Figure S1. Changes in bacteria concentration affect the lag time for onset of platelet aggregation. (A) Platelet aggregation reactions were performed in plasma with different concentrations of *S. sanguinis* 133-79 as indicated. Platelet-rich plasma (PRP) was 80% of the final volume and was kept constant in all reactions. (B) Platelet aggregation reactions were performed in plasma with different concentrations of *S. gordonii* DL1 as indicated. PRP was 80% of the final volume and was kept constant in all reactions. (C) Platelet aggregation reactions were performed in plasma by crosslinking FcγRIIA receptor. Platelets were pre-incubated for 3 min with 2 μg/mL or 4 μg/mL monoclonal antibody IV.3 followed by addition of 15 μg/mL or 30 μg/mL anti-mouse IgG F(ab’)2, respectively.
Figure S2. Related to Figures 1 and 2. Role of αIIbβ3, FcγRIIA and Src- and Syk-tyrosine kinases in bacteria-induced platelet activation. (A) Effect of αIIbβ3 inhibitor Integrilin on bacteria-induced platelet aggregation. Platelet-rich plasma (PRP) was incubated for 2 min with Integrilin (9 μM) or vehicle (PBS) prior to addition of bacteria or agonists, and platelet aggregation was monitored. Representative traces of three independent experiments per bacterial strain are shown. Lag times for the reactions shown are: S. sanguinis 133-79, 2 min 30 sec; S. aureus Newman, 4 min; S. gordonii DL1, 11 min; S. oralis CR834, 7 min 45 sec; S. pneumoniae IO1196, 6 min 30 sec. (B) Effect of anti-FcγRIIA mAb IV.3 on bacteria-induced platelet aggregation. PRP was incubated for 10 min with mAb IV.3 (10 μg/mL) or vehicle (PBS) prior to addition of bacteria or agonists, and platelet aggregation was monitored. Representative traces of a total of three independent experiments per bacterial strain are shown. Lag times for the reactions shown are: S. sanguinis 133-79, 3 min; S. aureus Newman, 2 min 30 sec; S. gordonii DL1, 7 min; S. oralis CR834, 9 min 30 sec; S. pneumoniae IO1196, 6 min. (C) Effect of the Src-tyrosine kinase inhibitor dasatinib on bacteria-induced platelet aggregation in plasma. PRP was incubated for 2 min with dasatinib (4 μM) or vehicle (DMSO)
prior to addition of bacteria or agonists, and platelet aggregation was monitored. Representative traces of three independent experiments per bacterial strain are shown. Lag times for the reactions shown are: S. sanguinis 133-79, 2 min 30 sec; S. aureus Newman, 2 min; S. gordonii DL1, 14 min; S. oralis CR834, 7 min 15 sec; S. pneumoniae IO1196, 8 min. (D) Effect of Syk-tyrosine kinase inhibitor PRT-060318 on bacteria-induced platelet aggregation in plasma. PRP was incubated for 2 min with PRT-060318 (10 μM) or vehicle (DMSO) prior to addition of bacteria or agonists, and platelet aggregation was monitored. Representative traces of three independent experiments per bacterial strain are shown. Lag times for the reactions shown are: S. sanguinis 133-79, 2 min 10 sec; S. aureus Newman, 6 min; S. gordonii DL1, 10 min; S. oralis CR834, 2 min; S. pneumoniae IO1196, 11 min.
Figure S3. Related to Figure 4. Effect of secondary mediators on bacteria-induced platelet aggregation. Platelet-rich plasma was incubated for 2 min with COX-inhibitor indomethacin (10 μM), ADP-receptor P2Y12 inhibitor cangrelor (1 μM), or vehicle (DMSO) prior to addition of bacteria or crosslinked mAb IV.3, and platelet aggregation was monitored. Representative traces of three independent experiments per bacterial strain are shown. Lag times for the untreated reactions shown are: *S. sanguinis* 133-79, 3 min; *S. aureus* Newman, 3 min; *S. gordonii* DL1, 11 min; *S. oralis* CR834, 3 min 30 sec; *S. pneumoniae* IO1196, 9 min 10 sec.
Figure S4. Related to Figure 7. Effect of heparin on bacteria-induced platelet aggregation. Aggregation reactions were performed in plasma in the presence of varying doses of unfractionated heparin (UFH): 6 µg/mL (1.25 U/mL), 600 µg/mL (125 U/mL), 3000 µg/mL (625 U/mL). UFH was pre-incubated with platelets for 2 min before adding *S. gordonii* DL1 (A), TRAP (B), or buffer (C). Representative traces of six independent experiments for *S. gordonii* DL1 and three independent experiments for TRAP and buffer are shown.
Figure S5. Proposed model for human platelet aggregation in response to adhesive bacteria. **Adhesion step.** Binding of bacteria to the platelet surface is mediated by a variety of strain-specific pathways and usually involves multiple molecular interactions (A). **Activation step.** A shared pathway is involved in subsequent platelet activation that includes engagement of both FcγRIIA and αIIbβ3 receptors (B). While FcγRIIA engagement is achieved by plasma IgG bound to bacteria, the way in which the initial activation of αIIbβ3 takes place is strain-specific. For most bacteria, “inside-out” activation of αIIbβ3 might be achieved by IgG-dependent FcγRIIA signaling, as well as by signaling initiated from other strain-specific molecular interactions. In the case of *Streptococcus gordonii*, αIIbβ3 engagement is facilitated by direct binding of the bacterial protein PadA1. A third example is that of *S. aureus* Newman, in which the bacterial protein ClfA/B is known to bind to fibrinogen and hIgG simultaneously, which then leads to crosslinking of αIIbβ3 and FcγRIIA2,4. In all these cases, initial engagement of FcγRIIA and αIIbβ3 leads to weak activation, most likely via cooperative integrin/FcγRIIA ITAM signaling5,6, with granule secretion and secondary mediators release. At this stage, feedback mechanisms are key in order to achieve full activation and aggregation. In some bacteria, FcγRIIA-mediated activation is reinforced by a pathway that depends on PF4.
secretion from α-granules (C.1). Although the detailed mechanism is unknown, several possibilities exist such as reinforcement of FcγRIIA signaling by bacteria-bound PF4/IgG complexes, synergy with direct interaction of PF4 with platelet receptors, or charge interactions promoting bacteria-platelet cell contact. In this context, platelet secondary mediators ADP and TxA2 might still contribute to activation by reinforcing “inside-out” αIIbβ3 activation and α-granule secretion, leading to a strong positive feedback cascade. For some bacteria such as Streptococcus oralis, aggregation seems to be independent of PF4. This strain might be using secondary mediators ADP and TxA2 very efficiently to feedback on αIIbβ3 activation and FcγRIIA signaling (C.2.). **Aggregation step.** While initial activation of αIIbβ3 only takes place on those platelets that are binding to bacteria, for aggregation to occur, secondary mediators ADP and TxA2 are essential for αIIbβ3 “inside-out” activation in the neighboring (bacteria-free) platelets. Activated αIIbβ3 then binds soluble fibrinogen that bridges platelets forming an aggregate (D). Therefore, the release of secondary mediators starts a process in which integrin αIIbβ3 activation and release of ADP/TxA2 feedback on each other. At this stage, FcγRIIA ITAM signaling is being reinforced (in bacteria-free platelets) as a consequence of integrin αIIbβ3 engagement and contributes to a cooperative integrin/ITAM signaling⁵,⁶.


