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| Glycoprotein Ib and integrin  IIb 3 contribute to GPVI-dependent vWF-collagen induced thrombus formation under flowAbstract number: P1306Kuijpers\* M. J. E., Oury† C., Schulte‡ V., Feijge\* M. A. H., Hoylaerts† M. F., Lindhout\* T., Nieswandt‡ B., Heemskerk\* J. W. M.*†KU Leuven, Belgium; \*University of Maastricht, Netherlands; ‡University of Wuerzburg, Germany*Vessel wall damage causes exposure of thrombogenic collagen fibers, to which platelets adhere directly via their collagen receptors and indirectly via collagen-bound vWF. Initial tethering of the platelets to immobilized vWF/collagen via GPIb and collagen receptors (GPVI and integrin  IIb 3) is followed by platelet activation, aggregate formation and surface exposure of procoagulant phosphatidylserine (PS). Earlier we established that, under high shear conditions with mouse blood, GPVI is a prerequisite for stable platelet adhesion to collagen and all subsequent activation responses, while integrin  2 1 serves to facilitate and potentiate the GPVI-induced signalling. In the present study, we investigated the involvement of GPIb and  IIb 3 in vWF-collagen induced thrombus formation in flowing whole blood. Using video fluorescence microscopy, single platelet adhesion and activation as well as aggregate formation were monitored real-time during high shear perfusion experiments with a parallel-plate flow chamber. When control mouse blood was perfused over a vWF/collagen surface, platelets tethered, adhered to the surface and assembled into aggregates, with the adherent cells responding by a rapid increase in cytosolic [Ca2+]*i*. These platelets also showed an activated morphology and exposed procoagulant PS, as apparent from staining with Oregon Green 488-labelled annexin V. Delayed and reduced adhesion was observed when in wildtype blood the GPIb–vWF interaction was blocked with anti-GPIb antibody, p0p/B. The remaining adherent platelets formed small aggregates, were increased in [Ca2+]i and exposed PS. In the presence of saratin, a leech protein blocking the binding of vWF to collagen, similar results were obtained. When  IIb 3-mediated events were inhibited by a Fab fragment against  IIb 3 (JON/A), the number of adherent and subsequently activated platelets was substantially reduced, indicating that  IIb 3 is involved in stable adhesion to the surface. The platelet activation events were all GPVI-mediated, as blocking of the GPVI receptors with Fab fragments of JAQ1 antibody resulted in only few adherent platelets that were all low in [Ca2+]i without PS exposure. These data confirm the key position of GPVI in platelet adhesion to collagen and subsequent activation. In addition, they indicate that platelet–vWF interaction accelerates and potentiates the GPVI-mediated activation events which are dependent on both GPIb and integrin  IIb 3. |