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COMMENTARY

Another piece of knowledge in the puzzle of procoagulant COAT platelets

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Factor XIII (FXIII) is a transglutaminase circulating in human plasma as a tetramer consisting of two zymogen A subunits and two protective/inhibitory B subunits.[1] Interestingly, FXIII is also present as dimer of its A-subunit in the cytosol of human platelets at a concentration that is 150-fold greater than in plasma.[2] The existence of this pool of cytosolic FXIII (cFXIII) is intriguing because platelet activation by single agonists does not elicit its secretion[3] and its intracellular functions are not clearly elucidated yet.[1,4] Some light was recently shed by Mitchell et al.,[5] who showed for the first time that cFXIII is externalized and retained on the membrane of platelets when they are simultaneously activated by collagen and thrombin.

In this issue of the *Journal of Thrombosis and Haemostasis*, Somodi et al.[6] set out to explore the mechanisms underlying cFXIII externalization on platelets and platelet-derived microparticles. First, they showed that a prolonged high cytosolic free calcium ($[Ca^{2+}]_{cyt}$) level is necessary but not sufficient for translocating cFXIII to the surface of activated platelets. In fact, while upon activation by the combined action of convulxin (a specific agonist of the collagen receptor glycoprotein GPVI) and thrombin, platelets externalized cFXIII on their surface, this was not observed after calcium ionophore, which induced even higher $[Ca^{2+}]_{cyt}$. Second, exploring signaling pathways downstream of the collagen and/or thrombin receptors that are not involved in modulating $[Ca^{2+}]_{cyt}$, Somodi et al.[6] found that inhibition of RhoA prevents surface exposure of cFXIII. RhoA signals downstream of PAR-associated $G\alpha_{12/13}$ and is involved in orchestrating cytoskeletal dynamics.[7]

The key information of Somodi et al.'s work[6] (i.e., that a high and prolonged $[Ca^{2+}]_{cyt}$ is necessary but not sufficient to expose cFXIII on the platelet surface) parallels the observation made with surface retention of α-granule factor V/Va on procoagulant collagen-and-thrombin-activated (COAT) platelets.[8] COAT platelets, also referred to as "coated platelets" are a phenotype of procoagulant platelets characterized by high exposure of phosphatidylserine.[9] The phenotype of COAT platelets is distinct from that of aggregating platelets and the difference in platelet phenotype becomes apparent about 2 min following activation by collagen/convulxin plus thrombin in experiments performed with platelet suspensions.[8,10] COAT platelets show a progressively decreased binding of the PAC-1 antibody that recognizes the activated form of integrin αIIbβ3,[10–12] can assemble a functional prothrombinase complex on their surface,[8] retain various α-granule proteins on the procoagulant surface in a serotonin- and transglutaminase-dependent mechanism[11] after having depolarized mitochondria,[13] and reach a sustained elevated $[Ca^{2+}]_{cyt}$ in the micromolar range independently from apoptotic pathways.[10] The works of Mitchell et al.[5] and Somodi et al.,[6] showing surface expression of cFXIII, add another piece of knowledge in the puzzle of procoagulant COAT platelets. However, these data prompt further questions.

First, which surface receptors are necessary to achieve this activation endpoint? Both groups activated platelets using the combination of convulxin and thrombin, which engage the collagen receptor GPVI and the three thrombin receptors PAR1, PAR4,

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and GPIb. The possible involvement of GPIb is most intriguing because it plays a peculiar role in thrombin-induced platelet procoagulant activity[14] via a nonredundant signaling through 14–3-3ζ,[15] in cytosolic calcium mobilization,[16] and, apparently, cFXIII exposure as well. In fact, figure 5 of Mitchell et al.[5] suggests that the combination collagen-plus-TRAP6 is a weaker inducer of surface exposure of cFXIII compared with collagen plus thrombin. Researchers are warmly encouraged to verify the role of GPIb in their systems, for instance, by analyzing the effect of thrombin versus the combined use of PAR1 and PAR4 agonists in addition to collagen/convulxin.

Second, which mechanisms are responsible for externalizing cFXIII from the cytosol onto the platelet membrane? Somodi et al.[6] point to a key role for RhoA, which is not a major regulator of $[Ca^{2+}]_{cyt}$ but rather of cytoskeletal rearrangement.[7] This information suggests that surface exposure of cFXIII is linked to

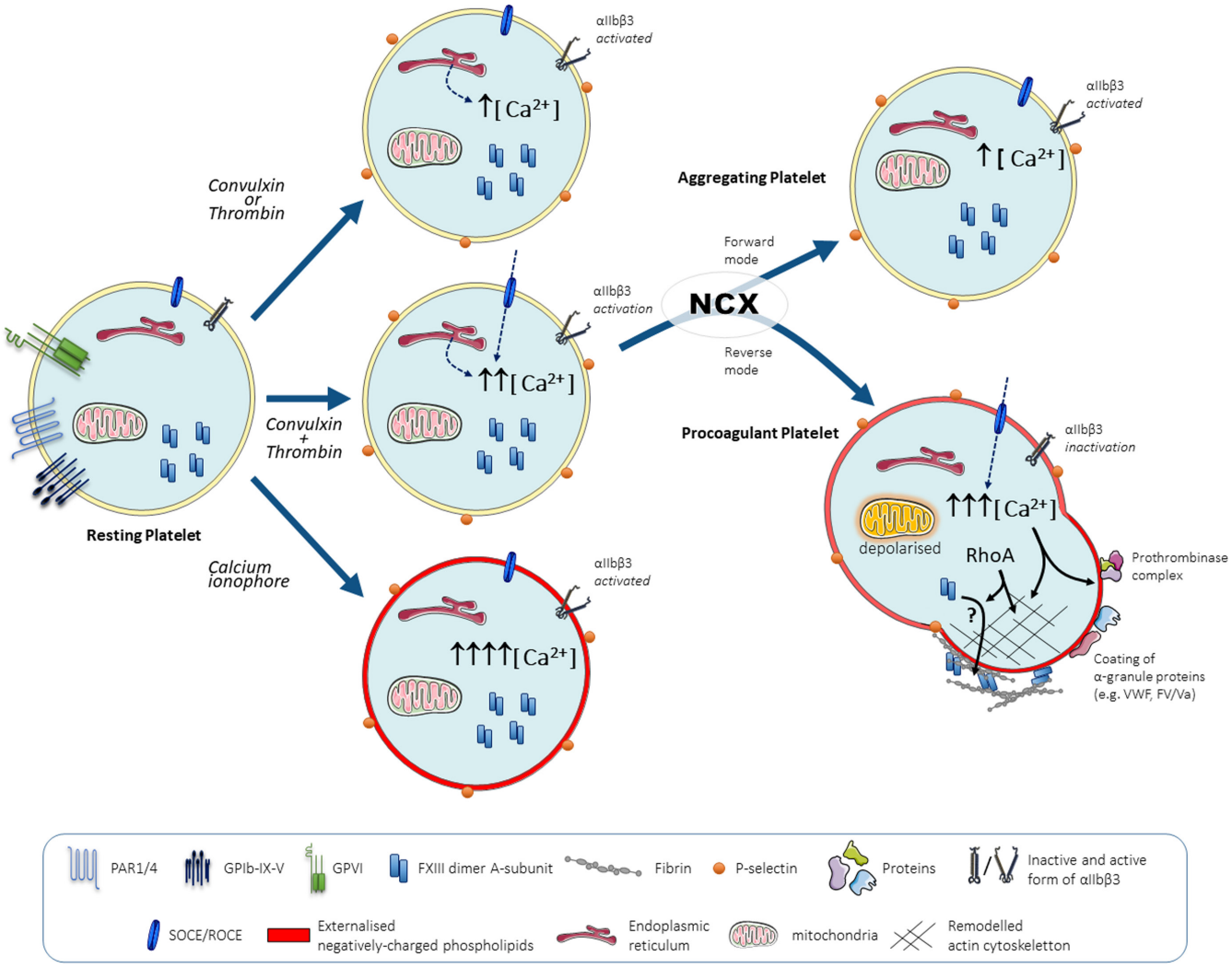

FIGURE 1 Agonist-dependent platelet activation phenotypes. Platelet activation by thrombin or convulxin/collagen as single agonists induces a moderate increase in cytosolic calcium ($[Ca^{2+}]_{cyt}$) and "typical" endpoints, such as activation of the fibrinogen receptor integrin αIIbβ3 and granule secretion. Under these conditions, only minimal expression of negatively charged phospholipids and no externalization of cytosolic FXIII-A are observed. Calcium ionophore is able to induce very high $[Ca^{2+}]_{cyt}$, thus mediating externalization of phosphatidylserine. However, no cytosolic FXIII-A is present at the platelet surface. The strong activation with convulxin-plus-thrombin generates a subpopulation of procoagulant COAT platelets, which begins to differentiate from aggregating ones about 2 min after activation. Differentiation between COAT and non-COAT platelets relies on the action of the sodium-calcium exchanger (NCX). Non-COAT platelets use forward-mode NCX, pumping calcium out of the platelet cytosol and moving sodium in, whereas COAT platelets rely on reverse NCX function, which pumps additional calcium into the cytosol, by extruding sodium.[17] The COAT platelet subpopulation progressively downregulates activated αIIbβ3, depolarizes mitochondria, reaches sustained and elevated $[Ca^{2+}]_{cyt}$ resulting in surface expression of substantial quantities of phosphatidylserine to eventually assemble a functional prothrombinase complex, and retains on its surface α-granule proteins (in a serotonin- and transglutaminase-dependent mechanism) as well as cytosolic FXIII-A. According to the publication by Somodi et al.,[6] surface externalization of cytosolic FXIII-A depends on high $[Ca^{2+}]_{cyt}$ and a functioning RhoA. This figure was created using templates from Servier Medical Art (CC BY 3.0 license). COAT, collagen-and-thrombin-activated; F, factor.

cytoskeletal modifications. This is plausible because, for instance, the reverse mode of the sodium/calcium exchanger that results in influx of additional calcium into the cytosol, by extruding sodium and is required to generate COAT platelets,[17] appears to depend, among other variables, on a functional actin cytoskeleton.[18] However, because RhoA is already engaged downstream of thrombin alone, it is puzzling that cFXIII externalization did not occur under single-agonist conditions. This leads to the hypothesis that RhoA activation may be necessary but not sufficient for externalizing cFXIII. For instance, surface exposure of cFXIII may depend both on RhoA and a peculiar, possibly high $[Ca^{2+}]_{cyt}$-dependent cytoskeletal modification required to form procoagulant COAT platelets. Moreover, because in megakaryocytes, inhibition of RhoA prevents surface exposure of phosphatidylserine,[19] it will be interesting to evaluate its role and more broadly the role of cytoskeletal rearrangement in the formation of procoagulant COAT platelets and surface exposure of cFXIII.

Third, which are the functions of surface-retained cFXIII? Work of other groups explored possible functions of activated cFXIII (cFXIIIa) expressed on the surface of platelets activated by collagen and thrombin. cFXIIIa is exposed within the "cap" region rich in phosphatidylserine of procoagulant platelets. This localizes important amounts of active FXIII-A directly at sites of thrombus formation.[4] Indeed, Mitchell et al.[5] demonstrated that platelet FXIII-A exerts an antifibrinolytic function by cross-linking α2-antiplasmin into the fibrin network adjacent to platelet aggregates. Subsequently, Mattheij et al.[20] showed that on the surface of the platelet subpopulation expressing negatively charged procoagulant phospholipids upon activation by collagen and thrombin, fibrinogen bound to activated αIIbβ3 and cross-linked by cFXIIIa is responsible for the formation of so-called "star-like fibrin fiber formation",[21] thought to be involved in the anchorage of these platelets within the thrombus and eventually stabilization of the platelet-fibrin clot. These publications, which investigated cFXIII in platelets activated by the combined action of convulxin and thrombin, lead us back to the work on procoagulant COAT platelets, which had demonstrated that this subpopulation of platelets is able to retain α-granule proteins on its surface in a transglutaminase-dependent mechanism.[8,11,22] Thus, surface-exposed platelet cFXIIIa appears to exert at least three different functions.

The publications of Mitchell et al.[5] and Mattheij et al.,[20] and the work of Somodi et al. published in this issue of the *Journal of Thrombosis and Haemostasis* [6] enrich our picture of procoagulant COAT platelets, showing their ability to expose functional cFXIIIa on their surface. Further studies are still needed to elucidate the precise mechanisms involved in the externalization of cFXIII on activated platelets and all its functions. This new piece of knowledge in the puzzle of procoagulant COAT platelets provided by Somodi et al.[6] will stimulate the search for adjacent fitting pieces of the puzzle yet to be discovered.

CONFLICT OF INTEREST

The authors have no conflict of interest.

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AUTHOR CONTRIBUTIONS

Alessandro Aliotta and Lorenzo Alberio wrote the manuscript and developed Figure 1.

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