Assessing Antibody Effects on Platelet Function: *In Vitro* Assays for De-Risking in Antibody Discovery and Development

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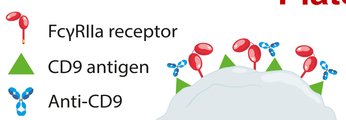
Background

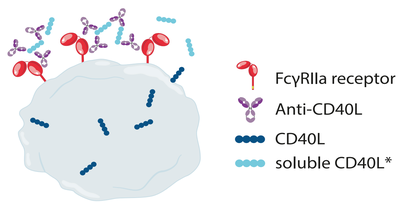
- Therapeutic antibodies (mAbs, ADCs, bispecifics, fragments) dominate new drug pipelines. Some antibodies and IgG-containing immune complexes can activate platelets via FcγRIIa, increasing thrombotic risk.
- **We evaluated which *in vitro* platelet assays are most applicable for detecting antibody- and immune complex-mediated effects on human platelet function.**

Mechanisms of Antibody Effects on Platelets



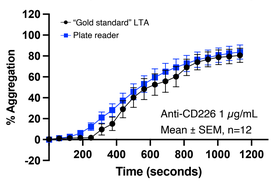
Human platelets express FcγRIIa. When an antigen is present, **IgG** antibodies such as anti-CD9, bind and **crosslink FcγRIIa** leading to platelet activation.

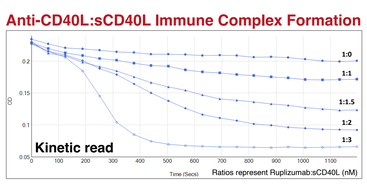
IgG-containing immune complexes, such as anti-CD40 with soluble CD40L, can also activate platelets through FcγRIIa signalling.



Methods

- Blood was collected from healthy human volunteers.
- Platelet activation was measured in whole blood (WB) and washed platelets (WP) by flow cytometry (P-selectin expression).
- Platelet aggregation was measured in platelet-rich plasma (PRP) and WP by changes in light absorbance over time.
- All assays were carried out on 96-well plates and measurements were performed in duplicate.





1 Results: Effects of Anti-CD9, Anti-CD226, and Anti-CD47 on Aggregation in Platelet Rich Plasma

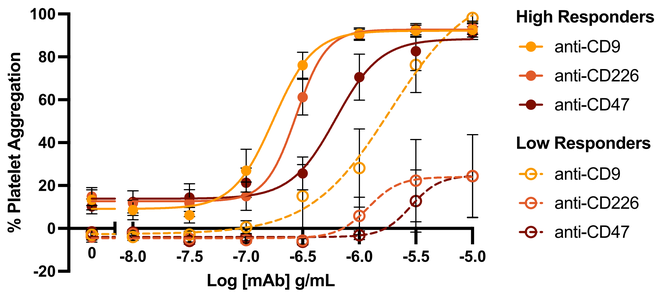


Figure 1. Aggregation assessed in PRP with anti-CD9, anti-CD226 and anti-CD47 (n=7-9; mean ± SEM).

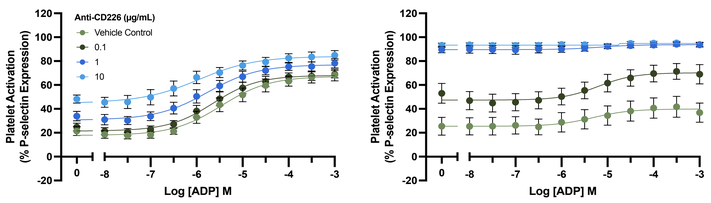
- Volunteers were classified as ***high*** or ***low*** responders based on aggregation with 1 μg/mL of anti-CD226: >80% aggregation = "***high responders"*** (Based on data from 36 volunteers).
- The three antibodies induced aggregation with varying potency; relative potency was consistent between *high* and *low* responders.

2 Results: Anti-CD226-Induced Platelet Responses Across Platelet Assays and Matrices

Platelet Activation

Whole Blood

Washed Platelets



Platelet Aggregation

Platelet-Rich Plasma

Washed Platelets

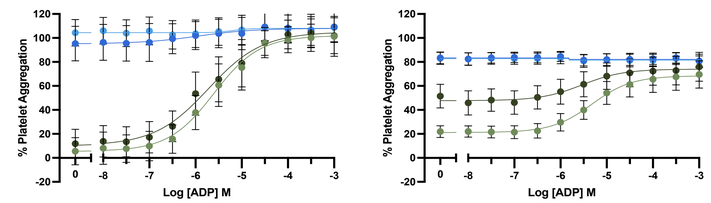


Figure 2. Activation (WB, WP) and aggregation (PRP, WP) co-stimulated with ADP and anti-CD226 in *high responders* (n=5-6; mean ± SEM).

- **Activation:** Anti-CD226 induced a modest effect in WB but a stronger, concentration-dependent activation response in WP.
- **Aggregation:** Both PRP and WP showed concentration-dependent aggregation.
- **Adenosine diphosphate (ADP):** Included to increase assay sensitivity, but little to no potentiation of anti-CD226-induced platelet aggregation and activation was observed.

3 Results: Anti-CD40L:sCD40L Immune Complexes Induce Platelet Aggregation

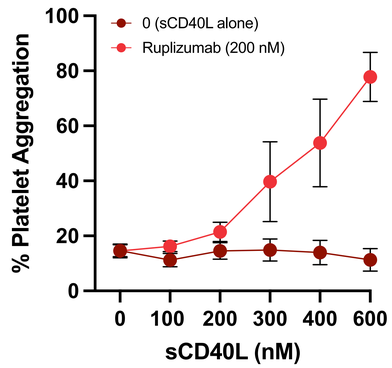


Figure 3. Anti-CD40L:sCD40L immune complex effects on platelet aggregation in WP obtained from *high responders* (n=5-7; mean ± SEM).

- Ruplizumab (an anti-CD40L), discontinued in clinical development due to thromboembolic risk *(Kawai et al., 2002)*, forms immune complexes (ICs) with soluble CD40L (sCD40L).
- These ICs induced concentration-dependent platelet aggregation in washed platelets, with the highest response observed at a 1:3 molar ratio. In contrast, no clear aggregation was observed in platelet-rich plasma (data not shown).

Conclusion

- Platelet activation and aggregation **assays performed in a 96-well plate format** can clearly demonstrate antibody-induced platelet responses; **anti-CD226** showed **concentration-dependent effects,** most pronounced in **washed platelets.**
- Platelet reactivity via **FcγRIIa** varies substantially among human volunteers; **anti-CD9, anti-CD226 and anti-CD47** showed **volunteer-dependent responses,** suggesting that recruiting ***"high responders"*** improves the detection of secondary pharmacology effects of antibodies on platelets.
- **Ruplizumab (anti-CD40L) and soluble CD40L IC, induced platelet aggregation**, reflecting the proposed FcγRIIa-mediated mechanism underlying its reported thromboembolic risk.
- ***In vitro* platelet assays can support early de-risking during antibody discovery and development.**

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