

P008 The role of human $\alpha_2\beta_1$ in platelet-collagen interaction under flow

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The two-site-two-step model of platelet activation by collagen suggests that $\alpha_2\beta_1$ is the primary adhesive collagen receptor, preceding glycoprotein (GP) VI-dependent platelet activation. However, GPVI may activate $\alpha_2\beta_1$, and $\alpha_2\beta_1$ or GPVI knockout studies have questioned the need for $\alpha_2\beta_1$ in either adhesion or signalling.

Our aim was to clarify the role of $\alpha_2\beta_1$ in human thrombus formation in relation to GPVI and GPIb-V-IX, by specific blockade of each receptor in flowing blood. Thrombus formation was assessed morphometrically, and procoagulant status of the deposited platelets was measured by Annexin V binding. Intracellular calcium ($[Ca^{2+}]_i$) was measured from single fluo-3 labelled collagen-adherent platelets.

Blocking α_2 (using mAb 6F1 or peptide GFOGER) and GPIb each diminished surface coverage ($-30 \pm 11\%$ and $-50 \pm 14\%$, respectively) while blocking GPVI (with scFv 10B12) eradicated aggregate formation, although a layer of single platelets remained. Blocking either collagen receptor reduced both $[Ca^{2+}]_i$ and annexin V-binding of adherent platelets, with GPVI contributing more to these signals. Finally, complete eradication of platelet deposition required combined blocking of both $\alpha_2\beta_1$ and GPVI, or GPIb and GPVI.

Together these data show that $\alpha_2\beta_1$ has both an adhesive and an activatory role in human thrombus formation.