Platelet functional responses and signalling: the molecular relationship. Part 1: responses.

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Abstract

Blood platelets are small anucleated cells whose main function is to form a plug upon vascular damage to stop bleeding. This role involves a number of functional responses induced by different agonists and coordinated by an intricate network of signal transduction pathways. Understanding this network is vital from both basic research point of view and for the purposes of drug target identification in thrombosis and hemostasis. This review series will focus on the regulation of platelet signalling, on tracking the molecular relationship between receptor activation and functional responses, and on the networking aspects of these pathways. The present paper, first one out of two, focuses on the description of platelet functional responses and of the conditions for their triggering.

Keywords: hemostasis, thrombosis thrombus formation, platelet activation, platelet intracellular signaling.

Introduction

The process of hemostasis is a spatiotemporally regulated physiological response [1] aiming to stop bleeding upon vascular injury. Blood platelets play a critical role in this response. They actually have a number of critical roles in human physiology, which include formation of the hemostatic plug upon injury [2], acceleration of the membrane-dependent reactions of blood coagulation [3,4], maintenance of vascular integrity [5–7], modulation of immune responses [8–10], tissue growth and regeneration [11,12]. In order to perform these urgent functions when and where it is needed, platelets rely on their huge network of receptors and signal transduction pathways.

The functional responses of platelets to stimulation are numerous [13] (Fig. 1), and the number of positive and negative stimuli to be processed by the

Figure 1. Scheme of the main platelet functional responses to activation (all of them could be blocked by inhibition). Blue ovals represent functions; yellow ovals represent blood coagulation factors, that can assemble on the surface of procoagulant platelets; GPIIb-IIIa is the main (but not only) platelet integrin, which determines platelets aggregation; GPIb represents receptor complex for von Willebrand factor, which determines the initial adhesion of platelets to the site of injury.

cellular signalling networks in order to make correct decisions is likewise not small [14]. Additionally, a number of platelet inhibitory signals has been identified in recent years [15,16]. As a result, the network of platelet signal transduction that is initiated by at least ten major receptors, goes through multiple interconnected pathways and ends up with various responses of different degree might appear quite terrifying (Fig. 2).

When these pathways are reviewed and discussed, they are usually analyzed not as a network but rather as a simpler cause-and-effect chain. For example: activator X stimulates receptor Y leading to an increase of a secondary messenger Z that mediates further downstream events, including responses “1”, “2”, and “3”. The interplay of different receptors that could work cooperatively or downregulate each other is often overlooked. Interestingly, in other fields like immunology, terms like “co-activator” or “co-receptor” have already become accepted [17,18]. Likewise, transformation of the signal along the

signalling pathway, different dose-dependence for different responses to the same agonist caused by it, principles of signal encoding and de-coding are rarely discussed even beyond the platelet field [19] and almost never for platelets.

Another not usually discussed point in platelet receptor-response relationship is the mechanosensitivity of these cells, whose whole life is spent in fast streams of blood. There is enough evidence that platelets can “sense” the shear rate of the blood stream and even turbulence of the flow [20–23]. However, this question will be discussed in the next part of this review, as here our goal is to address issues of responses rather than receptors. We shall highlight the main principles of platelet signal processing and decision-making, with the focus not on the description of signalling pathways but rather on the attempt to characterize how they work together to achieve different sets of physiologically relevant responses depending on the situation.

Here we shall mostly focus on the platelet responses relevant to hemostasis, because they are believed to be more comprehensively studied. The minimal set of main platelet functions implemented in response to injury usually includes integrin $\alpha IIb\beta 3$ activation [24], dense granule release [25], alpha-granule release [26], thromboxane A2 synthesis, shape change, procoagulant activity [27], and contraction [28] (Fig. 1). The initial adhesion to von Willebrand factor mediated by glycoprotein Ib is not included among them because it does not require platelet activation [29]. It should be noted that these functions are probably not equally important, or rather not all of them were reliably shown to contribute to hemostasis.

Integrin activation is probably the one response

believed to be the most vitally important as demonstrated by severe bleeding in their deficiency, Glanzmann's thrombasthenia [30]. Platelets have about 100000 integrin $\alpha\text{IIb}\beta 3$ molecules per cell that are capable to radically increase their affinity to von Willebrand factor and fibrinogen thus directly mediating aggregate formation [24]. All platelet agonists, including biomechanical platelet activation via the vWf-glycoprotein Ib axis, are believed to cause integrin activation to some degree [31]. This response is gradual, probably with the intermediate activation states [21]. The immediate cause of the integrin transition between the states is formation of a large cytoskeleton-associated complex of proteins at the cytosolic side of the plasmatic membrane. The critical structural role in this complex is played by proteins talin-1 [32] and kindlin-3 [33], while the signal transduction switch triggering its formation is believed to be small soluble cytosolic GTPase, Rap1-GTP [31,32,34]. In addition to $\alpha\text{IIb}\beta 3$, platelets have a number of other integrins specific to collagen, fibronectin, laminin and other molecules; their significance is less certain, while the activation mechanisms appear to be similar [31,35,36].

Dense granule release

Dense (or delta-, δ -) granules are platelet-specific organelles released upon stimulation [25,37,38]. They contain mostly low-molecular-weight compounds, the major of them being ADP able to activate other platelets [39]. Another component that has been recently attracting a lot of attention is polyphosphate reported to have a number of potential roles in the coagulation cascade [40,41]. The functional role of other dense granule components such is ATP, serotonin, calcium is much less clear [41,42]. In contrast to integrin activation, dense granule release is not easily mediated by all activators; for example, ADP itself causes it only to a very minor degree [43]. The intracellular mechanism of differential dense granule release is still controversial [37,38] and will be discussed in the next part of this Review. The essential signal transduction switch mediating these processes is believed to be various isoforms of protein kinase C [44,45] and phosphatidylinositol content of their membranes [46,47].

Alpha granule release

Alpha (α -) granules are another type of platelet not necessarily spherical [48] intracellular vesicles released upon activation, which have specific cargo composed mostly of proteins including fibrinogen, von Willebrand factor, factor V, C1 inhibitor, growth factors, and other molecules [26,49]. Membranes of alpha-granules have additional integrins and P-selectin. Some of the studies suggest that contact-pathway-activating components of platelets are associated with alpha granules, and not dense

granules [50]. There is evidence that there are subtypes of alpha-granules with different content released upon different stimulation [51]. The proteins in alpha-granules are involved in platelet aggregation, coagulation, angiogenesis, immunity, inflammation, vessel wall integrity maintenance/repair and other vital processes [5,52,53]. However, the only component of alpha granules whose hemostatic function is well established is factor V, which is present there in significant quantities and in partially activated form, and whose importance is confirmed by patient and animal studies [54,55]. Alpha-granule proteins remain associated with procoagulant platelets [56] by forming a fibrin polymerization-dependent and transglutaminase-dependent "cap" [57]. Its function is not completely clear, although they were shown to mediate procoagulant platelet attachment to aggregates [58]. The release of alpha granules is believed to be produced by almost all platelet activators [59–61].

Procoagulant activity.

Acceleration of the membrane-dependent reactions of blood coagulation occurs on the surface of the specific platelet subpopulation, called procoagulant platelets; recent data suggest that there is even a special dedicated cell structure for this [62]. Therefore, this response is a bit different from other responses, which are more uniformly distributed among platelet. Interestingly, integrins are inactivated in this subpopulation at some timepoint of procoagulant platelet formation [63]. The procoagulant platelets are formed upon strong stimulation with thrombin or glycoprotein VI agonists as a result of necrosis process [64], caused by accumulation of calcium by the mitochondria [65,66]. However, other activators such as ADP are capable of fine-tuning procoagulant platelet formation [67].

Other responses.

The earliest platelet response to any activation, even very slight one, is shape change from an ellipsoid to a sphere associated with reorganization of peripheral microtubule ring [68,69]. However, functional importance of this transition is not clear. The next level of shape change is formation of lamellipodia and filopodia leading to increased surface of contact with other platelets and vascular wall [70]. These stages are caused by actin cytoskeleton rearrangements [70,71], potentially induced by thrombin, followed by ADP, but less by collagen [71]. Platelets ability to change their shape significantly affects packing of platelets within thrombus, and results in increased platelet density and decreased porosity [72]. However, the necessity of this response is unclear, as the disregulated actin polymerization in patients with Wiscott-Aldrich syndrome cause smaller platelet formation rather than platelet dysfunction

Figure 3. Platelet functional responses within arterial thrombus. The thrombus is conditionally divided into two regions, the “core” and the “shell” [86]. Platelet cytosolic calcium concentration is shown by shades of red, circles indicate oscillations. The intensity of calcium signaling is supposed to reflect the level of platelet activation. Cell trajectories in the bloodstream are demonstrated by means of gradient yellow. (A) Thrombus “core” is composed of degranulated platelets that have formed filopodia and lamellipodia and slightly less activated platelets closely adjacent to them. These activated platelets release the contents of dense and alpha granules (shown by arrows) and thromboxane (shown in purple dots) in the lumen between the cells. Integrins bind fibrinogen, thus forming “bridges” between neighboring platelets (orange lines). (B) Procoagulant platelets provide their surface for membrane-dependent reactions of plasma coagulation ultimately catalyzing thrombin (IIa) formation. Over time, they’re pushed up to the “shell” by the “core” platelets. (C) “Shell” platelets are disc-shaped and contain a large number of granules of both types. They’re the last ones that have settled on the thrombus and are connected to it by integrin-fibrinogen-integrin bridges. Abbreviations: plt – platelet, TxA2 – thromboxane A2, IIa – blood coagulation factor IIa.

[66,73].

Another early and easily induced response is thromboxane A2 synthesis mediated by phospholipase A2, cyclooxygenase (mostly, COX-1) and thromboxane synthase [74]. Thromboxane A2 is a lipid eicosanoid acting upon platelets (like ADP) [75]. It is still not clear whether thromboxane is important for the same platelet that has released it. At the first step, arachidonic acid is produced from different membrane phospholipids, and the second step is that of thromboxane A2 formation. Interestingly, the regulated step is the first one, that of phospholipase A2. That is why resting platelets may produce thromboxane A2 upon addition of just arachidonic acid [76]. Platelets have two phospholipases A2, C- and I- isoforms. The former is activated by slight increase of cytosolic calcium concentration, and that is probably why all classic platelet activators cause thromboxane synthesis, while the latter is activated independent of calcium and its role in platelets is not yet established [77]. COX-1 is inactivated by aspirin and is one of the main targets for anti-aggregation

therapy, suggesting importance of thromboxane A2 in arterial thrombus formation [78].

Platelet contraction, sometimes called “retraction”, occurs at a later stage of plug/thrombus formation, and is mediated by classic actin/myosin mechanisms [79]. Contraction is observed in platelets activated by a variety of agonists. The studies of signal transduction pathways leading to clot contraction are lacking, although integrin “outside-in” signalling is known to be crucial for this process [80]. Also, a calcium-dependent enzyme, myosin II light chain kinase (MLCK) is involved during clot retraction [81]. Recent studies suggested that contraction may play a role in re-arranging thrombus architecture, e.g. expelling procoagulant platelets to the platelet thrombus periphery to form fibrin there [27], organizing ischemic thrombi [82], or increasing platelet concentration at the fibrin clot periphery, also mechanically increasing local fibrin density [83].

Conclusions

Some of the platelet responses are critical for

platelet plug formation (integrins, dense granules, thromboxane A₂), while others are involved in blood coagulation (procoagulant activity, alpha-granules); yet other responses while being important have less clearly defined area of applicability, such as contraction and shape change.

These responses are spatiotemporally organized: they occur at different stages in different parts of the thrombi (Fig. 3). Integrin activation of different degree is responsible for forming the whole body of the thrombus, both the dense parts of highly activated platelets and loosely activated external regions; dense granule release is vital for the activation of platelets distal from the damaged region; alpha-granules and procoagulant surfaces are vital for fibrin formation and thrombus solidification.

In order to achieve this, platelet activation responses form a hierarchy of strength: some of them are induced by all or most agonists (alpha granule release, weak integrin activation), while others require potent stimulation (procoagulant activity), with all shades of gray in-between. Some of the responses are gradual meaning that the response is gradually increased within a wide range of activation conditions (dense granules), while others are trigger-like meaning that they either completely implemented or not at all (alpha granules).

The in-depth analysis of the signalling pathways interaction, encoding and decoding of information, which give rise to these properties, are considered in the next part of this review. As a post-note it is worth mentioning that the computational biology approach [14,84,85] greatly facilitates our understanding of the platelet receptor-function relationship.

Author Contributions

A.N.S. drew the figures, wrote the text and edited the paper; M.G.S. drew the figures; M.A.P. supervised the project, wrote the text and edited the paper. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest

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