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Review

Platelet populations and priming in hematological diseases

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ABSTRACT

In healthy subjects and patients with hematological diseases, platelet populations can be distinguished with different response spectra in hemostatic and vascular processes. These populations partly overlap, and are less distinct than those of leukocytes. The platelet heterogeneity is linked to structural properties, and is enforced by inequalities in the environment. Contributing factors are variability between megakaryocytes, platelet ageing, and positive or negative priming of platelets during their time in circulation. Within a hemostatic plug or thrombus, platelet heterogeneity is enhanced by unequal exposure to agonists, with populations of contracted platelets in the thrombus core, discoid platelets at the thrombus surface, patches of ballooned and procoagulant platelets forming thrombin, and coated platelets binding fibrin. Several pathophysiological hematological conditions can positively or negatively prime the responsiveness of platelet populations. As a consequence, *in vivo* and *in vitro* markers of platelet activation can differ in thrombotic and hematological disorders.

1. Introduction

Individual platelets interact in numerous ways with the vessel wall or adherent blood cells. This versatility is fundamental to the role of platelets in a wide range of (patho)physiological processes, ranging from vascular repair, hemostasis and thrombosis, to inflammation progression, innate immunity and tumor metastasis. In the past years, evidence has been accumulating that circulating platelets are markedly heterogeneous in properties, which has led to the suggestion that identifiable populations of platelets with specialized response spectra are best suited for specific roles. In the present paper, we resume the current evidence for heterogeneity in terms of composition and functions of platelets during their formation from megakaryocytes, when circulating over time in the circulation, and once adhered to a vessel wall. We further describe how various physiological and pathophysiological conditions can change or prime the responsiveness of circulating platelets, and hence alter the distribution of platelet populations. We finally define how *in vitro* and *in vivo* markers of platelet activation phenotypes can be judged in relation to thrombotic and hematological disorders.

2. Intrinsic factors of platelet heterogeneity

Platelets from a given subject, healthy or diseased, greatly vary in receptor expression levels and markedly diverge in responsiveness once activated. Different populations of activated platelets can be distinguished, which can differently interact with the inflamed or injured vessel wall and differently support hematological processes. This heterogeneity is explained by several intrinsic factors, including variability of clonal megakaryocytes, unequal division of megakaryocyte-derived proplatelets, and modifications upon ageing of the newly formed platelets.

2.1. Heterogeneity between megakaryocytes and platelets

Heterogeneity between megakaryocytes, whether or not linked to a specific niche in the bone marrow or lungs, is a likely cause of inter-platelet variability, although there is only limited literature available on this subject. Several authors have described that polyploid megakaryocytes, either cultured from $CD34^+$ hematopoietic stem cells or derived from immortalized cell lines, considerably differ from cell to cell, in terms of levels of cytoplasmic and membrane proteins, as well as in agonist-induced Ca^{2+} transients [1–3]. Recently, this was demonstrated also for megakaryocytes derived from single-cell clones of

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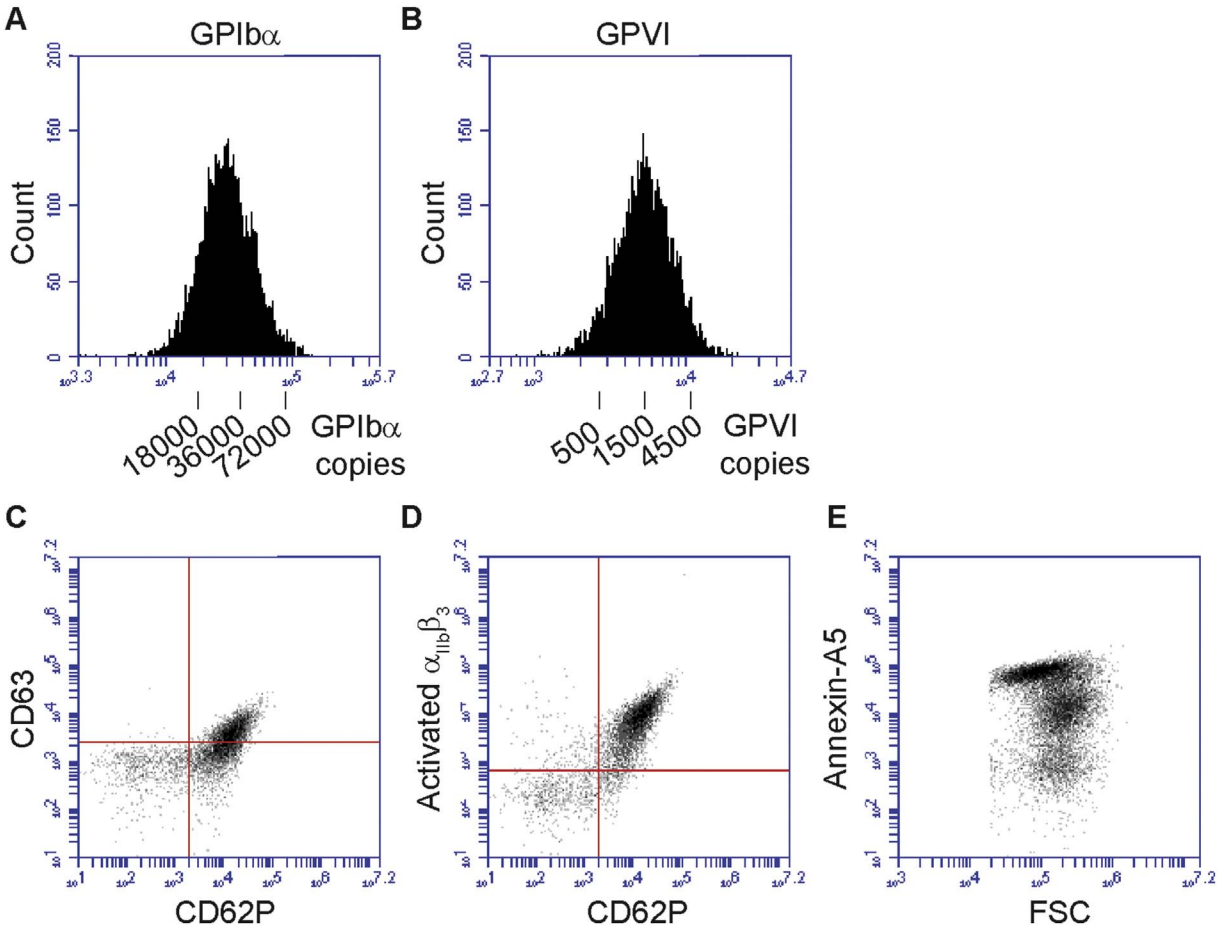

Fig. 1. Intrinsic heterogeneity in platelet composition and functions.
(A, B) Differential expression of key adhesive receptors in the total platelet population, immunologically stained for GPIbα or GPVI. Indicated in the flow cytometric histograms are the estimated copy numbers, based on proteome analysis [128]. (C, D) Platelets stimulated with a GPVI agonist immunologically stained for CD62P in combination with CD63; or CD62P in combination with a marker for activated integrin $\alpha_{IIb}\beta_3$. Shown is the presence of different platelet populations with alpha granule secretion ($CD62P^+$) with or without dense granule secretion or $\alpha_{IIb}\beta_3$ activation. (E) Two apoptotic platelet populations after stimulation with BH3 mimetic ABT-737, displaying low or high phosphatidylserine (PS) exposure.

forward-programmed human pluripotent stem cells. After re-programming, individual megakaryocytes of the same clone showed a 100-fold difference in expression levels of common receptor proteins, such as glycoprotein (GP)Ibα, GPVI and integrin $\alpha_{IIb}\beta_3$, as assessed by flow cytometry [4]. No studies are yet available to demonstrate whether distinct megakaryocytes (in the bone marrow) also yield different types of platelets. However, one can expect that single platelets 'inherit' at least in part their expression profiles of receptors and other signaling molecules from their precursor megakaryocyte. It has been shown that when the environment of megakaryocytes changes (*e.g.* upon inflammation or diabetes), the transcriptome of platelets is influenced as well [5,6]. For instance, patients diagnosed with the autoimmune disease systemic lupus erythematosus (SLE) may have an altered platelet transcriptome. The changes in platelet mRNA and protein levels were linked to increased procoagulant activity and platelet-monocyte interactions in SLE patients [7].

Proplatelets are formed from the demarcation compartment of megakaryocytes by pinching off the cytosol containing cell membrane compartment and organelles [8]. Although studies are lacking, it can be conceived that part of the heterogeneity between platelets may come from unequal retention or re-distribution of the cellular components from the mother cell, including surface receptors, actin and tubulin cytoskeletons, signalosomes, internal membrane vesicles, secretory granules, ribosomes, and mitochondria.

Clear evidence for heterogeneity in platelet size and volume comes from flow cytometric profiles and blood count histograms. Forward side scatter plots (flow cytometry) point to a considerable variation in size, which is in agreement with the relative large distribution width of the platelet volume (blood cell count). Sizable heterogeneity between platelets is also detectable by quantifying the abundantly expressed receptors. Thus, flow cytometric profiles of platelets that are stained with fluorescent antibodies against integrin β_3 (CD61) point to a large inter-platelet variation in expression levels of this integrin [9,10]. Similarly, staining for GPIbα or GPVI gives histograms, which show an about 10-fold difference in fluorescence intensity (indicative of expression level) of platelets in the lower and upper 10% percentiles (Fig. 1A–B). Evidence for signaling heterogeneity comes from recordings of the Ca^{2+} responses of single platelets from an arbitrary donor, which appear to vary greatly in the type of transient Ca^{2+} fluxes after stimulation *via* ADP, thrombin or collagen receptors [11].

2.2. Populations of activated platelets

Platelets, upon activation by agonists, are known to form populations with different surface properties [10]. For instance, platelets stimulated with a maximal dose of a GPVI agonist showing CD62P expression (marker of α-granule secretion) only display CD63 expression to a certain degree (marker of δ-granule and lysosome release), while the majority of platelets that express CD62P do have activated integrin $\alpha_{IIb}\beta_3$ (Fig. 1C–D). Inter-platelet heterogeneity in granule release has also been observed by electron microscopy [12]. Thus, secretion of granule content can occur in two different ways: single granule