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REVIEW ARTICLE

Coated-platelets: an emerging component of the procoagulant response

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Summary. Coated-platelets, formerly known as COAT-platelets, represent a subpopulation of cells observed after dual agonist stimulation of platelets with collagen and thrombin. This class of platelets retains on its surface high levels of several procoagulant proteins, including fibrinogen, von Willebrand factor, fibronectin, factor V and thrombospondin. Coated-platelets also express surface phosphatidylserine and strongly support prothrombinase activity. Retention of α-granule proteins on the surface of coated-platelets involves an unexpected derivatization of these proteins with serotonin and an interaction of serotonin-conjugated proteins with serotonin binding sites on fibrinogen and thrombospondin. This review will also detail experimental systems where coated-platelets are generated as a result of other agonist(s). Finally, the putative physiological consequences of coated-platelet formation will be discussed.

Keywords: COAT-platelet, fibrinogen, platelet, serotonin, thrombospondin, transglutaminase.

Introduction

Platelets have two critical functions: adhering at a site of vasculature injury, and providing a negatively charged cell surface for thrombin generation. Most investigators have assumed that all platelets are equally capable of fulfilling either essential role; and with regard to adhesion/aggregation, there is no reason to doubt that assumption. However, recent observations suggest that some platelets are more capable concerning the other function: promoting thrombin generation. The purpose of this review is to present recent data on coated-platelets, a subpopulation of activated platelets which are 'coated' with a number of strongly anchored, procoagulant proteins, resulting in an unparalleled ability to promote thrombin generation. As discussed below, the nomenclature for coated-platelets is still unsettled, and several aliases exist in the literature. Hopefully, this review will both emphasize the shared characteristics of these variously activated platelets and justify the proposed common nomenclature. Two recent reviews have also examined coated-platelets [1,2].

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History of multiple platelet classes

The first suggestion that platelets were not homogeneous was provide in 1965 by Webber and Firkin [3], who showed morphological differences among hypotonically treated platelets. Behnke and colleagues, however, were the first to describe biochemical differences among platelets that delineated clear subpopulations of cells [4,5]. These workers observed that histological stains for a cytoplasmic phosphatase activity identified two populations of platelets, and subsequent experiments suggested that the phosphatase was more specifically a phospho-tyrosine phosphatase. The absence of this phosphatase activity appeared to correlate with more reactive platelets.

Additional examples of platelet heterogeneity are present in the literature. For example, Heemskerk *et al*. [6] observed that approximately 50% of platelets adherent to a collagen matrix expressed phosphatidylserine (PS) as monitored by annexin-V binding. Inclusion of thrombin in the adhesion reaction increased the percentage of PS-positive cells to more than 80%. Similarly, Pasquet *et al*. [7] observed that stimulation of platelets with collagen plus thrombin resulted in two populations of platelets relative to PS exposure, and when PS-positive platelets were isolated by fluorescent-activated cell sorting, they were found to have decreased levels of tyrosine phosphorylation. Also, Feng and Tracy [8] in 1998 reported in an abstract that stimulation of platelets with high doses of thrombin resulted in two distinct populations of cells when analyzed for surface-bound factor (F)Va or factor (F)Xa, and platelets expressing both FVa and FXa represented ~ 38% of the total population.

One very recent example of multiple platelet classes was the description of 'vanguard' platelets by Patel *et al*. [9]. These authors used videomicroscopy to examine the deposition of single platelets onto a collagen surface. A subpopulation of cells, the vanguard platelets, was first to adhere and spread on the collagen surface and then to serve as a nexus for the adhesion and accumulation of additional platelets. Blockade of

the fibrinogen receptor, glycoprotein (GP) IIb/IIIa, affected the second wave of deposition but not the initial vanguard binding. The relationship, if any, of vanguard platelets to the following discussion of coated-platelets is unknown.

COAT-FV platelets

The debate over multiple platelet classes was reinvigorated by a report in 2000 from Alberio *et al.* [10]. This study examined the impact of dual agonist activation of gel-filtered platelets with thrombin plus collagen or thrombin plus convulxin, an agonist for the collagen receptor GP VI [11]. A subpopulation of activated cells expressed high levels of α-granule FV on their surface (Fig. 1), and they were called COAT-FV platelets, an acronym for *co*llagen *a*nd *t*hrombin activated platelets with FV. This subpopulation represented on average 30% of the total population when platelets were activated with near maximal levels of thrombin and convulxin, and decreasing levels of either agonist resulted in lower percentages of COAT-FV platelets [10]. As mentioned, convulxin is fully able to substitute for collagen in generation of these cells, suggesting that GP VI stimulation and thrombin activation are essential for COAT-FV platelet formation. Both conclusions were premature, as discussed below.

Further examination of COAT-FV platelets demonstrated that they also expressed high levels of surface PS on the same subset of cells with bound FV [10]. More importantly, the bound FV and expressed PS were functional in their ability to bind FXa and generate an active prothrombinase complex [10]. In fact, the prothrombinase levels generated by COAT-FV platelets approximated that of A23187 ionophore-treated platelets, the gold standard of prothrombinase activity. One other noteworthy finding was that newly synthesized platelets, as characterized by the ability to bind the mRNA dye thiazole orange, were hyperreactive in their ability to form COAT-FV platelets. While the total population of platelets formed about 30% COAT-FV platelets, the thiazole orange-positive cells formed approximately 70% COAT-FV platelets [10], indicating that younger, hyperreactive platelets were more capable of generating these unusual cells.

COAT-platelets

In 2002, Dale *et al.* [12] demonstrated that dual agonist activation of gel-filtered platelets with convulxin plus thrombin resulted in more than FV binding and PS exposure; additional α-granule proteins, including fibrinogen, von Willebrand factor, thrombospondin, α_2-antiplasmin and fibronectin, were

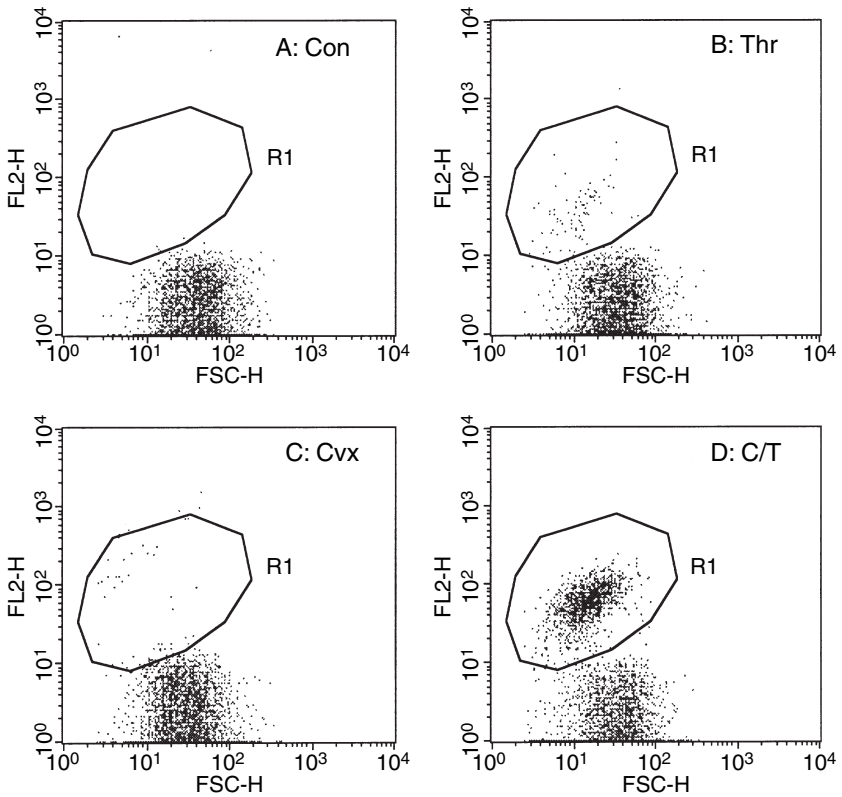

Fig. 1. Coated-platelets bind α-granule factor (F)V. Gel filtered platelets were activated with the agonists identified in each panel and then stained with a mAb recognizing FV light chain (vertical axis); horizontal axis depicts forward light scatter (FSC). Region R1 in (D) represents COAT-FV platelets as originally described by Alberio *et al.* [10] but now referred to as coated-platelets. (A) Control (Con) platelets. (B) Thrombin-activated (Thr) platelets. (C) Convulxin-activated (Cvx) platelets. (D) Convulxin plus thrombin (C/T) activated platelets. For this blood donor, 28.4% of platelets were converted into coated-platelets (D, region R1). Single agonist activation by thrombin or convulxin resulted in a negligible number of events in the R1 region.

also retained on the surface of the same subpopulation of cells as were FVa and PS. At this point, it was apparent that COAT-FV was too limiting a name, and these platelets were then referred to as COAT-platelets, again emphasizing the two agonists (*co*llagen *a*nd *t*hrombin) used in their formation.

The first indication that there was something unusual about the retention of α-granule proteins on COAT-platelets was the inability of PAC-1 to displace or prevent the binding of fibrinogen [12]. PAC-1 is a monoclonal antibody which recognizes the activated conformation of GP IIb/IIIa with an affinity 50 × greater than does fibrinogen, and PAC-1 can actually be used as a GP IIb/IIIa antagonist to prevent platelet aggregation [13]. Not only was PAC-1 unable to inhibit fibrinogen retention on COAT-platelets [12], but subsequent studies demonstrated that other GP IIb/IIIa antagonists were equally ineffective in preventing this interaction [14] even though experiments with LIBS-6 indicated that GP IIb/IIIa molecules on COAT-platelets were occupied [12]. These observations led to the conclusion that fibrinogen, and perhaps other α-granule proteins, were being retained on the platelet surface with an exceptional affinity.

One characteristic shared by all the α-granule proteins present on COAT-platelets was that each was a known transglutaminase substrate, and this seemed to offer a plausible explanation for the strong affinity of these proteins for the platelet surface. We therefore examined the impact of transglutaminase inhibitors on COAT-platelet formation and found an attenuation [12]. More importantly, it was also demonstrated that a synthetic transglutaminase substrate CP-15, a 15-residue peptide serving as a glutamine donor, was incorporated into COAT-platelets [12], thereby providing positive evidence that a transglutaminase was active during COAT-platelet formation.

The CP-15 transglutaminase substrate was also used to address the question of which platelet component(s) served as the amino-donor in this transglutaminase reaction. While the anticipated result was that a platelet membrane protein would be the anchoring site for these transglutaminase substrates, the actual molecule conjugated to CP-15 was serotonin [12]. This unexpected finding was corroborated by the demonstration that fibrinogen recovered from COAT-platelets had covalently bound serotonin [12]. No other α-granule proteins from COAT-platelets have been examined with this very laborious assay; however, inclusion of biotin-pentylamine during the formation of COAT-platelets indicates that multiple proteins are labeled with this transglutaminase substrate (G. L. Dale, unpublished observation).

The identity of the transglutaminase responsible for coupling serotonin to α-granule proteins is still uncertain. Platelets have significant levels of the factor (F)XIIIa subunit in their cytoplasm [15] as well as a tissue transglutaminase [16]. Immunochemical studies of COAT-platelets identified both transglutaminases on the surface of these cells [12], and an anti-FXIII antibody was able to inhibit COAT-platelet formation [12]. However, Jobe *et al.* [17] report in an abstract that FXIIIa knockout mice are unaffected in their ability to produce COAT-platelets. While this point remains unresolved, it is noteworthy that Walther *et al.* [18] have recently demonstrated that small, cytoplasmic GTPases are derivatized with serotonin via a transglutaminase activity during platelet activation; this derivatization has been termed serotonylation [18].

The observation that serotonin was attached to α-granule proteins on COAT-platelets presented a dilemma as to how attachment of a small molecule like serotonin could possibly impact the apparent affinity of α-granule proteins for the COAT-platelet surface. Since it was known that albumin derivatized with multiple serotonins, BSA-$(HT)_6$, was able to bind to COAT-platelets [12], Szasz and Dale [19] used this as a probe to identify possible serotonin-binding sites on COAT-platelets. Early experiments demonstrated that not only was the putative binding site(s) a protein, but the binding site was expressed only by activated platelets. Subsequent studies using photo crosslinking of BSA-$(HT)_6$ to COAT-platelets demonstrated that both fibrinogen and thrombospondin were capable of binding serotonin-derivatized proteins [19].

These data allowed a preliminary model of COAT-platelet formation and structure to be proposed. This model is based upon four primary findings: several α-granule proteins are coordinately retained on the COAT-platelet surface; fibrinogen is bound to the COAT-platelet with an exceptional affinity; serotonin is conjugated to at least some of these α-granule proteins; and fibrinogen and thrombospondin have binding sites for serotonin-derivatized proteins. The proposed model (Fig. 2) addresses all four findings. With this model, α-granule proteins bind to their traditional receptors, e.g. fibrinogen to GP IIb/IIIa and FVa to PS. In addition, serotonin-derivatized α-granule proteins are also able to interact with serotonin-binding sites on neighboring fibrinogen and/or thrombospondin molecules. For example, serotonin-derivatized fibrinogen

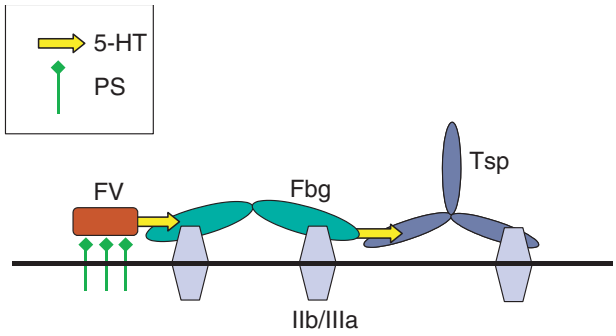

Fig. 2. Model of proposed structure for coated-platelet. This model reflects four key findings relevant to the generation of coated-platelets. Procoagulant proteins bind to their traditional receptors [e.g. factor (F)V to phosphatidylserine (PS), fibrinogen (Fbg) to GP IIb/IIIa]. Second, α-granule proteins on coated platelets are covalently derivatized with serotonin (5-HT; yellow arrow) via a transglutaminase reaction. In this figure both FV and Fbg are derivatized with serotonin. Third, Fbg and thrombospondin (Tsp) have binding sites that recognize conjugated serotonin; in this figure, the yellow arrowhead is binding to serotonin-binding sites on Fbg and Tsp. Fourth, the multiple interactions of α-granule proteins with surface-bound molecules (e.g. Fbg-5-HT with GP IIb/IIIa and Fbg or Tsp) result in a stabilized network of proteins on the coated-platelet surface.

on COAT-platelets is not only bound to GP IIb/IIIa, but also to neighboring fibrinogen or thrombospondin molecules as a result of serotonin-dependent interactions. The consequence of these multivalent interactions is stabilization of the surface-bound complex.

Coated-platelets: nomenclature and definition

Two significant roadblocks hinder further study of COAT-platelets: nomenclature and a working definition. While COAT is an acronym for the *co*llagen *a*nd *t*hrombin agonists initially used to generate this subpopulation of cells, it is apparent that an identical subpopulation of cells can be produced by other agonist pairs or, in some cases, by single agonists (see following section). On this basis alone, the COAT acronym is inappropriate. There is, however, an additional problem, and that is the non-COAT-platelet population. As indicated in Fig. 1D, the COAT-platelet subpopulation present in region R1 exhibits all the characteristics which are the focus of this review. However, the other 70% of platelets present in Fig. 1D were similarly activated with collagen plus thrombin, but they did not form the activated product referred to as COAT-platelets. As a result, I propose a more universally applicable nomenclature of 'coated-platelets'. In this instance, coated is used as an adjective to describe the 'coating' of procoagulant proteins retained on the surface of these activated platelets. This nomenclature is independent of the agonists utilized to produce these cells, and it also allows differentiation between the coated-platelets expressing procoagulant proteins and those platelets which were similarly activated but did not become coated-platelets (Fig. 1D; cells outside R1).

The second obstacle is a working definition of coated-platelets. As discussed above, it seems prudent to focus on the final product in establishing this definition, and the parameters which characterize coated-platelets include bound α-granule proteins [10,12], non-displaceable fibrinogen [12,14], incorporated serotonin [12], attenuation by transglutaminase inhibitors [12], exposed PS [10], and permeability to calcein [20]. There may be examples of coated-platelets which do not express all of these characteristics, but that circumstance has yet to present itself. In addition, the very recent nature of the coated-platelet field suggests that new, and perhaps better, markers of coated-platelets may be discovered.

Current experience indicates that identification of coated-platelets in a purified system is straightforward. Platelets with bound α-granule proteins, exposed PS and calcein permeability are clearly coated-platelets. It is important, however, that identification of coated-platelets utilizes as many of these characteristics as possible. For example, platelets activated with calcium ionophore A23187 alone will express surface PS but none of the other hallmarks of coated-platelets [10], thereby indicating that PS exposure alone is not sufficient to identify coated-platelets. On the other hand, more complex situations, e.g. activated whole blood, present problems for identification of coated-platelets. In such a setting, essentially all platelets may have bound adhesive and procoagulant proteins, and it is then necessary to rely on other markers of coated-platelets, such as PS-exposure and calcein permeability [20,21], to identify these cells.

Agonist requirements for production of coated-platelets

As mentioned, there is more than one way to make a coated-platelet. While recent interest in platelet subpopulations was the result of dual agonist activation with thrombin plus collagen, other agonists produce a subpopulation of cells with similar characteristics. Batar and Dale [22] showed that engagement of the Fc receptor on platelets in conjunction with thrombin stimulation produced a product indistinguishable from coated-platelets, although these cells were initially termed FcRT-platelets to identify the agonists engaged [22]. Fc receptor engagement was accomplished with ALB6 or ML13, antibodies against CD9 that simultaneously engage Fc receptors. Similarly, Fc receptor activation could be achieved with an anti-Fc receptor antibody (IV.3) and crosslinking of receptor–antibody complexes by a secondary antibody [22]. Signaling by activated FcγRIIa receptors utilizes many of the same second messenger pathways as does the collagen GP VI receptor [23], so the production of similar activation products should not be too surprising.

As noted, Feng and Tracy [8] reported a subpopulation of platelets which bind FVa and FXa upon stimulation with high concentrations of thrombin. The 50 nM thrombin utilized by these investigators is 10-fold higher than this laboratory routinely uses (5 nM) in conjunction with convulxin activation [10]. While Feng and Tracy have not reported additional procoagulant proteins present on their subpopulation of cells, we have utilized high levels of thrombin and found a subpopulation that is indistinguishable from convulxin plus thrombin-stimulated platelets (G. L. Dale, unpublished observations). Additional studies by London *et al.* [24] found that high-dose thrombin (20 nM) or thrombin receptor agonist peptide resulted in a small subpopulation of platelets binding factor (F)IXa and expressing PS. While no other markers of coated-platelets were analyzed, the studies of Kempton *et al.* [25], demonstrating colocalization of FV, factor (F)VIII, FIX, FX and PS on the same subpopulation of dual-agonist stimulated platelets, suggest it is likely that London *et al.* [24] are observing coated-platelets.

A review of the literature suggests other agonists which are capable of producing coated-platelets. For example, Kulkarni and Jackson [26] have investigated collagen-adherent platelets and identified a class of platelets they call SCIP (sustained calcium-induced platelet morphology). SCIP bind to immobilized collagen, express PS and are inhibited by FXIIIa antagonists. Only a fraction of adherent platelets become SCIP, and that fraction can be potentiated with additional fluid-phase agonists [26]. Thus, SCIP share several characteristics with coated platelets, including PS exposure, sensitivity to FXIII inhibitors and fractional expression. In addition, Heemskerk *et al.* [6] reported that platelets adherent to immobilized collagen are heterogeneous with respect to

expression of surface PS with ~50% binding FITC-annexin. More recently, these same investigators examined the role of several platelet receptors in promoting PS exposure in adherent platelets and found a strong influence of GP VI [27].

Our laboratory has performed similar experiments with flowing whole blood in a parallel plate chamber coated with collagen, and we also found heterogeneity of PS exposure among collagen-adherent platelets (G. L. Dale, unpublished observation). Importantly, the PS-positive platelets were also permeable to calcein, a low-molecular-weight fluorescent dye which has been shown to be lost from coated-platelets [20]. These findings are particularly noteworthy as another example of a single agonist, adherent collagen, that is apparently capable of producing coated-platelets.

Physiological aspects of coated-platelets

The physiological significance of coated-platelets is unknown, although inspection of the prothrombotic proteins present on the surface of these cells leads to speculation that they could be significant contributors to thrombotic processes. Most remarkable, of course, are the presence of an active prothrombinase complex and the availability of surface PS. While there are observations which suggest an important role for coated-platelets, hard data on this subject are lacking.

One of the more intriguing findings is that coated-platelet potential varies among donors. While several reports indicate that normal donors average approximately 30% of coated-platelets upon stimulation with thrombin plus convulxin [10,14], the range of coated-platelet values for normals is considerable. In our laboratory this range is 15–53% (Fig. 3A) with a mean of 33.0 ± 9.3% (1 SD; $n = 50$). Furthermore, the coated-platelet value an individual produces is relatively stable, as indicated in Fig. 3B, where coated-platelet levels in several individuals were determined repeatedly over a 6-week period.

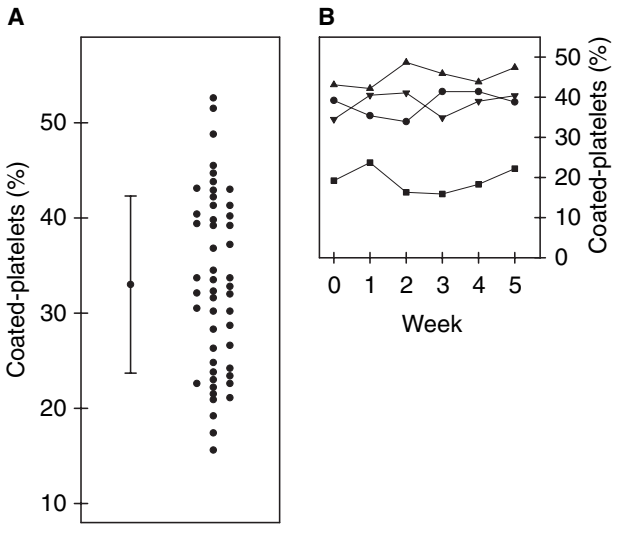

Fig. 3. Coated-platelet values for normal blood donors. The ability to produce coated-platelets was determined for 50 normal controls. (A) The distribution of coated-platelet values ranged from 15.6% to 52.6% with a mean of 33.0 ± 9.3% (1 SD). (B) Four individuals were repeatedly assayed for coated-platelet potential over 6 weeks. Data demonstrate only a modest fluctuation in coated-platelet potential.

The range of individual coated-platelet values (Fig. 3A) is quite extraordinary for a potentially prothrombotic marker and suggests that examination of coated-platelet synthesis in prothrombotic clinical settings could be informative. Along these lines, trials investigating coated-platelets in acute coronary syndrome (ACS), stroke, diabetes and systemic lupus erythematosus are underway. In an abstract, Holmes and colleagues [28] observed that the subpopulation of FXa-binding platelets observed upon activation with high levels of thrombin (probably coated-platelets) varies among patients with myeloproliferative disorders (range 2–55%), and those patients with a history of arterial or venous thrombosis had a significant trend toward higher levels within this range.

Experimental animals, including mouse, dog and baboon, also produce coated-platelets upon dual agonist stimulation with convulxin plus thrombin, and no differences have been observed when comparing the surface-bound proteins on coated platelets from these species compared with humans. In addition, these experimental animals provide an opportunity to examine parameters affecting coated-platelet production. For example, dogs receiving a booster vaccination with attenuated parvo virus were found to produce increased levels of coated-platelets, actually doubling coated-platelet potential during the time frame from 4 to 20 days post vaccination (G. L. Dale, unpublished observation). This finding indicates that immune stimulation is one modulator of coated-platelet potential and opens the possibility that these cells may serve as a link in the well-documented association of inflammation and thrombosis [29].

The synthesis of coated-platelets involves transglutaminase-mediated addition of serotonin to α-granule proteins [12]. Serotonin is present at high levels in the platelet dense granule, and the serotonin transporter present in the platelet cytoplasmic membrane is inhibited by selective serotonin reuptake inhibitors (SSRIs) used in the treatment of chronic depression. As a result, individuals taking SSRIs have decreased platelet serotonin levels [30,31], a mild tendency for bleeding [32,33], and decreased ability to generate coated-platelets (G. L. Dale, unpublished observation). However, the critical observation is that patients on SSRI medications are also at decreased risk of a myocardial infarction. Initially, Sauer *et al.* [34] demonstrated this connection for a first myocardial infarction among smokers taking an SSRI; subsequently, that observation was extended to a more general population [35]. No molecular hypothesis has been widely accepted for this protective effect of SSRI medications, but an impact on coated-platelet potential is a reasonable addition to the discussion. A dosing trial examining the impact of SSRI medications on coated-platelet synthesis is in progress (S. Hamilton, personal communication).

Another hint at the pathological significance of coated-platelets comes from an *in vitro* observation and a clinical conundrum. Hamilton *et al.* [14] demonstrated that GP IIb/IIIa inhibitors increased by 5–35% the level of coated-platelets

produced *in vitro*. While the biochemical mechanism responsible for this change is uncertain, the physiological consequence may be relevant to a perplexing observation in the literature. Orally available GP IIb/IIIa inhibitors were conceived and produced with the expectation that they would be strong antithrombotics; however, these drugs were found actually to increase all-cause mortality [36]. Several proposals have been put forward as to why orally available GP IIb/IIIa inhibitors were not beneficial [36], but this remains an open question. The ability of GP IIb/IIIa antagonists to potentiate the basal level of coated-platelet production offers another possible explanation for the increased thrombotic risk associated with these drugs [37].

One final observation concerns GP VI-deficient platelets, generated either genetically [38] or immunologically [39]. *In vivo*, GP VI-deficient platelets fail to produce thrombi upon a collagen challenge [39] even though tail bleeding times are only modestly affected. *In vitro*, GP VI-deficient platelets do not generate thrombi on a collagen surface [38]. While these observations do not directly bear on coated-platelets, a failure of GP VI deficiency to have a physiological impact would not have boded well for coated-platelets.

Additional studies with coated-platelets

Several investigators have ventured into the coated-platelet field, even if that name or a pseudonym is not used. Recently, Kjalke and Rojkjaer [40] reported in an abstract the strong binding of recombinant activated factor VIIa (rFVIIa) to convulxin plus thrombin-generated coated-platelets. This is one of the few examples of an exogenous protein being incorporated into coated-platelets. These authors also indicate that rFVIIa is a transglutaminase substrate, although it is not clear if that characteristic is important in retention of this coagulant factor on coated-platelets. Norris *et al.* [41] have observed that soluble phospholipase A_2 ($sPLA_2$), a component of the platelet α-granule, is also retained on the surface of coated-platelets. These authors speculate that bound $sPLA_2$ may alter membrane lipid composition of coated-platelets and perhaps result in generation of additional second messengers [41].

In a reconstituted system, Kempton *et al.* [25,42] measured the generation of thrombin in the presence of convulxin-activated platelets, tissue factor-expressing monocytes and appropriate coagulation factors. The level of thrombin generated was potentiated $\sim$2-fold by the addition of convulxin-activated platelets. These authors also observed by flow cytometry the production of two platelet populations in their system, with one population binding high levels of FV, FVIII, FIX and FX and expressing PS. This subpopulation of platelets represented $\sim$50% of the total. Alberio *et al.* [10] previously observed FV and FXa on coated-platelets in a simpler reaction mixture, but the inference is strong that similar processes are underway with the complex system utilized by Kempton and colleagues [25].

The laboratories of Di Paola and Lentz have utilized knockout (KO) mice to examine the role of specific proteins in generation of coated-platelets. Initially, Leo *et al.* [43] utilized KO lacking SLP-76, a key component in the GP VI signaling pathway. Mice lacking SLP-76 had an impaired response to collagen plus thrombin producing fewer cells positive for surface PS and an attenuated procoagulant response. More recently, Jobe *et al.* [17] have reported in an abstract the specific characteristics of coated-platelets from the mouse. Analogous to the human, these platelets retain fibrinogen and thrombospondin and also express surface PS. Additional KO models were examined, and mice deficient in FcRγ chain, another critical component for GP VI signaling, were not able to produce coated-platelets in response to thrombin plus convulxin. However, a more surprising observation was that FXIIIa KO mice were able to generate coated-platelets. As mentioned above, this finding raises doubts about earlier reports on the role of transglutaminases in coated-platelet formation [12]. Either there are significant species differences in the mechanism of coated-platelet formation, or a transglutaminase other than FXIIIa is involved, or the earlier studies are in error.

Another question of special interest for coated-platelets is why only a fraction of platelets are converted into this product. Little information is available. As mentioned, young platelets have an increased ability to form coated-platelets [10], but it is unlikely this is the sole explanation. The work of Pasquet *et al.* [7] referred to above used collagen plus thrombin to generate PS-positive platelets. The decreased level of phospho-tyrosine these authors observed in the PS-positive population (i.e. coated-platelets) deserves closer scrutiny as a determining factor in coated-platelet production.

Perhaps most perplexing is the 'all or nothing' nature of coated-platelet production. This is illustrated in Fig. 1D, where the coated-platelet population binds high levels of FV while the remainder of platelets bind very little FV; most surprisingly, there is no intermediate population. This type of discrete demarcation suggests that a gradual change in platelet reactivity, as might be expected for age-dependent processes, is an unlikely explanation for why only some platelets become coated-platelets. Another 'all or nothing' aspect to coated-platelet production reaction has recently been reported [20]. Remenyi *et al.* [20] observed that loss of mitochondrial potential ($\Delta\Psi_m$) correlated with generation of coated-platelets. This loss of $\Delta\Psi_m$ is analogous to that observed during apoptosis [44] and results from generation of the mitochondrial permeability transition pore (MPTP) in the mitochondrial membrane. In keeping with the close association of MPTP formation with coated-platelet formation, inhibitors and activators of MPTP had similar effects on coated-platelet formation. Interestingly, MPTP formation has also been observed to be an 'all or nothing' process [44], opening the possibility that MPTP formation might serve as a gate keeper in determining which cells become coated-platelets.

Summary

This review has summarized current knowledge concerning coated-platelets. While considerable progress has been made in

understanding the reactions involved in generating these cells as well as the likely structure of the final product, more questions remain unanswered than answered. The most important of these is the physiological significance of coated-platelets. As mentioned, there are few specific data relating to this question, although considerable conjecture on this point has been presented. While the overwhelming impression conveyed by the coated-platelet model presented in Fig. 2 is of a cell designed to promote thrombosis, future studies will hopefully be able to address this question unambiguously.

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References

1 Szasz R, Dale GL. COAT-platelets. *Curr Opin Hematol* 2003; **10**: 351–5.
2 Alberio L, Clemetson KJ. All platelets are not equal: COAT platelets. *Curr Hematol Rep* 2004; **3**: 338–43.
3 Webber AJ, Firkin BG. Two populations of platelets. *Nature* 1965; **205**: 1332.
4 Behnke O, Forer A. Blood platelet heterogeneity. Evidence for two classes of platelets in man and rat. *Br J Haematol* 1993; **84**: 686–93.
5 Behnke O. Blood platelet heterogeneity: a functional hierarchy in the platelet population. *Br J Haematol* 1995; **91**: 991–9.
6 Heemskerk JWM, Vuist WMJ, Feijge MAH, Reutelingsperger CPM, Lindhout T. Collagen but not fibrinogen surfaces induce bleb formation, exposure of phosphatidylserine, and procoagulant activity of adherent platelets: evidence for regulation by protein tyrosine kinase-dependent Ca^{2+} responses. *Blood* 1997; **90**: 2615–25.
7 Pasquet JM, Dachary-Prigent J, Nurden AT. Microvesicle release is associated with extensive protein tyrosine dephosphorylation in platelets stimulated by A23187 or a mixture of thrombin and collagen. *Biochem J* 1998; **333**: 591–9.
8 Feng P, Tracy PB. Not all platelets are equivalent procoagulants. *Blood* 1998; **92**: 350a (Abstract).
9 Patel D, Väänänen H, Jirousková M, Hoffmann T, Bodian C, Coller BS. Dynamics of GPIIb/IIIa-mediated platelet–platelet interactions in platelet adhesion/thrombus formation on collagen *in vitro* as revealed by videomicroscopy. *Blood* 2003; **101**: 929–36.
10 Alberio L, Safa O, Clemetson KJ, Esmon CT, Dale GL. Surface expression and functional characterization of alpha-granule factor V in human platelets. Effects of ionophore A23187, thrombin, collagen and convulxin. *Blood* 2000; **95**: 1694–702.
11 Polgar J, Clemetson JM, Kehrel BE, Wiedemann M, Magnenat EM, Wells TNC, Clemetson KJ. Platelet activation and signal transduction by convulxin, a C-type lectin from *Crotalus durissus terrificus* (tropical rattlesnake) venom via the p62/GPVI collagen receptor. *J Biol Chem* 1997; **272**: 13576–83.
12 Dale GL, Friese P, Batar P, Hamilton SF, Reed GL, Jackson KW, Clemetson KJ, Alberio L. Stimulated platelets use serotonin to enhance their retention of procoagulant proteins on the cell surface. *Nature* 2002; **415**: 175–9.
13 Shattil SJ, Cunningham M, Hoxie JA. Detection of activated platelets in whole blood using activation-dependent monoclonal antibodies. *Blood* 1987; **70**: 307–15.
14 Hamilton SF, Miller MW, Thompson CA, Dale GL. Glycoprotein IIb/IIIa inhibitors increase COAT-platelet production *in vitro*. *J Lab Clin Med* 2004; **143**: 320–6.
15 Devine DV, Bishop PD. Platelet associated factor XIII in platelet activation, adhesion and clot stabilization. *Sem Thromb Hemost* 1996; **22**: 409–13.
16 Puszkin EG, Raghuraman V. Catalytic properties of a calmodulin-regulated transglutaminase from human platelet and chicken gizzard. *J Biol Chem* 1985; **260**: 16012–20.
17 Jobe SM, Leo L, Dicknite G, Lentz SR, Di Paola J. Murine platelets retain high amounts of alpha-granule proteins on their surface upon stimulation with multiple agonists. *Blood* 2004; **104**: 61b (Abstract).
18 Walther DJ, Peter JU, Winter S, Holtje M, Paulmann N, Grohmann M, Vowinckel J, Alamo-Bethencourt V, Wilhelm CS, Ahnert-Hilger G, Bader M. Serotonylation of small GTPases is a signal transduction pathway that triggers platelet alpha-granule release. *Cell* 2003; **115**: 851–62.
19 Szasz R, Dale GL. Thrombospondin and fibrinogen bind serotonin-derivatized proteins on COAT-platelets. *Blood* 2002; **100**: 2827–31.
20 Remenyi G, Szasz R, Friese P, Dale GL. Role of mitochondrial permeability transition pore in coated-platelet formation. *Arterioscler Thromb Vasc Biol* 2005; **25**: 467–71.
21 Remenyi G, Szasz R, Friese P, Dale GL. Extruded zinc promotes COAT-platelet formation. *Blood* 2003; **102**: 291a (Abstract).
22 Batar P, Dale GL. Simultaneous engagement of thrombin and Fc-gamma-RIIA receptors results in platelets expressing high levels of procoagulant proteins. *J Lab Clin Med* 2001; **138**: 393–402.
23 Anderson CL, Chacko GW, Osborne JM, Brandt JT. The Fc receptor for immunoglobulin G (Fc gamma RII) on human platelets. *Semin Thromb Hemost* 1995; **21**: 1–9.
24 London FS, Marcinkiewicz M, Walsh PN. A Subpopulation of platelets responds to thrombin- or SFLLRN-stimulation with binding sites for factor IXa. *J Biol Chem* 2004; **279**: 19854–9.
25 Kempton CL, Hoffman M, Monroe DM. Platelet heterogeneity: variation in coagulation complexes on platelet subpopulations. *Arterioscler Thromb Vasc Biol* 2005, in press.
26 Kulkarni S, Jackson SP. Platelet factor XIII and calpain negatively regulate integrin alphaIIbbeta3 adhesive function and thrombus growth. *J Biol Chem* 2004; **279**: 30697–706.
27 Kuijpers MJE, Schulte V, Oury C, Lindhout T, Broers J, Hoylaerts MF, Nieswandt B, Heemskerk JWM. Facilitating roles of murine platelet glycoprotein Ib and alpha IIb beta 3 in phosphatidylserine exposure during vWF-collagen-induced thrombus formation. *J Physiol-London* 2004; **558**: 403–15.
28 Holmes CE, Bouchard BA, Barbick RR, Safdar H, Tracy PB. The existence of platelet subpopulations in patients with myeloproliferative disease: preliminary correlation with clinically abnormal hemostasis. *Blood* 2003; **102**: 787a (Abstract).
29 Libby P. Simon DI. Inflammation and thrombosis—the clot thickens. *Circulation* 2001; **103**: 1718–20.
30 Hergovich N, Aigner M, Eichler HG, Entlicher J, Drucker C, Jilma B. Paroxetine decreases platelet serotonin storage and platelet function in human beings. *Clin Pharmacol Therapeutics* 2000; **68**: 435–42.
31 Maurer-Spurej E, Pittendreigh C, Solomons K. The influence of selective serotonin reuptake inhibitors on human platelet serotonin. *Thromb Haemost* 2004; **91**: 119–28.
32 Humphries JE, Wheby MS, Vandenberg SR. Fluoxetine and the bleeding time. *Arch Pathol Lab Med* 1990; **114**: 727–8.
33 Pai VB, Kelly MW. Bruising associated with the use of fluoxetine. *Ann Pharmacother* 1996; **30**: 786–8.
34 Sauer WH, Berlin JA, Kimmel SE. Selective serotonin reuptake inhibitors and myocardial infarction. *Circulation* 2001; **104**: 1894–8.
35 Sauer WH, Berlin JA, Kimmel SE. Effect of antidepressants and their relative affinity for the serotonin transporter on the risk of myocardial infarction. *Circulation* 2003; **108**: 32–6.
36 Cox D. Oral GPIIb/IIIa antagonists: what went wrong? *Current Pharmaceut Design* 2004; **10**: 1587–96.

37 Editorial. 'COAT' platelets: a clue to the lack of clinical efficacy of glycoprotein IIb/IIIa inhibitors. *J Lab Clin Med* 2004; **143**: 266.
38 Kato K, Kanaji T, Russell S, Kunicki TJ, Furihata K, Kanaji S, Marchese P, Reininger A, Ruggeri ZM, Ware J. The contribution of glycoprotein VI to stable platelet adhesion and thrombus formation illustrated by targeted gene deletion. *Blood* 2003; **102**: 1701–7.
39 Nieswandt B, Schulte V, Bergmeier W, Mokhtari-Nejad R, Rackebrandt K, Cazenave JP, Ohlmann P, Gachet C, Zirngibl H. Long-term antithrombotic protection by *in vivo* depletion of platelet glycoprotein VI in mice. *J Exp Med* 2001; **193**: 459–69.
40 Kjalke M, Rojkjaer R. Colocalization of recombinant human factor VIIa with its substrates on highly procoagulant COAT-platelets. *J Thromb Haemost* 2003; (Suppl. 1): P1265 (Abstract).
41 Norris JW, Walker NJ, Oliver AE, Looper SA, Crowe JH, Tablin F. Kinetics of sPLA2 binding to platelets and microparticles due to thrombin and convulxin stimulation: correlation with phosphatidylserine flip-flop in respective membranes. *Blood* 2003; **102**: 292a (Abstract).
42 Kempton CL, Roberts HR, Monroe D. Calcium ionophore A23187 and convulxin increase Xa generation in a cell-based model of hemostasis. *J Thromb Haemost* 2003; (Suppl. 1): OC460 (Abstract).
43 Leo L, Di Paola J, Judd BA, Koretzky GA, Lentz SR. Role of the adapter protein SLP-76 in GPVI-dependent platelet procoagulant responses to collagen. *Blood* 2002; **100**: 2839–44.
44 Halestrap AP, Mcstay GP, Clarke SJ. The permeability transition pore complex: another view. *Biochimie* 2002; **84**: 153–66.