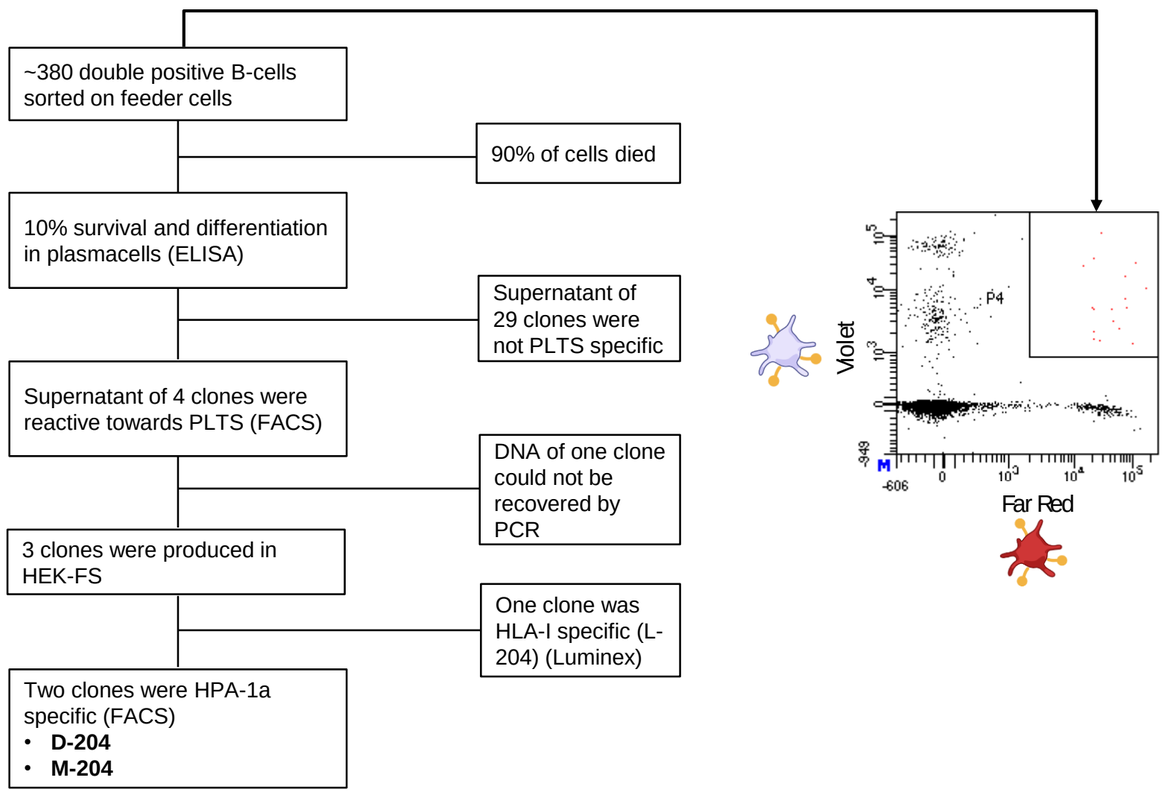


Figure 1 | Overview of single cell sort of platelet reactive memory B-cells and antibody cloning

Flowchart describing the process starting with double positive B-cell sorting (sorting gate depicted on the left, named P4) and ending with the generation of two novel HPA-1a antibody clones.

D-204 and M-204 are binding to HPA-1a in the context of both αIIbβ3 and αVβ3

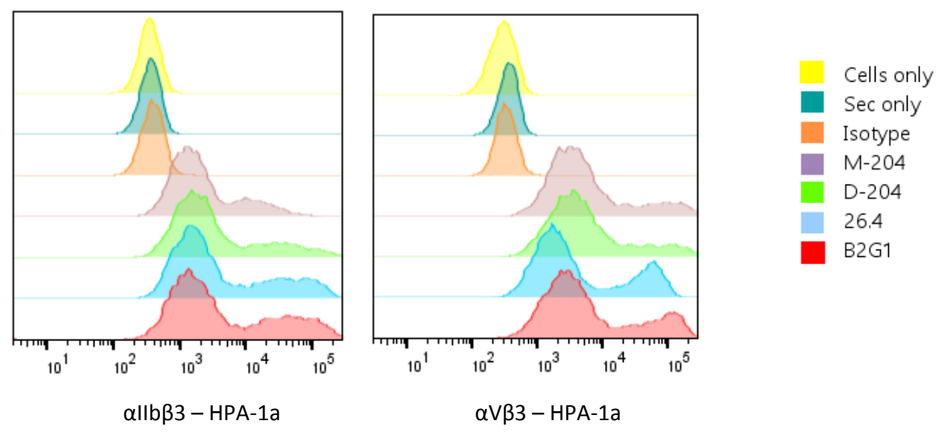


Figure 2 | D-204 and M-204 bind to HPA-1a in the context of αIIbβ3 and αVβ3

HEK293T cells were transiently co-transfected with either αIIbβ3 or αVβ3. After 48h, cells were incubated with two existing HPA-1a monoclonal antibodies B2G1, 26.4 and D-204, M-204 at a concentration of 10µg/ml. MaH IgG FITC was used as secondary antibody for visualization by flow cytometry.

No binding observed of D-204 and M-204 to HPA-1b

Figure 3 | D-204 and M-204 do not bind to HPA-1b

HEK293T cells were transiently co-transfected with either αIIbβ3 or αVβ3 both carrying the HPA-1b epitope. After 48h, cells were incubated with two existing HPA-1a monoclonal antibodies B2G1, 26.4 and D-204, M-204 at a concentration of 10µg/ml. MaH IgG FITC was used as secondary antibody for visualization by flow cytometry.

Conclusions

1. **Two novel HPA-1a specific antibodies are cloned from single B-cells using dual labeled HPA-1a⁺ PLTS**
2. **This approach could be adapted for other conformational epitopes**