Trends in Endocrinology & Metabolism

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REVIEWS

Review

Platelets as Potent Signaling Entities in Type 2 Diabetes Mellitus

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Type 2 diabetes mellitus (T2DM) is a multifactorial disease with a dysregulated circulating inflammatory molecule tendency. T2DM is closely associated with systemic inflammation, endothelial dysfunction, cardiovascular risk, and increased clotting susceptibility. Platelets have fundamental roles in the development and propagation of inflammation and cardiovascular risk. They signal through membrane receptors, resulting in (hyper)activation and release of inflammatory molecules from platelet compartments. This review highlights how circulating inflammatory molecules, acting as platelet receptor ligands, interact with platelets, causing platelets to be potent drivers of systemic inflammation. We conclude by suggesting that focused platelet research in T2DM is an important avenue to pursue to identify novel therapeutic targets, and that platelets could be used as cellular activity sensors themselves.

Highlights

T2DM has a multifactorial nature, and systemic inflammation, endothelial dysfunction, with resulting hypercoagulation and platelet dysfunction, are key disease elements.

Increased circulating inflammatory markers are present in T2DM, and they originate from various sources, including the platelets themselves.

Platelets have important cellular crosstalk functions in T2DM, and they become proinflammatory entities, driving inflammation in this condition.

Platelets react to, for example, collagen and von Willebrand factor, and interfere with endothelial integrity.

Focused platelet research may be an important avenue to pursue to find novel therapeutic targets or to be used as cellular activity sensors themselves.

Diabetes as an Inflammatory Disorder

The prevalence rate of obesity, together with type 2 diabetes mellitus (T2DM) has increased throughout the world [1], and it is now described as a global pandemic, affecting over 20% of people aged >65 years [2]. One of the important considerations in the disease etiology is the presence of comorbid conditions [2]; including cardiovascular disease (and vascular calcification), atherosclerosis [3–5], microvascular disease [6], increased risk of myocardial dysfunction [7], coronary artery calcification [8]. Cardiovascular risk, together with dysregulated glucose and an increase in circulating inflammatory molecules, suggests that the condition should be classified as a true systemic inflammatory disorder.

Platelets have fundamental roles in both the development and the propagation of sustained inflammation, as well as cardiovascular risk, and this is also true for T2DM. Platelets signal through various membrane receptors (after ligand binding), followed by activation of downstream signaling pathways, resulting in the release of molecules from various platelet compartments (e.g., the dense or alpha granules, or the dense tubular network; Box 1 and Figure 1). These released platelet molecules themselves have inflammatory functions in circulation, by signaling to nearby platelets, as well as endothelia and other cells in circulation, (e.g., erythrocytes). Erythrocytes can promote thrombus formation and impact thrombus stability [9], and can themselves act as cellular markers for T2DM [10]. Furthermore, it has been shown that interactions between platelets and erythrocytes are enhanced in T2DM because of increased surface phosphatidylserine [11]. In T2DM, erythrocytes show high aggregability, together with enhanced platelet activation [12]. However, persistent and unregulated inflammatory molecule presence is one of the most important causes of platelet (hyper)activation, aggregation, and spreading, and may result in the platelets themselves acting as drivers of inflammation. (See Table 1.)

Platelet number, mean volume, and functional analysis are determined by well-known techniques used in diseases associated with thrombosis and abnormal clotting. Mean platelet volume and platelet distribution width values are easy-to-measure platelet parameters that have been associ-

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Box 1. Platelet Signaling in a Nutshell

Upon vessel wall damage, platelets are exposed to collagen, which activates platelets via outside-in signaling. Exposed collagen binds to GPVI/FcRγ (chain), resulting in activation of the signaling pathway that involves spleen tyrosine kinase (Syk), phospholipase Cγ2 (PLCγ2), and phosphatidylinositol 3-kinase (PI3K). This activation leads to cytoplasmic calcium mobilization, protein kinase C (PKC) activation, platelet shape change, and granule release [104]. Molecules released by platelets, such as ADP and thromboxane A2 (TXA2), not only amplify the platelet activation, but also recruit more platelets from circulation to the developing thrombus [104]. The thromboxane receptor (TP receptor) on platelets binds circulating TXA2. Fibrinogen binds to the integrin αIIbβ3, resulting in an association or complex between αIIbβ3 and FcγRIIa, followed by aggregation of adjacent platelets [21,126]. Cytoplasmic signals also cause resting, low-affinity integrin receptors to become active, resulting in a change to high-affinity conformations; these receptors have increased ligand binding affinity, and this is known as inside-out signal transduction. Ligand binding to active, high-affinity integrins further mediates and initiates a series of intracellular signaling events that result in morphological changes in platelets, with subsequent outside-in signaling [54]. The following associations (complexes) may result between the various platelet receptors: (i) FcγRIIa with integrin αIIbβ3 (e.g., fibrinogen binding) [21]; (ii) GPIb-IX-V with αIIbβ3 (e.g., vWF and thrombin binding) [21]; (iii) GPIb-IX-V and FcγRIIa and GPIb-IX-V with FcRγ (chain) [71]; and (iv) GPVI with FcRγ (chain) [fibrin(ogen) [73,97,101,102], collagen, CRP, and thrombin binding] [21].

ated with cardiovascular disease [13]; while platelet function devices that measure platelet activation and platelet aggregation are well-known techniques to determine abnormalities [14]. However, platelet bioenergetics and biomechanics data from such devices and how the

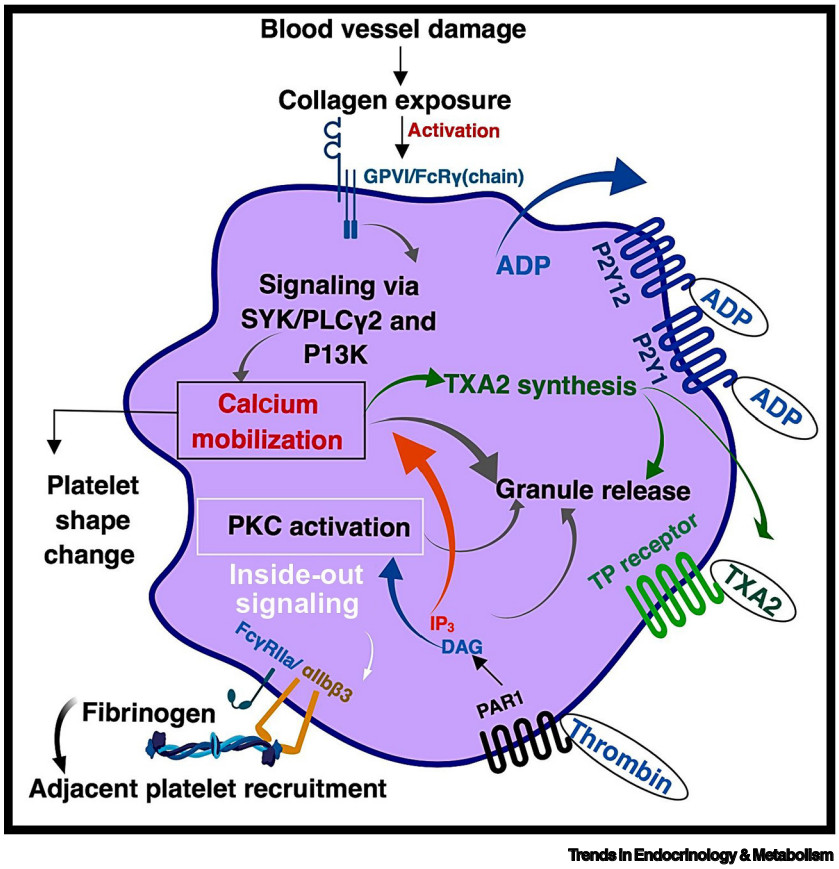

Figure 1. Platelet Signaling in a Nutshell. A simplified diagram of platelet activation and signaling after ligand binding. Figure created using BioRender (https://biorender.com/). Abbreviations: DAG, diacylglycerol; Ip_3, inositol trisphosphate; PI3K, phosphatidylinositol 3-kinase; PAR1, protease-activated receptor 1; PKC, protein kinase C; PLCγ2, phospholipase Cγ2; SYK, spleen tyrosine kinase; TP, thromboxane; TXA2, thromboxane A2.

Table 1. Well-Known Platelet Membrane Receptors and Their Functions

Receptor type	Membrane receptor and function	Refs
Receptors associated with antigen presentation	CD40 and CD40L (CD154): activation receptors, upregulated in thrombosis, inflammation, and atherosclerosis Major histocompatibility complex (MHC) class I: platelets directly activate naive T cells in a platelet MHC class I-dependent manner Fc receptor for IgG, (FcγRIIa or CD32): engagement by circulating ligands results in immune complexes that trigger intracellular signaling events, activation and aggregation; complexes include FcγRIIa/ Integrin αIIbβ3 and FcγRIIa / GPIb-IX-V	[20–23]
Activating and modulating platelet receptors	CD63: after platelet activation and granule exocytosis, CD63 translocates to the platelet membrane, where it colocalizes with the αIIbβ3-CD9 complex and is incorporated into the cytoskeleton Platelet C-type lectin-like receptor 2 (CLEC2) and Glycoprotein VI(GPVI): both induce platelet activation	[24]
Adhesion receptors	GPIb-IX-V (also known as GPIb-V–IX or GP1b-IX): both an adhesion and a major signaling receptor; the GPIbα subunit bears the binding site for vWF, leukocyte integrin αMβ2, P-selectin, and coagulation factors (e.g., thrombin, XI, XII, and Mac-1) p-selectin: after platelet activation, it mediates rolling of platelets and leukocytes on activated endothelial cells CD147 (EMMPRIN): localized to the open canalicular system (OCS), and potentially within α-granules, because its stimulated expression coincides with CD62P release to platelet surface	[25–29]
Integrins	Integrin αVβ3: assembles fibronectin fibrils on platelets and mediates cell adhesion to extracellular matrix Integrin αIIbβ3: binds fibrinogen and vWF, causing activation and spreading Integrin α2β1: binds collagen	[30–34]
GPCRs	Protease-activated receptors PAR1 and PAR4: binding of thrombin and ADP causes activation P2Y1 and P2Y12: binding of ADP causes activation and aggregation tpα and tpβ receptors: binding of thromboxane A2 (TXA2) causes activation	[35–38]

relationship between parameters should be applied as part of clinical interpretations, and also in translational research, are frequent points of debate [15]. Efficiency of treatment regimens can also be analyzed using platelet function tests. Furthermore, various treatment options for when platelet dysfunction is diagnosed are available to clinicians [14,16]. A recent paper discussed and assessed the usefulness of nine different platelet function tests to determine dose responses to aspirin in T2DM [17].

Recently, the development of user-friendly point-of-care methods to assess platelet reactivity has increased the frequency of platelet function testing in clinical practice. However, prospective, randomized trials have 'failed' to demonstrate that personalized antiplatelet therapy based on point-of-care assessment of platelet function is effective in reducing ischemic events [16]. The main confounding factor of platelet function tests is the fact that platelets are activated easily and, thus, the time from platelet collection to laboratory analysis is crucial. However, an important consideration is to use translational approaches to identify innovative instruments to study mechanisms of platelet release [18] and to find novel point-of-care devices to be used in precision and/or personalized medicine. For such goals to succeed, platelet receptor presence and number, as well as

(inflammatory) molecules derived from platelets or in circulation that (hyper)activate platelets, might be new avenues to focus on, instead of conventional platelet function tests.

In this review, we show how dysregulated circulating inflammatory molecules in T2DM may interact with platelet receptors and how this interaction, in turn, perpetuates systemic inflammation and might have a central role in the cardiovascular risk that accompanies the disease. The presence of these circulating molecules and platelet receptors might be better indicators of platelet health than current platelet indices and function tests.

Recent studies confirmed that vascular calcification is associated with inflammatory status and is enhanced by inflammatory cytokines [5] in the circulation; in addition, a plethora of cytokines are implicated in the development of vascular dysfunction and vascular disease [19]. Evidence of the presence of various risk factors that accompany T2DM suggests that circulating biomarkers known to be present in cardiovascular disease, vascular calcification, myocardial dysfunction, and microvascular disease should be looked at more closely. Platelets have various receptors on their membranes that interact and complex with each other. These include four main types: receptors associated with antigen presentation; activation and modulating receptors; adhesion receptors and integrins; and G-protein-coupled receptors (GPCRs) (see Table 1 for a brief explanation of the main types of receptors and their functions). In inflammatory conditions and T2DM, these receptors interact with dysregulated circulating biomarkers that act as ligands, resulting in platelet hyperactivity, spreading, and aggregation.

Dysregulated Circulating Biomarkers in T2DM

Various dysregulated circulating biomarkers are found in T2DM and act as ligands to stimulate and (hyper)activate platelets, resulting in an inflammatory milieu which indicates that platelets have a fundamental role in driving cardiovascular risk in T2DM. Dysregulated and circulating inflammatory molecules known to be present in T2DM, but that are also implicated in cardiovascular disease, include tumor necrosis factor-α (TNF-α), interleukin (IL)-1, and IL-6 [39–45]. TNF-α, IL-1, and IL-6 can stimulate the release and expression of procoagulant molecules, such as von Willebrand factor (vWF), plasminogen activator inhibitor 1 (PAI-1), and tissue factor (TF), and inhibit the expression of anticoagulant molecules, such as thrombomodulin, by endothelial cells [40]. Together with dysregulated thrombin and fibrin(ogen) levels, these cytokines are prominent participants in driving inflammation in T2DM.

IL-1β and Thrombin as Signaling Molecules

IL-1β is particularly well studied in T2DM and is known to be increased in circulation [44,46–48]. Both IL-1α and IL-1β may also originate from the platelets themselves [49,50]. Platelets are also known to express the receptor IL-1R1, and IL- 1α and IL-1β both stimulate heteronuclear IL-1β splicing and translation of newly made mRNA in platelets [51]. Furthermore, platelets respond to the IL-1β that they synthesize themselves, which is exclusively associated with shed microparticles; this cytokine also further acts as a platelet agonist that promotes its own synthesis [51]. mRNA encoding IL-1β is present in platelets and associated with polysomes; IL-1β is also synthesized by activated platelets due to exposure to thrombin, resulting in signaling with endothelial cells [50]. Thrombin and IL-1β release are also closely linked. The platelet signaling process, in which ligand binding (in this case thrombin) is involved, results in bi-directional activation via IL-1β. This is a complex process and it involves both inside-out and outside-in signaling processes.

It is known that thrombin levels are dysregulated in T2DM, and thrombin generation potential in patients with T2DM is also associated with increased fasting insulin and insulinogenic index [52]. Furthermore, in T2DM, increased thrombin formation is associated with hypofibrinolysis and a prothrombotic fibrin clot phenotype [53]. When increased levels of thrombin are present, there is

also the potential for (cleaved) thrombin to bind to the protease-activated receptor 1 (PAR1), which is on the platelet membrane. The activation process is discussed in detail elsewhere [54,55]. Cleaved thrombin binding leads to phospholipid hydrolysis, which results in the generation of inositol trisphosphate (IP_3) and diacylglycerol (DAG), and an increase in cytosolic free Ca^{2+}. The elevated concentration of Ca^{2+} and DAG, which in turn activates CALDAG-GEFI and protein kinase C (PKC), converts RAP1 from a GDP-bound to a membrane-attached GTP-bound form. Activation of RAP1 leads to recruitment of its effectors, RAP1-GTP-interacting adaptor molecule (RIAM), and its binding partner, talin 1, to the plasma membrane. This facilitates access of talin to the integrin β3 tail and talin-induced activation of integrin αIIbβ3. Therefore, thrombin binding can ultimately result in membrane exposure of activated adhesive proteins, including αIIbβ3, αvβ3, α2β1, and P-selectin [29]. Following activation of, for example, αIIbβ3, transmitted outside-in signals can result in translational mechanisms that regulate IL-1β synthesis [50]. Integrins such as αIIbβ3 behave like traditional signaling receptors in transmitting information into cells by outside-in signaling; binding of integrins to their extracellular ligands changes the conformation of integrin and, because many of the ligands are multivalent, this contributes to integrin clustering [55].

Therefore, increased circulating thrombin in T2DM has the potential to bind to platelets, activate integrins, and also result in the release of IL-1β; this released IL-1β, in turn, acts as a platelet agonist, resulting in platelets shedding microparticles that contain IL-1β. An increase in circulating IL-1β (together with other dysregulated circulating cytokines) induces endothelial adhesiveness [50]. Microparticles can activate platelets through the IL1R1 that they express, and IL1β-containing microparticles activate endothelium; thus, these platelet microparticles have the potential to act on the surrounding vasculature [51]. As mentioned earlier, human platelets also express IL1R1 themselves, the receptor for IL-1β, and it was suggested that IL-1R1/IL1β cause platelet activation [56], thereby promoting a prothrombotic environment during infection and obesity; potentially contributing to the development of atherothrombotic disease [57]. This supports the importance of both platelet-generated IL-1β, as well as circulating IL-1β (originating from other sources) and increased levels of thrombin, in T2DM. Box 1 and Figures 1 and 2 provide further discussion and simplified diagrams of a possible route of thrombin (and collagen) signaling in T2DM platelets.

The GPIb-IX-V complex also binds thrombin (Figure 2) and is important for low-dose thrombin-induced platelet activation [21,58]. GPIb-IX-V complex signaling induced by either vWF or thrombin both require the binding of 14-3-3ζ to the cytoplasmic domain of GPIbα, a Src family kinase (SFK) (Lyn and possibly Src)-Rac1 signaling pathway, and downstream activation of the phosphoinositide 3-kinase (PI3K)-Akt and cGMP-dependent protein kinase (PKG), mitogen-activated protein kinase (MAPK) (ERK1/2 and p38) and LIM kinase 1 (LIMK1) pathways. It is interesting that the role of LIMK1 in stimulating vWF and low-dose thrombin-induced platelet activation is specific for the GPIb-IX-V complex signaling pathway, because LIMK1 appears to have a negative role in platelet activation induced by GPIb-IX-V-independent platelet agonists. LIMK1 promotes vWF-stimulated activation of cytosolic phospholipase A2 (cPLA2), as well as thromboxane A2 (TXA2) production [21] (Figure 2).

IL-6 as a Signaling Molecule

IL-6, and its downstream product serum C-reactive protein (CRP), is also a well-known inflammatory molecule that is dysregulated in T2DM and present in circulation. IL-6 exerts its biological activities through two molecules: IL-6 receptor (IL-6R) and the membrane-bound β-receptor glycoprotein 130 (gp130) [59,60]. Transduction of the signal is mediated by gp130 and by *trans*-signaling, where IL-6 binds to soluble forms of IL-6R (sIL-6R). Resting platelets express gp130 on their membranes and, in the presence of IL-6 (produced by stressed endothelial cells), platelet-derived IL-6 *trans*-signaling occurs, which could be crucial in the development of

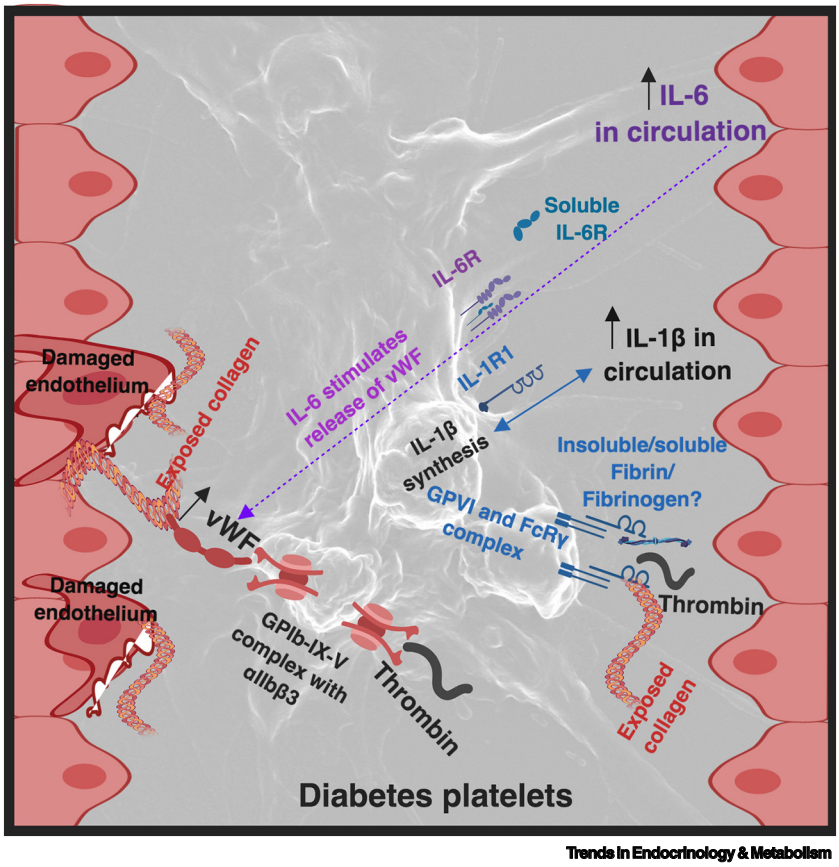

Figure 2. Collagen, Thrombin, and Fibrin(ogen) Signaling via Various Complexing Receptors in Type 2 Diabetes Mellitus (T2DM). For a discussion on the binding of soluble versus insoluble fibrin and fibrinogen see [101]. Adapted from [21,55,102]. Figure created using BioRender (https://biorender.com/). Abbreviations: IL, interleukin; vWF, von Willebrand factor.

inflammation within a damaged vessel [61] (Figure 2). Molecular events in IL-6 signaling are initiated by binding to its receptor subunit IL-6Rα (which has no intrinsic kinase activity) [62]. Receptor ligation can induce conformational changes in the cytoplasmic domains of gp130 that bring Janus tyrosine kinases (JAKs) into close proximity, leading to the activation of transcription factors of the signal transducers and activators of transcription (STAT) family. Another major signaling pathway for IL-6-type cytokines is the MAPK cascade [63]. IL-6 and CRP both actively modulate adaptive and pathological responses to cardiovascular stress [62]. The JAK2/STAT3 signaling pathway is also a prominent pathway in platelets [64] and is involved in collagen-induced platelet activation through the activation of JAK2-JNK/PKC-STAT3 signaling [65]. STAT3 signaling regulates collagen and thrombin-induced platelet activation and aggregation. Furthermore, STAT3 signaling is one of the important pathways mediating receptor signals and promoting αIIbβ3-talin interaction and integrin activation [66,67].

CRP also binds to the GPVI/FcRγ (chain) complex, causing shedding by metalloproteases, such as a-disintegrin-and-metalloproteinase 10 (ADAM10) [68]. Furthermore, GPVI can be shed from the platelet membrane by the release of soluble ectodomain fragments, which are then found in plasma [69]. This might have clinical implications for T2DM.

Collagen and vWF as Signaling Molecules

Collagen and vWF are two important signaling molecules and endothelial cells are attached to subendothelial collagen by vWF, which they produce. When the endothelial layer is disrupted, collagen is exposed and vWF binds to this exposed collagen, anchoring platelets to the subendothelium [70], causing platelet aggregation [31] and formation of a platelet plug [64]. vWF binding is mediated by GpIbα (which is part of GPIb-IX-V) and the integrin αIIbβ3 complex [71]. This αIIbβ3 receptor also binds fibrinogen (discussed later) and both fibrinogen and vWF work together to have critical roles in aggregation (Figure 3). GpIbα is abundantly expressed at the platelet surface [72]. Initial adhesion of platelets to the blood vessel wall requires the interaction between immobilized vWF on the surface of endothelium or in the subendothelial matrix with its platelet receptor, the GP1b-IX-V complex, while stable adhesion of platelets initiated by vWF/GPIb-IX-V complex binding requires the activation of integrin αIIbβ3 [21] (Figure 3).

The platelet receptor GPVI also interacts with collagen and several other adhesive macromolecules, and is involved in amplification of thrombin generation and further recruitment of circulating platelets to clots [73,74]. The signaling pathway involves the formation of a noncovalent complex between GPVI and FcRγ (chain) (Figure 2) and, upon collagen-induced clustering of GPVI,

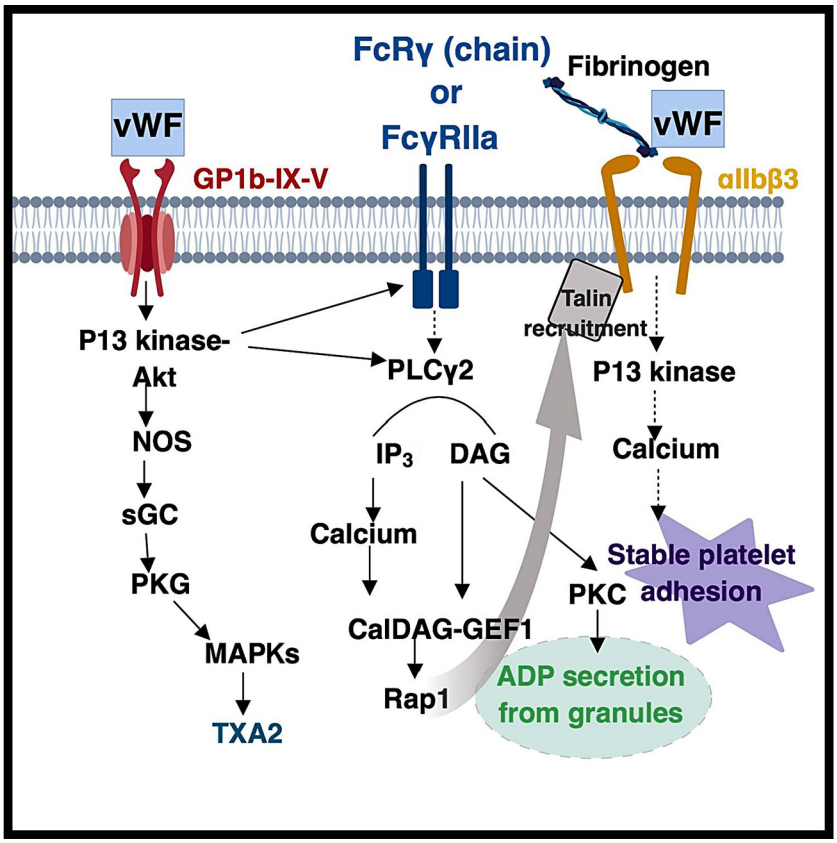

Figure 3. The (Possible) Signaling Pathway of von Willebrand Factor (vWF) and Fibrinogen via the GPIb-IX-V and Integrin αIIbβ3 Complex [71]. Figure created using BioRender (https://biorender.com/). Abbreviations: DAG, diacylglycerol; Ip_3, inositol trisphosphate; MAPK, mitogen-activated protein kinase; NOS, nitric oxide synthase; PI3, phosphoinositide 3; PKC, protein kinase C; PKG, cGMP-dependent protein kinase; PLCγ2, phospholipase Cγ2; sGC, soluble guanylate cyclase; TXA2, thromboxane A2; vWF, von Willebrand factor

immunoreceptor tyrosine based activation motif (ITAM) in the associated FcRγ (chain) is tyrosine phosphorylated by SFK, which enables binding of the tyrosine kinase Syk [21]. Activated Syk initiates a cascade of events and, ultimately, DAG and IP3 activate PKC and release calcium into the cytosol from intracellular stores, promoting thromboxane production and granule secretion, inside-out signaling, and integrin activation [21]. Therefore, in complex with the FcRγ (chain), GPVI is a major collagen signaling receptor on platelets. Engagement of GPVI activates intracellular signaling events, leading to platelet aggregation through αIIbβ3, and to activation of the collagen-binding integrin, α2β1, which stabilizes the interaction of platelets with fibrillar collagen [75].

As mentioned earlier, IL-6 in circulation can also stimulate the release and expression of vWF [40]. IL-6, collagen, and vWF are all increased in T2DM. Oxidative post-translational modified collagen, particularly type II collagen has also been shown in, amongst others, diabetic neuropathy [76]. It has also been found that type VI collagen formation predicts cardiovascular events, all-cause mortality, and disease progression in patients with T2DM and microalbuminuria [77]. Furthermore, α1-type IV collagen, α2-type IV collagen, γ1-laminin, and β2-laminin are also known to be significantly increased in T2DM [78], as is vWF [79,80]. The presence of increased levels of IL-6, collagen, and vWF in T2DM, and the ability of these molecules to bind to platelet receptors, resulting in platelet activation and participation in the inflammatory process, confirms the vital role of both these ligands and platelets in inflammation in T2DM.

As mentioned earlier, thrombin is also an important signaling molecule in inflammation and is involved in the terminal stages of the coagulation cascade, particularly in the conversion of soluble fibrinogen to insoluble fibrin; it has been suggested that thrombin promotes diet-induced obesity through fibrin-driven inflammation [81]. Thrombin also signals through PAR1 and PAR4 [82], both of which occur in platelets [37,38] and also signals via ADP [83]. When thrombin binds to PAR1, it forms heterodimers with PAR4 and this enhances PAR4 cleavage [21,84]. PAR4 and P2Y12 can also dimerize, and their interaction promotes PAR signaling, ultimately leading to MAPK activation and granule secretion (Figure 4) [21]. Thrombin is dysregulated in T2DM [53] and there is higher thrombin generation in patients with T2DM with cardiovascular disease compared with patients with T2DM but without cardiovascular disease [85].

TNF-α as a Signaling Molecule

TNF-α is an important signaling molecule that can interact with platelet receptors and is able to activate platelets through stimulation of the arachidonic acid pathway [86]; platelet CD40L is upregulated by TNF-α via a cyclooxygenase-1-independent, arachidonic acid-mediated oxidative stress mechanism [87]. Multiple platelet agonists, including collagen, thrombin, and ADP, induce exposure of CD40L from platelets. CD40L stimulates resting platelets by binding to constitutively expressed CD40 during direct cell–cell contact, enhancing proinflammatory responses [88]. Two TNF-α receptors are found on platelets (TNFR1 and TNFR2); these can also be shed in soluble form [87] and are known to cause platelet activation and clumping [89]. TNF-α is increased in T2DM [39,41,90], and TNF-α, its receptor TNF-R, and ICAM-1 are important markers of platelet abnormalities during the development of microvascular complications in T2DM [91].

ADP as a Signaling Molecule

ADP is another molecule that is involved in inflammation. The ADP receptors P2Y1 and P2Y12 [92,93] are involved in platelet activation and aggregation [35,94]. P2Y12 is physiologically activated by ADP, resulting in platelet aggregation [95]. ADP is also released from dense granules and requires both Gαq-coupled P2Y1 and Gαi-coupled P2Y12 signaling for platelet activation [21]. In T2DM, P2Y12 receptor expression is significantly increased and the receptor is constitutively activated; furthermore, P2Y12 expression correlates with ADP-induced platelet aggregation [96].

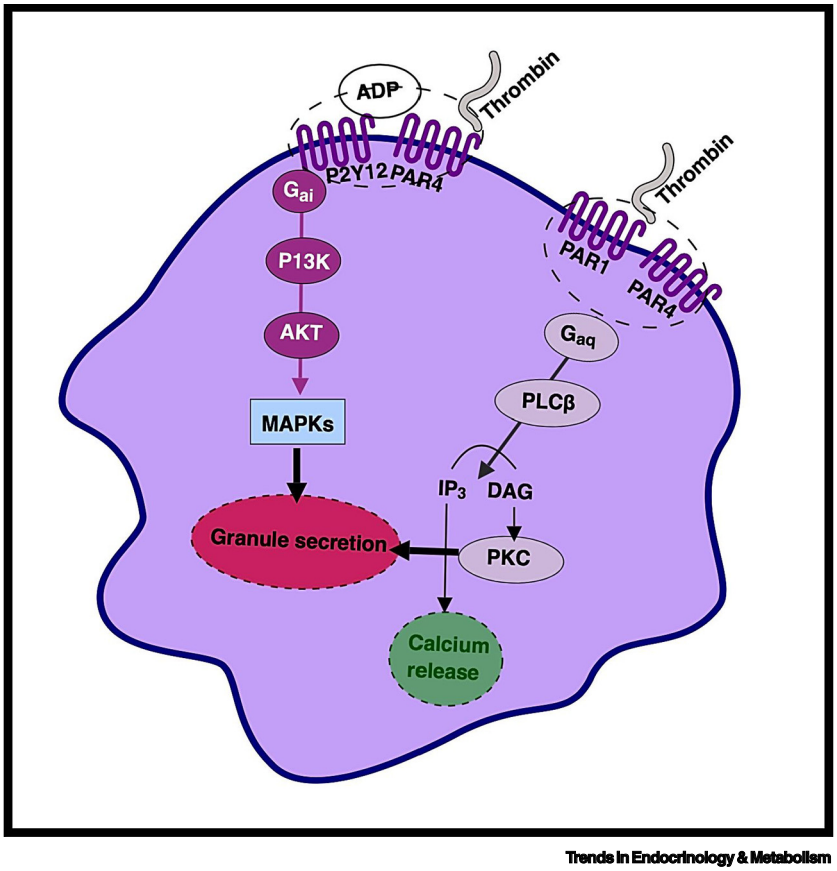

Figure 4. Protease-Activated Receptor (PAR)-1, PAR4, and P2Y12 Complex Formation after Signaling via Thrombin, Resulting in Protein Kinase C (PKC) and Mitogen-Activated Protein Kinase (MAPK) Pathways Involved in Granule and Calcium Release. Figure created using BioRender (https://biorender.com/). Abbreviations: DAG, diacylglycerol; Ip_3, inositol trisphosphate; PI3K, phosphatidylinositol 3-kinase; PLCβ, phospholipase beta.

Fibrin(ogen) as a Signaling Molecule(s)

Fibrin(ogen) is an important signaling molecule and is fundamental in causing hypercoagulation that ultimately results in vascular damage and cardiovascular disease. Fibrinogen levels are frequently elevated in patients with T2DM, particularly when comorbidities such as cardiovascular complications and diabetic nephropathy are present [97–99]. In T2DM, abnormally folded fibrin(ogen) clots have also recently been found, where the fibrin(ogen) molecules have a changed conformation in the presence of thrombin, rendering them amyloid in nature [100]. The structure of this amyloid fibrin(ogen), its precise role, whether fibrin molecules are partly soluble/insoluble, and which of these conformations might be the main culprit in abnormal clotting and platelet signaling, are not yet fully understood. Researchers have investigated whether soluble fibrinogen or insoluble/soluble fibrin (or both) binds via the GPVI/ FcRγ (chain); and it has been suggested that solving the structure of GPVI by mapping the binding epitopes and conformation of GPVI during binding with fibrin and/or fibrinogen, as well as collagen, will add valuable information [101]. Within a forming clot, polymerized (insoluble) fibrin is both a ligand and an agonist of platelet GPVI signaling [73]. Platelet GPVI binding to (insoluble) fibrin surfaces increases platelet procoagulant activity [102], and amplifies collagen-independent thrombin generation and platelet recruitment at the clot surface [68]. Lee and coworkers reported that fibrinogen can be activated to desA and desB soluble fibrin monomers, which immediately bind fibrinogen (an assembly also considered,

confusingly, to be a soluble fibrin monomer); however, the function of soluble fibrin on platelets in suspension is less well understood [68]. Therefore, soluble and/or insoluble fibrin or soluble fibrinogen all might act on the GPVI/FcRγ (chain) complex (Figure 2).

As mentioned earlier, αIIbβ3 is also an important receptor for fibrinogen [31] and this binding may have an essential role in platelet spreading [32]. Upon platelet activation, inside-out signaling pathways increase the affinity of αIIbβ3 for fibrinogen (and other ligands). Binding to active integrin αIIbβ3 receptors triggers outside-in signaling that is dependent on FcγRIIa ITAM/Syk/PLCγ2, and PI3K/Akt to amplify platelet activation and facilitate the formation of stable thrombi [103,104] (Box 1 and Figure 1). Here, both fibrin and fibrinogen also interact with αIIbβ3, although the interaction of fibrin and fibrinogen with αIIbβ3 is mediated through distinct epitopes: fibrinogen binds to αIIbβ3 via the C-terminal peptide sequence of the γC-peptide (GAKQAGDV), while fibrin binds to the integrin through a unique sequence in the γC-peptide, ATWKTRWYSMKK, which binds to the αIIb β-propeller [102].

Glucose as a Signaling Molecule

It is well known that hyperglycemia, glycemic variability, and insulin resistance are determinants and predictors of platelet activation [88]. Intraplatelet glucose concentration mirrors the extracellular concentration, and chronic hyperglycemia has been clearly identified as a causal factor for *in vivo* platelet activation and platelet hyper-reactivity in T2DM [105]. Aldose reductase (AR) is the first enzyme of the polyol pathway, which converts excess glucose to sorbitol accompanied by an increase in the cytosolic NADH/NAD+ ratio; therefore, signaling events downstream of GPVI are influenced by hyperglycemia, oxidative stress, and shear stress [106]. Both AR activity and expression are upregulated following GPVI-dependent platelet activation [107]. Thus, AR is essential for GPVI-dependent signal transduction, which includes increased PLCγ2/PKC/p38 MAPK activation, ultimately leading to increased TxA2 generation [108].

TXA2 is important in the presence of glucose and drives platelet activation. Inflammatory mediators derived from platelets, such as soluble CD40 ligand, and soluble receptor for advanced glycation-end-products (sRAGE), may be involved in platelet signaling [88]. As mentioned earlier, CD40 and CD40L are also important mediators of platelet activation. It was suggested that CD40L is released during TXA2-dependent platelet activation [22]. Furthermore, upregulation of CD40L is also involved in the advanced stage of cerebrovascular disease in T2DM and is associated with increased risk of cardiovascular events [109]. Platelets from patients with T2DM synthesize more TXA2 compared with normal platelets in response to, among other agonists, high circulating glucose, and enhanced platelet biosynthesis of TXA2 has been associated with cardiovascular risk factors [88]. In addition, high glucose levels were also found to enhance ADP- and thrombin receptor-activating peptide (TRAP)-induced platelet P-selectin expression, as well as TRAP-induced platelet fibrinogen binding [110]. Furthermore, mean platelet volume and mass have also been positively correlated with fasting glucose and HbA1c in T2DM [88]. Human platelets express both insulin and insulin-like growth factor-1 (IGF-1) receptors, and it has been shown that their subunits may randomly heterodimerize to form insulin/IGF-1 receptor hybrids, which avidly bind IGF-1 but not insulin [111]. Interestingly, insulin has minimal effects on platelet function, given that they have relatively low insulin receptor expression levels. However, an association between insulin receptor substrate-1 polymorphisms and high platelet reactivity with clopidogrel therapy in patients with coronary artery disease and T2DM has been found [112]. Platelet glycosylation may also be important in T2DM, because platelet glycosylation is closely related to platelet function, survival, high platelet reactivity, and ultimately increased risk of thrombosis and coronary heart disease in T2DM [113].

Concluding Remarks

Taken together, increased glucose levels, dysregulated circulating inflammatory marker presence, and the increased presence of molecules associated with (hyper)coagulation classify

Outstanding Questions

How can the research community effectively communicate with medical practitioners with regard to the importance of platelets, their receptors and signaling pathways, as well as the inflammatory molecules that platelets themselves produce?

How can we more actively pursue the use of precision and personalized medicine in T2DM research?

How can we increase machine-learning research and incorporate IOT in existing data analysis regimes?

T2DM as a true systemic inflammatory condition. These dysregulated circulating molecules both originate from, and participate in, platelet dysfunction, placing platelets as important players that are central to the abnormal blood clotting and increased cardiovascular propensity seen in T2DM.

Abnormal clot formation is a feature of T2DM [53,114–118]. Here, we have reviewed literature highlighting the physiological effects of these dysregulated molecules on platelets and their receptors, confirming that they are important facilitators in inflammatory cellular crosstalk. Therefore, platelets are central in driving abnormal clot formation in the presence of dysregulated inflammatory molecules, and these inflammatory molecules act as ligands that directly bind to the various platelet receptors [119]. This binding causes various pathways to be initiated, ultimately resulting in platelet activation, spreading, clumping, and microparticle formation. Although these processes are part of the healthy coagulation cascade and platelet activation during blood clot formation, the increased presence of dysregulated makers (that may act as platelet receptor ligands) results in atypical, increased, and persistent platelet (hyper)activation. These dysregulated processes drive increased cardiovascular risk, as well as chronic and systemic inflammation, ultimately resulting in an increased propensity for abnormal clotting. Furthermore, it appears that the precise structure of the fibrin(ogen) clot, the presence of various conformations, and their interactions with platelet receptors may have important roles in abnormal clotting due to hypercoagulation, and might be of particular importance in T2DM.

As with many inflammatory diseases, glucose intolerance, as well as the early and late stages of T2DM, with its various comorbidities, is multifactorial in nature. Therefore, both precision and personalized medicine together with disruptive technologies that identify novel biomarkers;, perhaps combined with the use of novel, cost-effective biosensors, machine-learning possibilities, and cloud-based data storage, might prove to be the only way to curb the current diabetes pandemic. The emergence of biomarker technologies has allowed more targeted therapeutic strategies (precision medicine) to be developed for diabetes prevention [120]; however, currently, the use of such technologies is predominantly confined to pharmacotherapy. Biomarker usage for personalized lifestyle recommendations in T2DM treatment is also possible [121]. Therefore, both personalized and precision medicine approaches in T2DM should be an important and urgent consideration [122]; furthermore, researchers should actively pursue machine learning and Internet of Things (IOT) with existing biomarkers and platelet data (see Outstanding Questions). A question that might also arise is whether genetics and the transcriptome have any roles in T2DM platelet changes in particular, given that several diseases are known to impact the platelet transcriptome [123]. Although there is research that points to potential mechanisms involving transcriptome and gene expression differences in T2DM [124], a recent paper noted that uncontrolled T2DM only had a small impact on different components of the platelet transcriptome [125].

Thus, focused platelet research in T2DM may be an important avenue to pursue to find novel therapeutic targets or to use platelets themselves as cellular activity sensors, given their extreme sensitivity to dysregulated circulating inflammatory markers.

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