IGFBP-6 REGULATES PLATELET PROCOAGULANT ACTIVITY AT THE INTERFACE BETWEEN INFLAMMATION AND COAGULATION

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Background and Aims. Insulin-like growth factor binding protein-6 (IGFBP-6) is a multifunctional protein expressed in several immune and stromal cell types that plays key roles in inflammation and tissue repair. We previously described its role in fibrosis, showing that IGFBP-6 promotes immune cell chemotaxis, reactive oxygen species (ROS) production, and fibroblast activation. However, IGFBP-6 also reduces vascular inflammation and atherosclerosis. Platelets, beyond their canonical hemostatic function, actively participate in thrombo-inflammatory conditions in particular through a specialized subpopulation expressing procoagulant activity. No studies so far have explored the role of IGFBP-6 in regulating platelet activation. We aimed to study whether IGFBP-6 modulates platelet activation and procoagulant activity.

Methods. Spatial proteomics of blood cell antigens was carried out by molecular pixelation. Platelet rich plasma was incubated with IGFBP-6 at three different concentrations (0.1 – 1 – 10 ug/ml). Expression of the major platelet glycoproteins, platelet P-Selectin expression and aIIbb3 activation were assessed by flow cytometry. Procoagulant platelets were detected by measuring phosphatidylserine (PS) exposure and tissue factor (TF) expression by flow cytometry. Platelet extracellular vescicles (EVs) were counted in the supernatants of platelets stimulated with collagen and thrombin by flow cytometry, morphological gate was set using a mix of fluorescent beads of varied diameters. Platelet-leukocyte aggregates were assessed by flow cytometry. ROS production by platelets was assessed by flow cytometry using the H2DCFDA fluorescent probe.

Results. Spatial proteomics revealed decreased CD41 expression after incubation of a leukocyte–platelet suspension with IGFBP-6 while the pre-incubation of platelet rich plasma (PRP) with IGFBP-6 did not affect the expression of the main platelet receptors, except for a slight decrease in GPIba (Figure 1A). Incubation with IGFBP-6 determined also an increase of P-Selectin expression (Figure 1B). IGFBP-6 induced an increase in Annexin V binding, indicating an enhanced exposure of procoagulant phosphatidylserine on the platelet surface (Figure 1C). Consistently, tissue factor expression was increased (Figure 1D), together with a higher release of extracellular vesicles in the supernatant (Figure 1E).Finally, IGFBP-6 significantly increased platelet-dependent FXa generation, thereby enhancing the procoagulant activity of platelets (Figure 1F). Pre-incubation with IGFBP-6 increased ROS levels in platelets (Figure 1G), consistent with a cellular stress response known to favor the development of the procoagulant platelet phenotype. Finally, we observed a significant increase of platelet-neutrophil aggregates (Figure 1H).

Conclusions. Our findings identify IGFBP-6 as a novel regulator of platelet procoagulant activity, supporting a model in which IGFBP-6, at concentrations similar to those found at sites of inflammation, contributes to the fine-tuning of the platelet thromboinflammatory responses. Pre-incubation with IGFBP-6 increased ROS levels in platelets, consistent with a cellular stress response known to favor the development of the procoagulant platelet phenotype. By enhancing platelet–neutrophil crosstalk, IGFBP-6 may contribute to the amplification of thromboinflammatory responses at sites of vascular injury or inflammation.

