Chapter

Molecular Aspects of Pathophysiology of Platelet Receptors

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Abstract

Receptor is a dynamic instrumental surface protein that helps to interact with specific molecules to respond accordingly. Platelet is the smallest in size among the blood components, but it plays many pivotal roles to maintain hemostasis involving its surface receptors. It (platelet) has cell adhesion receptors (e.g., integrins and glycoproteins), leucine-rich repeats receptors (e.g., TLRs, glycoprotein complex, and MMPs), selectins (e.g., CLEC, P-selectin, and CD), tetraspanins (e.g., CD and LAMP), transmembrane receptors (e.g., purinergic—P2Y and P2X1), prostaglandin receptors (e.g., TxA2, PGH2, and PGI2), immunoglobulin superfamily receptors (e.g., FcRγ and FcεR), etc. on its surface. The platelet receptors (e.g., glycoproteins, protease-activated receptors, and GPCRs) during platelet activation are over expressed and their granule contents are secreted (including neurotransmitters, cytokines, and chemokines) into circulation, which are found to be correlated with different physiological conditions. Interestingly, platelets promote metastasis through circulation protecting from cytolysis and endogenous immune surveillance involving several platelets receptors. The updated knowledge about different types of platelet receptors in all probable aspects, including their inter- and intra-signaling mechanisms, are discussed with respect to not only its (platelets) receptor type but also under different pathophysiological conditions.

Keywords: platelet receptors, thrombus, cancer, aging, cardiovascular disease, viral infection

1. Introduction

Platelets including other blood components were first time drawn by George Gulliver in 1841 (though it was not named platelet at that time) when he was working with a newly made compound microscope with twin lens [1]. Giulio Bizzozero at the end of the nineteenth century [2] first time coined the term and identified as platelets. Blood platelet in its form is found in mammals, but in birds and amphibians, it is present in circulation as intact mononuclear thrombocytes [3]. Platelets are not true cells, and it is classified as cell fragments (from megakaryocyte by the megakaryocytopenisis) having no nucleus inside. Platelets circulate in the bloodstream and remain alive for 7–10 days. They (platelets) principally survey the inner lining of blood vessels. If they detect any breaches, they seal them in the vasculature by the creation of thrombi [4]. Platelets generally remain in inactive state.
| Sl. no. | Classes of receptors | Types of receptors | Family | Ligands | Involvement | References |
|--------|----------------------|--------------------|--------|---------|-------------|------------|
| 1.     | GP                   | GPIb-IX-V complex  | Type I membrane spanning GP and leucine-rich repeat | vWF, thrombin, P-selectin, αMβ2, and Mac-1 | Initiation of platelet recruitment | [5–22] |
|        | GPVI                 | Immunoglobulin (Ig) |        |         | Platelet aggregation | [23–26] |
|        | GPVI-FcRγ            | Transmembrane      |        |         | Adhesion with collagen | [7, 27, 28] |
|        | Integrins (αIIbβ3, αvβ3, α2β1, α5β1, and α6β1) | Transmembrane | Fibrinogen or vWF, vitronectin, collagen, fibronectin, and laminin, respectively | | Platelet aggregations | [7, 29–35] |
| 2.     | C-type lectin receptor | CLEC-2            | Type II membrane protein C-type lectin receptor | Podoplanin and rhodocytin | Platelet aggregation | [36, 37] |
| 3.     | Thromboxane         | TP (α and β)       | Transmembrane and GPCR | TXA₂ | Platelet aggregation | [38–40] |
| 4.     | Prostaglandin (PG)  | PGE2 and PGI2      | GPCR   | PG      | Aggregation as well as inhibition of aggregation | [41–45] |
| 5.     | Thrombin            | PAR-1 and -4       | Transmembrane | Thrombin | Adhesion, spreading, and secretion | [19, 46] |
| 6.     | Ephrin kinase       | EphA and EphB      | Tyrosine kinase | Ephrins | Inhibition in aggregation | [47, 48] |
| 7.     | Purinergic          | P2Y1 and P2Y12     | Transmembrane and GPCR | ADP | Amplification of aggregation | [49–51] |
|        | P2X1                 |                    |        | ATP     |             |            |
| 8.     | TAM                  | TAM tyrosine kinase | Gas6 | | Stabilization of aggregation | [7, 52, 53] |
| 9.     | Tetraspanin         | CD151              | Tetraspanin | Fibrinogen | Aggregation, stabilization of aggregates | [7, 54–65] |
|        |                      | TSSC6              | | Not known |              |            |
|        |                      | TLT-1              | | Not known | |            |
|        |                      | CD36               | | vWF, oxPL, TSP1, and oxLDL | |            |
|        |                      | PEAR1              | | FcεR1α | |            |
| 10.    | P-selectin          | P-selectin        | Selectin | PSGL-1 | Clot formation with leukocytes | [7, 65] |
and get activated only when a blood vessel is damaged. But hemostasis or blood coagulation is not the sole function of platelets; rather, it is employed in several multifunctional attributes monitoring the homeostasis of the body. The advancement of understanding of platelet pathology has led scientists to go through the receptors for its instrumental role in different pathological conditions. There are 16 different classes of platelet receptors. Each class of some of these receptors has subtypes (Table 1). Platelet receptors (Figure 1), the surface proteins, are at the forefront of the recent research, and major advances have been made in understanding their molecular functions as well as their downstream signaling pathways. The experimental strategies with pharmacological inhibition and knocking out

| Sl. no. | Classes of receptors | Types of receptors | Family | Ligands | Involvement | References |
|---------|----------------------|--------------------|--------|---------|-------------|------------|
| 11. ITIM | PECAM-1 | Transmembrane and Ig | PECAM | Inhibition in thrombus formation |
|         | G6b-B | Transmembrane and Ig | HS | Platelet production and activation |
|         | VPAC1 | Transmembrane and Ig | PACAP | Inhibition in platelet activation |
| 12. TLR | TLR-1, -2, -3, and -4 | Lipoprotein | Peptidoglycan and pathogen | Inflammatory response |
| 13. Serotonin (5-HT) | 5-HT 2A and 5-HT 3 | Transmembrane | Serotonin | Thrombus formation, vasodilation |
| 14. Leucine-rich receptors | GPIb-IX-V complex | Transmembrane and Ig | vWF, thrombin, P-selectin, αMβ2, and Mac-1 | Initiation of platelet recruitment |
|         | TLRs | Lipoprotein | Peptidoglycan and pathogen | Inflammatory response |
| 15. Complement receptors | CR2, CR3, CR4, C3aR, C5aR, gC1qR, and cC1qR | Complement | Pathogens | Antimicrobial host defense |
| 16. DC-SIGN | DC-SIGN | Non-integrin | Viral pathogens | Binding platelet with viral pathogens |

Table 1. Classes of platelet receptors and their involvement in the maintenance of physiological hemostasis.

GP: glycoprotein; vWF: von Willebrand factor; TP: TXA2/PGH2 receptor; CLEC-2: C-type lectin-like type II transmembrane receptor; GPCR: G protein-coupled receptor; TX: thromboxane; PAR: protease-activated receptors; Eph: ephrin; TAM: Tyro 3, Axl, and Mer; TSSC6: tumor suppressing STF cDNA 6; Gas: growth arrest specific; TLT: TREM-like transcript-1; PEAR: platelet endothelial aggregation receptor-1; PECAM-1: platelet endothelial cell adhesion molecule; VPAC1: vasoactive intestinal peptide/pituitary adenylate cyclase-activating peptide receptor 1; PACAP: pituitary adenylate cyclase-activating polypeptide; TLR: Toll-like receptor; 5-HT: 5-hydroxytryptamine, serotonin; DC-SIGN: dendritic cell-specific ICAM3-grabbing non-integrin.
of nearly all known receptors and many signaling molecules have helped to reveal new mechanisms for how the thrombotic and hemorrhagic propensity of platelets is controlled in health and disease [79]. The firm adhesion of platelets to the injury site forms first a monolayer. This monolayer acts like a reactive site for other free moving platelets to replenish further. The accumulation of platelets is found to depend on vWF and fibrinogen that act like cross-linking adjacent to the platelets and promotes a stable aggregated platelets or clot [80, 81]. This process is essential for the formation of primary hemostatic clot and also for the development of pathological thrombi at site ruptured [82]. Further activation in developing thrombus converts platelets to a procoagulant phenotype, from a proaggregatory phenotype, which enables the assembly of the coagulation reaction complexes (the tenase and prothrombinase complex) on the platelet surface, necessary for thrombin and fibrin generation. These fibrins form a fibrin network and trap the RBCs and convert the white thrombus to the red clot [83]. This supports secondary hemostasis or red clot [83]. However, physiological thrombus formations or mural thrombi are found to be tightly regulated to avoid the excessive platelet accumulation at the site of injury. The vascular obstructions that are the principal pathological processes, to some extent, cause heart attacks and/or ischemic stroke as well [84]. During initiation, progression, and termination of this physiological thrombus formation, several receptors of platelet get involved as soon as the damage on the blood vessel wall happens. There are different surface receptors on the platelet which are involved not only in blood coagulation process but also in other immunogenic and/or pathogenic responses, even in neurodegeneration. In this chapter, the molecular aspects of different classes of platelet receptors and their contribution in different pathophysiological conditions are discussed.

2. Glycoprotein (GP) receptors

On the membrane of platelet, a class of glycoprotein (GP) receptors is present which plays a key role in hemostasis. In fact, the blood vessel wall damage initiates the event of interaction of GP receptors with the extracellular matrix (Figure 1).
The GP receptors include:

- GPIb-IX-V complex,
- GPVI,
- GPVI-FcRγ, and
- integrins.

### 2.1 GPIb-IX-V complex

The GPIb-IX-V complex consists of glycoproteins (GP) Ibα, Ibβ, IX, and V, which are known as GPIbα (~135 kDa), GPIbβ (~25 kDa), GPIX (~20 kDa), and GPV (~85 kDa), respectively. The GPIbα is linked with a disulfide bond to GPIbβ and non-covalently associated with GPIX and GPV in a ratio of 2:2:1 [5, 6]. GPIbo, GPIbβ, and IX are present at almost 25,000 copies per platelet, whereas GPV at 12,500 copies which can be calculated easily by their abundance ratio in a complex. GPIbα, GPIbβ, and GPIX are found to be closely associated and the arrangements of these subunits are essential for an efficient bioavailability of GPIb to its ligand. Lack of any of the single subunits is significantly able to decrease the surface expression of the whole complex (GPIb), whereas GPV is more loosely associated with the complex and its absence does not interfere with the expression of GPIb as well as its interaction with its ligand, vWF. GPIV has been found to be active only during the binding of thrombin to GPIbα subunit. It (complex) is found to be assembled in the megakaryocyte in the bone marrow (the origin site of platelet) as a functional unit [7]. These are type I membrane spanning GP and are members of the leucine-rich repeat family, with one or more approximately 24-residue leucine-rich repeats, with their N- and C-terminal disulfide-looped flanking sequences, in their extracellular domains [5]. The four subunits are encoded by genes mapping to chromosomes 17p12 (GPIBA), 22q11.2 (GPIBB), 3q29 (GP5), and 3q21 (GP9) [7]. GPIbo, the major ligand-binding subunit, has a globular N-terminal ligand-binding domain elevated from the cell surface by a sialomucin core [5]. This N terminal (282 residues) contains the leucine-rich repeats (LRR), the flanking sequences, and an anionic sequence (residues 269–282) with the sulfation (Tyr 276–279, except 277). The 1–282 sequence contains overlapping, but distinct binding sites for (a) vWF, (b) leukocyte integrin αMβ2, and (c) P-selectin, a granule-membrane receptor expressed on the surface of activated platelets or activated endothelial cells [5, 8, 9]. The adhesion of vWF to GPIbα under shear stress involves electrostatic interactions between a negative patch (centered on residues 59–128) within the leucine-rich repeats of GPIbo and a complementary positive patch (centered on residues 496–709) on the vWF-A1 domain [5, 10, 11]. The residues of N- and C-terminal to this region directly contact vWF in a co-crystal structure of a GPIbα fragment which lacks N-linked glycosylation sites at Asn21 and Asn159 [12]. The point mutations in either GPIbα (Met239/Val) or vWF-A1 (Arg543/Gln) alter their conformation producing subtle differences in their ligation as gain of function [12, 13]. More precisely, in the case of vWF-A1, the mutation is 415A from a GPIbα contacting sequence, emphasizing the sensitivity of interaction to the conformational regulations [12]. The gain-of-function mutation at Arg543/Gln and Met239/Val enhances affinity for GPIbα by virtue of almost 6-fold. The Met239/Val gain-of-function mutation of GPIbα is within a b-hairpin structure in the C-terminal flank, which undergoes significant structural alteration in a complex with vWF-A1. This Met239/Val gain-of-function

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mutation stabilizes the \( \beta \)-hairpin and increases the affinity of vWF binding [12].

These conformational changes of native GPIb \( \alpha \) and vWF with isolated ligand and receptor fragments might regulate the affinity of the adhesive interaction and result in an on-rate/off-rate for platelet adhesion in either rolling or firm adhesion in flowing blood, though the in vivo study is still under progress to understand. Thrombin binding to GPIb \( \alpha \) subunit presents the thrombin to its receptor, the protease activated receptor 1 (PAR-1). GPV acts as a negative modulator (as GPV cleaves by thrombin) of thrombin-induced platelet activation, which unmasking GPIb-IX complex and facilitates the binding of thrombin to GPIb \( \alpha \) [13]. GPIb interacts with vascular P-selectin, pointing to its function in inflammatory platelet pathways [9].

Depending on the orientation of the active site on GPIb-bound thrombin, thrombin-mediated proteolysis of platelet surface substrates (including GPV or PAR-1) may be regulated by interaction with GPIb \( \alpha \). GPIb \( \alpha \) is a cofactor for PAR-1 activation [9]. In turn, thrombin can regulate GPIb-IX-V signaling directly by engagement of GPIb \( \alpha \) under conditions where GPV is absent [9–15]. GPIb \( \alpha \)-associated thrombin also regulates Factor XI; the latter also binds to GPIb \( \alpha \) [16].

The extracellular domain comprises the binding site for vWF, P-selectin, and Mac-1 (Table 1). In addition to its dynamic role in platelet recruitment onto vWF, the GPIb membrane complex functions as a receptor for coagulation factor XII [17], XI [18], thrombin [19, 20], and HK (high-molecular-weight kininogen) [21]. Hence, GPIb is a receptor linking primary and secondary hemostasis [7]. The Src and Lyn of the Src family kinases (SFKs) are associated with the GPIb \( \alpha \) subunit to initiate the inside-out signaling. Binding of vWF to the extracellular region of GPIb \( \alpha \) subunit induces SFK activation and phosphorylation of downstream substrates. These include immunoreceptor tyrosine-based activation motif (ITAM)-containing FcR \( \gamma \)-chain (FcR \( \gamma \)) and FcR \( \gamma \)IIA. Both of them are found to be acted as high-affinity docking sites for the tandem SH2 domain-containing protein-tyrosine kinase Syk. Fc receptors (FcR \( \gamma \) chain and FcR \( \gamma \)IIA) may also be associated with GPIb-IX-V on platelets and contribute to GPIb-IX-V-dependent platelet activation. Intracellular signals emanates from GPIb-IX-V [5, 6, 11, 22]. It promotes elevation of cytosolic Ca\( ^{2+} \), cytoskeletal changes, secretion of agonists such as ADP (that activates G protein-coupled receptors, P2Y1 and P2Y12), and activation of the integrin \( \alpha I b \beta 3 \) that binds vWF or fibrinogen and mediates platelet aggregation. In vivo, plasma vWF is not recognized by GPIb-IX-V on resting platelets, thus preventing platelet aggregation in the normal circulation, with the interaction being triggered when sub-endothelial vWF is exposed following injury. GPIb-IX-V complex, in fact, plays a leading role in the elimination of high-stress injury. However, GPIb-IX-V can be induced to bind vWF in plasma at high pathological shear stress (e.g., coronary artery is blocked by atherosclerotic plaque) [7].

### 2.2 GPVI

Platelet GPVI is a member of the T cell receptor family and immunoglobulin superfamily. It has two extracellular Ig domains: (a) mucin-like domain, a transmembrane domain, and (b) cytoplasmic tail (Table 1). These are expressed constitutively on platelets and are engaged during the exposure of collagen in the subendothelial matrix after disruption of the endothelium in vivo. Ligands for GPVI including collagen, cross-linked collagen-related peptides, and the snake toxin, convulxin [85] may bind distinct sites of the Ig domains [23, 24]. GPVI can signal in response to collagen or other ligands by FcR \( \gamma \) receptor-dependent or receptor-independent pathways. The dependent pathway involves activation of Syk, and the independent pathway is regulated by calmodulin (via Ca\( ^{2+} \) signaling pathways) or Fyn/Lyn kinase [7].
The interaction of calmodulin with GPVI produces a soluble GPVI fragment [25]. Engagement of GPVI, the platelet integrins along with the collagen-binding receptors α2β1 and αIIbβ3 are found to be upregulated in the process of platelet aggregation [26]. The interaction between GPVI and collagen may be involved in the initiation of thrombus formation either at low or high shear rates. Further in the latter case, this thrombus formation possibly supports platelet aggregation, mediated by the GPIb-IX-V complex and the vWF [26]. These signaling pathways result in secretion of agonists such as ADP and inside-out activation of platelet integrins, primarily αIIbβ3 that binds fibrinogen or vWF and mediates platelet aggregation [26].

2.3 GPVI-FcRγ

Glycoprotein VI (GPVI), a transmembrane protein (63 kDa) consisting of two Ig-like domains in the extracellular region in platelets (Table 1). It connects to a highly glycosylated linker (a transmembrane domain) and a cytoplasmic tail. GPVI is expressed exclusively in platelets and megakaryocytes. In platelets, it expressed with around 3700 copies per platelet. The transmembrane adapter protein FcRγ is found to be associated with this. Surface expression of GPVI depends on FcRγ stabilization. This stabilization occurs through a salt bridge between the GPVI transmembrane domain residue Arg272 and FcRγ (Asp residues). The FcRγ is a covalent-linked homodimer containing one copy of an ITAM in each chain. It has two YxxL sequences separated by seven amino acids [27]. Phosphorylation of the ITAM motif by two Src kinases (Fyn and Lyn) associated with GPVI initiates platelet signaling, leading to potent platelet activation. SFKs for signal transmission are either associated with or in close proximity to their cytoplasmic tails. SFKs’ downstream effectors (adaptors, enzymes, and cytoskeletal proteins) collectively coordinate cytoskeletal remodeling, degranulation, membrane flipping, and integrin activation, and hence platelet activation. It has also been found that the SFKs act via the GPCRs. The Gq, coupled with PAR-1 and PAR-4, and the G1 coupled with ADP receptor, P2Y12, which synergizes with the primary activation, signal to maximally activate platelets. GPVI is expressed in platelets as a mixture of monomers and dimers, with a stoichiometry of one GPVI to each FcRγ-chain covalent dimer. The dimeric GPVI forms a unique conformation with higher affinity for collagen to mediate activation at the physiological concentrations of collagen, but not the monomeric GPVI due to its low affinity toward collagen [28]. F(ab’)2 fragments of antibodies to this structure induce platelet activation, while Fab fragments block activation by collagen, indicating a minimal signaling model in which activation is achieved through cross-linking of two GPVI dimers [7]. Ligand-mediated clustering of different platelet receptors (GPIb-IX-V complex, GPVI-FcRγ, integrins α2β1 and αIIbβ3, hemi-ITAM-containing podoplanin receptor CLEC-2, ITAM-containing low-affinity immunoglobulin receptor FcγRIIA) trigger transmission of primary activation signals through the phosphorylation of downstream tyrosine residues in proteins. These receptors depend on the family of protein-tyrosine kinases, known as Src, but not on the intrinsic kinase activity.

2.4 Platelet integrins

Integrins play an important role in the cell metabolism of every cell, including platelets. The integrin ligands of platelets are partly extracellular matrix bound and insoluble, and partly soluble. Platelet adhesion and aggregation are mediated by the heterodimeric receptors, the β1 and β3 of integrins family. Integrins are expressed in a low-affinity state in the resting platelets, but to bind to their ligands efficiently in response to the cellular activation they (integrins) are found to be
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shifted to a high-affinity state [29]. The integrin receptor for collagen on platelets, the α2β1, bears important roles at their disposal. A major downstream consequence of engagement of primary platelet adhesive receptors such as GPIb-IX-V and GPVI does rapidly activate platelet integrins.

Platelet integrins (Table 1 and Figure 1) are:

- αIIbβ3 (binds fibrinogen or vWF),
- αvβ3 (binds vitronectin),
- α2β1 (binds collagen),
- α5β1 (binds fibronectin), and
- α6β1 (binds laminin).

The Ca2+-dependent “inside-out” activation of αIIbβ3 to bind vWF is critically involved in stable thrombus formation at high shear stress. In the absence of αIIbβ3, activation of other integrins (e.g., α5β1-binding fibronectin) can at least partly compensate for this role [7]. Recent structural studies provide insight into the molecular mechanism of integrin activation [30]. Simultaneous with these conformational changes, altered attachment of the cytoplasmic domain with cytoskeletal components (e.g., αIIbβ3 binding to talin) facilitates integrin-dependent cell adhesion, signaling, and contraction.

2.4.1 αIIbβ3

The αIIbβ3 is the most abundant and dominant surface-expressed integrin in platelets (Figure 1). It may vary from 40,000 to 80,000 copies per platelet. Additional pool of this receptor can be recruited from internal membranes upon agonist-induced platelet activation. It is also the major functional integrin receptor on the platelet surface. The mature αIIb and β3 subunits are 148 and 95-kDa proteins, respectively. The αIIbβ3 binds with several RGD (Arg-Gly-Asp) motif containing ligands including fibrinogen, fibrin, vWF, vitronectin, fibronectin, and thrombospondin. During ligand recognition on platelet surface through RGD tract, fibrinogen (the major platelet αIIbβ3 ligand) promotes cell attachment by initiating αIIbβ3 clustering and recruitment of intracellular proteins. The RGD motif subsequently acts as a molecular switch on the β3 subunit to induce a conformational change necessary for full cell spreading [31]. This integrin mediates platelet aggregation via the SFK signaling pathways through the binding of plasma fibrinogen. It serves as the principal receptor for platelet adhesion in vivo [7, 26] with the inside-out signaling. The shifting from a low- to a high-affinity state of integrin αIIbβ3 is being considered the “final common pathway” of platelet activation. It (shifting) is essential for platelet αIIbβ3 to interact with the fibrinogen during platelet adhesion. Needless to mention a well-known fact that fibrinogen itself is a ligand with two receptor interaction sites: (a) enabling interaction with separate platelets and (b) constituting the basis of platelet aggregation. At this stage of transmitting signals, the Src is found to be the most abundant SFK in human platelets and is essential for propagation of signals from the activated αIIbβ3 integrins.

2.4.2 αvβ3

The αvβ3 receptors express widely in endothelial cells, osteoblasts, smooth muscle cells, and leukocytes, and throughout the vascular bed. It is present in only
a few hundred copies per platelet. A distinguished difference lies between αvβ3 and αIIbβ3 in platelets. The αvβ3 can bind several RGD containing ligands, including osteopontin (a class of protein which involves diverse physiological functions) and adenovirus penton base (a major capsid protein of human adenovirus), but vitronectin (a glycoprotein, binds to integrin αvβ3, and thus promotes cell adhesion and spreading) is a preferred ligand for αvβ3 [7]. High-affinity αvβ3 can be induced by agonists (adenosine diphosphate, ADP) and by direct integrin modulators (dithiothreitol and MnCl2). This high affinity and activated αvβ3 on platelets can bind osteopontin in atherosclerotic plaques and in the wall of only injured arteries [27, 32].

2.4.3 α5β1

It has been found to be the crucial one to involve the resting platelet to bind with the fibronectin. The α5β1 is the principal platelet receptor which supports resting platelet to adhere with the matrix fibronectin through its RGD sequence in static conditions. However, this interaction is unable to promote calcium oscillation, tyrosine phosphorylation, and/or lamellipodia formation. The interaction of α5β1 with fibronectin is sensitive to shear stress (the tangential force of the flowing blood on the endothelial surface of the blood vessel), and it has been found that it loses its avidity quickly with the increase of the shear stress. Therefore, α5β1 may have the limited role to initiate the interaction of resting platelets with the fibronectin matrix. Especially during injuries in the larger blood vessels, where shear forces are low, it promotes the engagement of other subsequent integrins and also receptors to amplify platelet-induced responses [7].

2.4.4 α6β1

The α6β1, a principal laminin receptor of platelets, does not require any platelet activation in order to bind laminin (a fibrous protein present in the basal lamina of the epithelia, influencing cell differentiation and migration) to promote adhesion. Some cations (Mn2+, Co2+, and Mg2+) support adhesion, while few others (Ca2+, Zn2+, and Cu2+) do not help. Binding of platelets to laminin through α6β1 does not induce platelet aggregation but adherent of platelets to laminin triggers signaling pathways. These signaling pathways induce filopodia formation with PI3K and cdc42 activities in higher rate than in platelets which activates through αIIbβ3 involvement. Laminin has been known for many years to support adhesion of platelets through integrin α6β1, but it has the ability to activate GPVI. The interaction of laminin with GPVI depends on the initial interaction with integrin α6β1. This is just in contrast to the event of collagen interaction which initiates platelet activation through GPVI. This difference between these interactions of these two matrix proteins may reflect the lower affinity (approximately 10-fold) of laminin for GPVI or the presence of a subpopulation of constitutively active α6β1. The weak nature of the GPVI activation (by laminin) argues against a significant role in the prevention of major bleeds. It is suited ideally to facilitate vessel repair after minor damage without the risk of forming occlusive thrombi in the blood vessels [33].

Collagens are not only the most abundant proteins (20–40% of total proteins in the aorta) in the subendothelial extracellular matrix but also it is essential in platelet adherence and platelet plug formation to provide a mechanical strength to the blood vessel wall. There are nine types of collagen resides in the vasculature. Among those, only fibrillar collagens of types I, III, V, and VI and nonfibrillar collagens of types IV and VIII are thrombogenic. Although platelets have various types of receptors for collagen, the receptors (e.g., GPVI, α2β1, p65, p47, TIIICBP, GPIV, the integrin α2β1, and GPVI) are considered as its (platelets) major receptors for binding to collagens and activation of platelets [34].
2.4.5 α2β1

The integrin α2β1 (or VLA2, CD49b/CD29, GPIa/IIa) is a collagen receptor composed of a α2 chain (150 kDa) and a β1 chain (130 kDa). This α2β1 has the expression profile ranging from 900 to 4000 copies/platelet and is expressed per platelet at about 2000 copies. The α2, inserted with 200-residue sequence, is the only platelet subunit with I domain. The crystal structure depicts that this domain comprises (a) seven helices surrounding a core of five parallel β-strands, (b) a short anti-parallel β-strand, and (c) a C-terminal helix [27]. The α2β1 integrin of this I domain binds with collagen and preferably Mg^{2+}/Mn^{2+}, in presence of the metal ion coordinating residues (D151, T221, and D254) in this site. Several recognition sequences (GFOGER, GLOGER, GASGER, GROGER, and GLOGEN) have been identified in collagens I and III. Their recognition profile in this recognition sequences have hierarchy and affinity differently [35]. This interaction is dependent on Mg^{2+} and the GER sequence. α2β1 recognizes these sequences in the resting state, but platelet activation by classical agonists via intracellular signal transduction pathways activates α2β1 via a structural rearrangement of the α2β1 domains [36], causing them to upregulate their affinity for their preferred ligand sequences, stereochemically positioned at regular positions on bundled collagen fibrils [35].

3. C-type lectin-like receptor

Another type of adhesion receptor is C-type lectin (CLEC), a type of carbohydrate-binding protein domain. It requires calcium (that is why “C” comes) for binding. Proteins that contain C-type lectin domains have a diverse range of functions including cell-cell adhesion, immune response (to pathogens), and apoptosis. This kind of receptor is present on platelet to help in adhesion as another adhesion receptor.

3.1 CLEC-2

CLEC-2 is highly expressed in megakaryocytes and platelets, and at low level in mouse neutrophils. The CLEC-2 gene (on chromosome 12) codes for a type II membrane protein C-type lectin receptor family with an extracellular carbohydrate-like recognition domain (CRD-like). It has a cytoplasmic tail of 31 amino acids that contain a single conserved YxxL sequence (known as a hem-ITAM). CLEC-2 was discovered from the snake (the Malayan pit viper, Calloselasma rhodostoma) venom rhodocytin (Table 1), known as aggretin. Previously it (rhodocytin) was considered to be a platelet activator through the action of α2β1 and GPIbα. This concept was based on the ability of high concentrations of antibodies to block the activation. Now it has been found that rhodocytin does not bind to the recombinant α2β1, as it has the ability to activate platelets even in the deficiency of integrin α2β1, GPIbα, and GPVI. Thus, it is a proven fact now that this rhodocytin appears to activate platelets through a novel receptor, CLEC-2. The CLEC-2 antibody establishes CLEC-2 as a novel platelet activation receptor as it has the ability to induce the potent activation to human platelets [37]. Experimenting with ligand and anti-CLEC-2 antibody, it has been found that Syk mediates phosphorylation of CLEC-2 with Src family kinases plays a critical role in further downstream signaling [7]. The rhodocytin-induced platelet aggregation also previously known to depend on secondary mediators (e.g., thromboxane A2, TXA2, and ADP) acted as agonists for GPCRs on platelets. Recently, it has been found that CLEC-2-induced Syk and PLCγ2 phosphorylation potentiates by the TxA2 by playing a critical role in the most proximal event of
CLEC-2 signaling (i.e., CLEC-2 receptor tyrosine phosphorylation). In addition, it may be mentioned that the ADP receptors and protease-activated receptors can also potentiate CLEC-2 signaling during the process of thrombosis formation. The PLCβ-PKCα pathway possibly is regulating the activation of SFKs (mentioned earlier), which are crucial for initiation of CLEC-2 signaling by the Gq-coupled receptors, not other G-proteins.

Amplification of platelet activation by TXA2 synthesis and binding to the TXA2/prostaglandin H2 (TP) receptor are the process of the aspirin-sensitive platelet activation (Figure 1). On the other hand, prostacyclin (PGI2) and PGD2 are known to inhibit platelet aggregation, whereas PGE2 potentiates or inhibits platelet response in a dose-dependent manner [7]. In this context, it may be mentioned that bioactive lipid mediators, prostanoids, formed from arachidonic acid by the cyclooxygenase enzyme is known to liberate from the cell membrane. They are involved in numerous physiological activities, including platelet aggregation, local inflammatory response, leucocyte-endothelial cell adhesion, and vasorelaxation as well as vasoconstriction. So, the thromboxane as well as PGs are other types of platelet adhesion receptors to discuss with.

4. Thromboxane receptor

TXA2 is produced from its precursor arachidonic acid through the cyclooxygenase pathway [38]. The TP receptor or TXA2/PGH2 receptor (57 kDa) is a membrane-bound seven transmembrane spanning G protein-coupled (including Gq and G12/13) receptor and widely present in the cardiovascular system (Table 1). Human TP receptors (TPα and TPβ) are encoded by the same gene, but different from each other as they produce from alternative splicing and have different C-terminal intracytoplasmic regions. Both TPα and TPβ mRNAs are found in platelets, but in endothelial cells, only TPβ has been found to be expressed. TP receptors are also expressed in other cell types related to atherothrombosis (smooth muscle cells, macrophages, and monocytes) [39, 40].

5. Prostaglandin (PG) receptors

The PG receptors, especially PGE2 and PGI2, bear a pivotal responsibility in platelet aggregation phenomena to maintain hemostasis, as mentioned earlier.

5.1 Prostaglandin E2 receptors (PGE2)

The biosynthesis of PGE2 is enhanced by inflammatory mediators in vascular smooth muscle cells and macrophages (Table 1). PGE2 shows a biphasic, concentration-dependent effect on platelet aggregation as (a) high concentrations of PG inhibit platelet aggregation, whereas (b) lower concentrations enhance it. PGE2 activates four types (PGE2 type 1 or EP1, PGE2 type 2 or EP2, PGE2 type 3 or EP3, and PGE2 type 4 or EP4) of its G protein-coupled receptors. Each of these receptors has a distinct pharmacological signature and intracellular signal transduction. Stimulation of EP3 receptors results in elevation of free intracellular calcium levels, whereas stimulation of EP2 and EP4 receptors usually decreases intracellular calcium levels due to increase in intracellular cAMP levels through the activation of Gas (growth arrest-specific) protein [41]. It may further be mentioned that except EP2 receptor, the mRNA for EP1, EP3, and EP4 receptors is present in human platelets [41].
The activation of the EP3 receptor by leading to inhibition of the increase in cAMP increases the mobilization of calcium and elevates P-selectin expression in platelets ascribing the proaggregatory effect of PGE2. Lacking of this receptor has been found to show an increased bleeding tendency and a decreased susceptibility to thromboembolism [42], but PGE2 produced by atherosclerotic plaques can further facilitate arterial thrombosis via EP3 [43]. Interestingly, no defects in the EP3 receptor gene have been found in humans [7]. The platelet aggregation, calcium mobilization, and upregulation of P-selectin are found to be inhibited by the selective EP4 agonist, ONO AE1-329. Additionally, the EP4 antagonists, GW627368x and ONO AE3-208, have been found to repeal the inhibitory effect of ONO AE1-329 on platelet aggregation [44]. Thus, the EP4 receptors might play an important role mediating the inhibitory effect of PGE2 in the control of hemostasis by balancing out the proaggregatory effect of EP3 receptors. EP4 agonists might constitute a novel class of antithrombotic agents and also might be clinically useful in those cases where aspirin or ADP antagonists are not warranted or are insufficient, as the EP4 activation enhances the inhibitory effect of aspirin [44].

5.2 Prostaglandin I2 (PGI2) or prostacyclin receptor

Prostaglandin I2 (PGI2) or prostacyclin is a derivative of arachidonic acid, released by vascular endothelial cells (Table 1). It is an effective (a) vasodilator, (b) platelet aggregation inhibitor, and (c) moderator of vascular smooth muscle cell proliferation-migration-differentiation (anti-atherosclerotic). It acts through a specific membrane-bound receptor, the prostacyclin receptor (IP receptor). The IP receptor belongs to the prostanoit family of GPCR. The receptor (37–41 kDa, depending upon different states of glycosylation) is a class A rhodopsin-like GPCR. The glycosylation of the extracellular domain is necessary for (a) ligand binding, (b) receptor activation, and (c) membrane localization. A number of serine residues (S328 and S374) are thought to be phosphorylated by GPCR kinases or second-messenger-activated kinases (PKC and PKA) in the cytoplasmic domain. It might play a potential role in either agonist-induced phosphorylation or kinase-mediated receptor desensitization [45]. Cyclopentane ring and side chains are the two structural features of prostaglandins. Among these, the side chains are found to be recognized by their receptor to stabilize ligand binding, and the binding pocket of the receptor can accommodate the cyclopentane rings (PGI2, PGE1, and PGE2). The IP is the most common to be associated with the Gas subunit of the heterotrimeric G-protein. Upon receptor activation, it has been found to catalyze the formation of the second messenger, cAMP by stimulating the membrane-bound adenyl cyclase [7].

The discussion with GP, TX, and PG lead us to discuss with thrombin receptor as stimulation of thrombin activates the thrombin receptors in vivo.

6. Thrombin receptors

As explained earlier, platelet activation by thrombin partially depends on GPIb-IX-V, but is primarily assured by two protease-activated receptors (PAR), i.e., PAR-1 and PAR-4. Binding of thrombin (immobilized, proteolytically inactive) to GPIb induces platelet adhesion as well as spreading and secretion [19], being an enhancer (GPIlb) of the thrombin response (see Section 2.1). PAR-1 and PAR-4 (Table 1) are activated by a unique irreversible proteolytic cleavage (within the first extracellular loop exposing an N-terminus) by serving as a tethered ligand to GPIb. Short
synthetic peptide mimetic of the N-terminus sequences, corresponding to the new N-terminus, reproducing most of the action of thrombin on platelets upon cleavage by thrombin (SFLLR for PAR-1 and GYPGQV for PAR-4) can activate these receptors directly. This dual-receptor (PAR-1 and PAR-4) signaling for thrombin implies that PAR-1 is the primary mediator that activates platelets at low concentration, whereas PAR-4 as a back-up receptor is found to be activated at higher thrombin concentrations. For sustained optimal platelet responses to thrombin, the qualitative differences in the dynamics of PAR-1 and PAR-4 activation might be relevant. The signaling for PAR-4 at high thrombin concentrations acts very slowly, as the PAR-4-mediated Ca^{2+} mobilization is found to be slower and more prolonged than that of PAR-1, and also, this activity terminates more slowly. Interestingly, there is no such report with congenital deficiencies of PAR receptors in any individuals. The pharmacological invention of PAR-1 inhibitors will be very helpful to prevent the platelet-dependent thrombosis in the first stage [46].

On the surface of platelet membrane a type of kinase receptors are present which are specific to its ligand and associated with integrin receptors to act with, known as ephrin kinase receptor.

7. Ephrin (Eph) kinase receptors

Eph kinases are receptors expressed on the surface of cells. It activates in response to binding with Eph receptor-interacting proteins, ephrins. Eph kinases are known to be a member of receptor tyrosine kinases subfamily with an extracellular ligand binding domain and an intracellular tyrosine kinase domain (Table 1). The EphB is distinguished from the EphA subfamily by an insertion within the extracellular domain that helps to define the ligand preferences for the receptor. EphrinA to EphA interaction (Table 1) occurs typically with higher affinity than ephrinB (to EphB) interactions. This may be due to the fact that EphAs bind via a “lock-and-key” mechanism with little conformational change with less energy, in contrast to EphBs which utilizes an “induced fit” mechanism with a greater amount of energy to alter the conformation of EphBs for binding to ephrinBs [47]. The Eph kinase and ephrin interactions on adjacent cells play a pivotal role in neuronal patterning and vasculogenesis. Eph subtypes of EphA4, EphB1, and also ephrinB1 are found to be expressed by the human platelets. In both resting and activated platelets, the EphA4 is constitutively associated with αIIbβ3. Fine tuning between the Eph and ephrin is very much essential as clustering of either EphA4 or ephrinB1 causes platelet adhesion to immobilize fibrinogen, whereas by blocking this (Eph/ephrin) interaction, the clot retraction can be hampered. This may cause platelet aggregation inhibition at low agonist concentrations and may form smaller thrombi on collagen-coated surfaces during normal conditions of arterial flow. This can develop premature disaggregation. It acts partially due to the ability of ephrin B1 to activate Rap1 (a Ras family member)-mediated signaling which supports platelets activation, especially the integrin in platelets [48].

There are also purinergic (P) receptors, present on the platelet membrane, which are basically ADP or ATP dependents.

8. Purinergic receptors

These purinergic receptors reside in coupled form, that is why it represents as P2. These P2 (purinergic coupled) receptors are of mainly three types—P2Y1, P2Y12, and P2X1 (Table 1).
Platelets

8.1 P2Y1

The P2Y1 receptors (42-kDa, contain 373 amino acid residues) are widely distributed in many tissues (heart, blood vessels, smooth muscle cells, neural tissue, testis, prostate, and ovary) including platelets. About 150 P2Y1 receptor-binding sites are expressed per platelet, and it is also abundantly represented in membranes of α-granules and elements of the open canalicular system. The P2Y1 receptor is absolutely required for ADP-induced platelet aggregation. ADP is a more potent agonist than ATP, and its 2-methylthio derivatives are more potent than the parent compounds. ATP is a partial agonist for the P2Y1 receptor, and at the low levels of receptor expression, it acts as an antagonist. Overall, P2Y1 accounts for about 20–30% of the total ADP-binding sites on the platelet surface [49]. At the P2Y1 locus, a common genetic variant (dimorphism, 1622AG) exist which is associated with platelet reactivity to ADP. However, this can partly explain the interindividual variation in platelet's response to ADP and may have clinical implications in relation to the thrombus formation [7].

8.2 P2Y12

The purinergic P2Y12, the Gι-coupled platelet receptor, exists in platelets, smooth muscle cells, endothelial cells, and glial cells. This surface protein is expressed from the chromosome 3q21–25 containing 342 amino acid residues which includes extracellular Cys residues at four different locations (17, 97, 175, and 270, respectively). The Cys 97 and Cys 175 are found to be linked by a disulfide bridge, and this link is important for receptor expression in platelets. P2Y12 receptor exists predominantly on the platelet surface among the purinergic receptors as homo-oligomers placed in lipid rafts. The active metabolite of clopidogrel (which covalently inhibits P2Y12) application as treatment disrupts the homo-oligomers into nonfunctional dimers and monomers which are sequestered outside the lipid rafts [7]. ADP and its analogs (e.g., 2-methylthio-ADP and N-methanocarba-2-methylthio-ADP) stimulate the P2Y12 receptor, while ATP and its triphosphate analogs act as antagonists to it [7]. P2Y12 plays a central role for ADP in platelet function. The congenital P2Y12 defective patients display a mild to moderate bleeding diathesis with the conditions of mucocutaneous bleedings and postsurgical and posttraumatic excessive blood loss. Any defects of P2Y12 can also be thought of even when high concentrations (10 mM) of ADP is unable to induce full, irreversible aggregation of platelets [49].

8.3 P2X1

P2X1 is a widely distributed ligand-gated ion channel, highly expressed in human megakaryocytes and platelets. It is well known that ATP is the physiological agonist and ADP is an antagonist. Platelet dense granules release ATP upon its activation with an ion channel (cationic and/or anionic) present on platelets, i.e., P2X1. During platelet preparation under in vitro condition, a rapid desensitization of the P2X1 receptor occurs which made this receptor unnoticed. The P2X1 gene lies on the chromosome 17p13.2. It encodes 399 amino acids which are organized into two transmembrane domains (TM1 and TM2). These are separated by a large extracellular domain containing 10 cysteine residues. Three molecules of ATP bind to the extracellular domain of P2X1 and trigger conformational changes. This results in the opening of a cationic pore for monovalent and divalent cations (e.g., Ca\(^{2+}\), Na\(^{+}\), and K\(^{+}\)) allowing rapid changes in the membrane permeability. P2X1 receptor activation triggers transient shape change of a platelet from a discoid to
spherical shape; furthermore, in presence of low concentrations of other platelet agonists, it (activation of P2X1) amplifies platelet responses. The P2X1 receptor contributes equally to low and high levels of thromboxane A2 receptor activation. More significantly, P2X1 receptor activation has been shown to be essential for enhanced platelet adhesion and thrombus formation under high shear rates [50]. P2X1 is distinguished as a potential new drug target for antithrombotic therapy, especially for the mild long-term risk management. As inhibition of P2X1 causes mild effects on different platelet function, it (P2X1) seems to be a so-called “safe” target clinically [51].

As mentioned earlier (see Section 5.1), Gas receptors also have some important role in the stabilization of platelet aggregation to maintain internal hemostasis.

9. TAM receptor and Gas6

Gas6 has its interactions with Tyro 3, Axl, and Mer (TAM) tyrosine kinase receptors (Figure 1). It is a vitamin K-dependent protein implicated in cell growth, adhesion, and migration, through its TAM receptor. These three related protein receptors (TAM) were cloned (in 1991) as orphan receptors and have been found to be widely expressed in the vertebrate nervous system [7]. They (TAM receptors) have two N-terminal immunoglobulin domains, followed by two fibronectin-III-like domains to mediate binding to the ligand. These domains are found to be attached to an intracellular tyrosine kinase domain, via a single-pass α-helical transmembrane domain. The functional receptors form both the hetero- and homo-dimers, which is common with other receptor tyrosine kinases (Table 1 and Figure 1).

It (Gas6) is found in plasma and platelet granules, but in human, it is present predominantly in plasma. It is secreted upon platelet activation, as mentioned earlier. Deficiency in Gas6 or one of its receptors (TAM) in mice has shown abnormal platelet responses to agonists and prevents thrombosis, suggesting a major role of this Gas6-TAM receptor-coupled axis in thrombus formation as well as in vascular wall homeostasis. The role of Gas6 in human platelet function has been clarified with the fact that the Gas-6 reinforced αIIbβ3 integrin with the outside-in signaling by the activation of PI3K and Akt and therefore clot retraction by promoting the β3 phosphorylation [52]. These effects constitute in fact an enhancement and perpetuation of the thrombus-stabilizing role of ADP. The inhibition of Gas6 signaling has been proposed as an attractive target for novel antithrombotic drugs [53].

Many receptors are known of transmembrane family. There is also a unique tetraspan transmembrane receptors on the platelet membrane, known as tetraspanin receptors.

10. Tetraspanin receptors

Tetraspans, mean four transmembrane domains, called as tetraspanins belong to the transmembrane 4 superfamily (TM4SF) receptors. Usually, tetraspanins are found to act as scaffolding proteins. It has intracellular (N- and C-) termini and two extracellular domains (EC1 and EC2), arranged in a way of one short and one long with typically 100 amino acid residue long loop. Its EC2 domain with four or more cysteine residues are its main feature, among which two are in a highly conserved “CCG” pattern. It can anchor multiple proteins to one area of platelet cell membrane [7].
10.1 CD151

The CD151, a tetraspanin superfamily member, previously termed as PETA-3/SFA-1 has been found to express broadly in hematopoietic, vascular, and immune compartments, and especially abundant in cardiac muscle, endothelia, epithelia, megakaryocytes, smooth muscle, and the platelets. It is found to functionally link with the integrin trafficking, cell migration, cancer metastasis, neurite outgrowth, hemidesmosome formation, vascular morphogenesis, wound healing, immune responsiveness, and hemostasis. CD151 has been found to appear to regulate fibrinogen-binding proteins (e.g., integrin αIIbβ3) (Table 1). In addition, the absence of CD151 in vivo leads to smaller, unstable thrombi formation [54].

10.2 TSSC6

The TSSC6 or tumor-suppressing subchromosomal transferable fragment cDNA 6 also called as pan-hematopoietic expression (Phemx). It is a member of tetraspanin superfamily. Its C-terminal cytoplasmic domain is relatively large (33 amino acids in mouse and 99 amino acids in human) than the other members of the tetraspanin superfamily [55]. TSSC6 may modulate hematopoietic cell function specifically when expressed in hematopoietic organs and tissues. It is expressed on the surface of murine platelets and is upregulated by thrombin stimulation. The secondary stability of arterial thrombi formation (by regulating integrin αIIbβ3 outside-in signaling events) has been found to affect upon vascular injury during the lack of platelet TSSC6 receptors in vivo (Figure 1) [55]. The proliferation of T lymphocytes has also been observed in the TSSC6-deficient mice, due to the increase in interleukin 2 production following T-cell receptor stimulation, providing a clue to the negative regulation of peripheral T-lymphocyte proliferation by TSSC6.

10.3 CD36

CD36 (80–90 kDa) is known to be a scavenger receptor. This CD36 along with other receptors and their corresponding subunits (GPIIb, GPIV, GP88, FAT, SCARB3, or PASIV) are expressed on the surface of the platelets and other cells (e.g., monocytes, endothelial cells, smooth muscle cells, and cardiomyocytes). Approximately 10,000–25,000 molecules are present in a single platelet. Its gene is located on the chromosome 7, in case of human.

It consists of a single peptide chain (consisting of 474 amino acids). Its two transmembrane domains (one near the N-terminus and the other near the C-terminus) are configured like a “hairpin-like” structure. The domains of CD36 are separated by a large, glycosylated extracellular loop. It (CD36) was initially described as a collagen receptor of types I and III on platelets, but later, it has been found that it is not a primary collagen receptor as its binding to the nonfibrillar type V collagen has been documented [56]. The interaction of CD36 with its various ligands (e.g., thrombospondin 1 or TSP1, long chain fatty acids, oxidized phospholipids or oxPL, and oxidized low-density lipoprotein or oxLDL) is found to modulate the platelet activation (Table 1). TSP-1 is found to promote platelet aggregation through the modulation of an inhibitory signaling pathway. TSP-1 binding to its receptor CD36 prevents cAMP/protein kinase A (PKA) signaling. Indeed, TSP1 triggers CD36-dependent signals that reduce platelet sensitivity to PGE1. It (CD36-dependent signaling) diminished its (PGE1) ability to inhibit platelet aggregation and arrest under conditions of flow [57]. Other CD36 ligand, oxLDL, formed during hyperlipidemia and atherosclerosis can also activate platelets in
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a CD36-dependent manner, as stated in TSP1. The level of platelet CD36 surface expression is highly variable among individuals within general population with inheritance of specific genotypic polymorphisms at the CD36 locus (Figure 1) [58].

10.4 TLT-1

Another member of this tetraspan superfamily is the triggering receptors expressed in myeloid cells or TREMs, which are found to be involved in the activation of various cell types of the innate immune system. It includes platelets, monocytes, macrophages, microglia, and neutrophils. The family is characterized by a single V-set immunoglobulin (Ig) domain, a short cytoplasmic tail, and a charged residue in the transmembrane domain. TREM-like transcript-1 (TLT-1 or TREML-1) is a type I single Ig domain orphan receptor. It is specific to platelet and megakaryocyte alpha-granules. It only relocates to the platelet surface upon platelet stimulation. Its longer cytoplasmic tail carries a canonical ITIM (immunoreceptor tyrosine-based inhibition motif) which is capable of becoming phosphorylated and of binding to the Src homology-containing protein tyrosine phosphatase-1 (SHP-1). It (ITIM) can identify TLT-1 as the only putative inhibitory member of the TREM cluster. During storage, the ability of anti-TLT-1 scFv (single chain variable fragment) to block aggregation of washed platelets suggested that TLT-1 facilitates thrombosis by interacting with ligand(s) in activated platelets. TLT-1 acts in collaboration with αIIbβ3 to facilitate fibrinogen/platelet interactions and/or higher order platelet aggregation following the same signaling mechanism (Figure 1 and Table 1) [59, 60].

10.5 PEAR1

The PEAR1 or platelet endothelial aggregation receptor-1 (150 kDa) is known as multiple epithelium growth factor 12 (MEGF12) or Jedi-1. Interestingly, “Jedi” is not a scientific name here, which is being used by the scientists. It is a myth to describe the power and devotion of a knight, known as Jedi knights who respect all life by defending and protecting those who cannot do encounter for themselves, and in any altercations, they remain ready to encounter and fight only in self-defense and for the defense of those they protect. Based on this myth’s symbolic importance and strength of PEAR-1 receptor in the maintenance of homeostasis, it was named so.

It is a transmembrane protein of the MEGF-like domain protein family. It is mainly expressed in platelets, endothelial cells, and also in satellite glial cell precursors. During development, it is necessary for the clearance of apoptotic neurons via phagocytosis in the embryonic dorsal root ganglia (DRG). PEAR1 is composed of (a) an extracellular Emilin domain (EMI domain), (b) 15 extracellular EGF-like repeats, and (c) multiple cytoplasmic tyrosines and pralines, (d) intracellular domain structure, containing 5 proline-rich domains and an NPXY motive (serving as a phosphotyrosine-binding site and an internalization signal). During platelet aggregation, PEAR1 is phosphorylated at Tyr-925 and Ser-953/1029 in an αIIbβ3-dependent manner of signaling mechanism, as described previously. The PEAR1 has been hypothesized as a platelet-platelet contact receptor, due to its (PEAR1) αIIbβ3-independent phosphorylation [61]. The high-affinity immunoglobulin E receptor subunit α (FceR1α) has been found as PEAR1 ligand (Table 1). PEAR1 promoter-region variant (rs2768759) was associated with increased aggregation in PRP, most strongly in response to epinephrine, in both pre- and post-aspirin treatment conditions [62]. Increased expression of PEAR1 might be an important cause of hyperactivity [62]
and genetic variation within PEAR1, particularly rs41299597, seems to lead to an increased membrane expression of PEAR1 in activated platelets and elevated responsiveness to GPVI ligands [63]. A genome-wide meta-analysis linked the minor allele of the PEAR1 SNP (rs12566888) to a drop in aggregation response toward ADP and epinephrine in the European and African-ancestry sample [64].

Cell-cell adhesion is very important in the process of thrombus formation and obviously to extend the formation into proper stability until the proper clot forms. In this process, a specific adhesion molecule and its specific ligand have been found to play an important and interesting role to maintain hemostasis. P-selectin is a member of the selectin family of adhesion molecules. P-selectin glycoprotein ligand-1 (PSGL-1) on the plasma membranes of neutrophils or monocytes, which are key effector cells of the innate immune system, binds to P-selectin translocated to the surfaces (Figure 1 and Table 1) of the inflamed endothelial cells or activated platelets [65].

11. P-selectin

Selectins are a family of cell adhesion molecules (CAMs). It is also known as clusters of differentiation 62 or CD62. It is present in endothelium (as E-selectin, 58.6 kDa), leukocyte (as L-selectin, 30 kDa), and platelets (as P-selectin). P-selectin has a molecular weight of 86 kDa based on the prediction from its cDNA, but from reducing SDS-PAGE, it is about 140 kDa. The primary ligand for P-selectin is P-selectin glycoprotein ligand-1, known as PSGL-1 which is found constitutively on all leukocytes (Table 1). The transient interactions between P-selectin of activated platelets and PSGL-1 of leukocytes allow them to roll along the venular endothelium. During this activation of the coagulation cascade, the formation of a fibrin network is found to be a critical event in thrombus stability (Figure 1). A laser injury-induced thrombosis is found to express a low level of tissue factor (TF) in mice. It has also been shown that this fibrin formation depends on the monocyte-derived TF, carried by microvesicules, with minimal contribution of vessel wall TF. These microvesicles are captured onto the thrombus through the interaction between P-selectin and PSGL-1 (as mentioned before) present on microvesicles, hence delivering TF to the growing thrombus. Based on this observation, studies with mice, deficient in either PSGL-1 or P-selectin display thrombi with little TF and reduced thrombin generation, resulting in hampered thrombus size [7, 65]. Elevated plasma P-selectin (normal value 100 ng/ml in man) is a major predictive factor of cardiovascular events related to platelet turnover and its activation as well as function. So, the increase in P-selectin expression is expected to develop the artery diseases of peripheral tissues, stroke, and even the acute myocardial infarctions [7]. Being a dynamic process, a state of surface is preferable in the arterial thrombus formation to limit thrombus growth passively. In this context, it is not unreasonable to mention here that the essential roles of nitric oxide (NO) and PGI2 in the negative regulation of platelets to prevent uncontrolled thrombosis have been well established [7]. However, the inhibitory role of various receptors with and without immunoreceptor tyrosine-based inhibition motif (ITIM) domain for active thrombus formation is well recognized [7, 65]. It is very important to limit the thrombus formation within the blood vessels. These receptors are now in focus of our discussion.

12. ITIM-containing receptors

Immunoreceptor tyrosine-based inhibition motifs or ITIMs are defined by a consensus sequence of (L/I/V/S)-X-Y-X-(L/V); ITIM-containing receptors are
found in pairs, separated from each other by 15–30 amino acid residues, were originally identified by their ability to inhibit signaling by its activation counterpart (immunoreceptor tyrosine-based activation motif or ITAM) receptors. The ITAM and ITAM-like receptors, GPVI and CLEC-2 in the presence of thrombin (a GPCR agonist), cause mild inhibition of platelet activation by the platelet endothelial cell adhesion molecule or PECAM-1. The action of this inhibition by the thrombin, GPVI, CLEC-2, and PECAM-1 is similar to that of G6b-B in which it (G6b-B) can inhibit the SFK (Src and Syk) signaling to prevent unwanted platelet activation. This signaling mechanism also shows the inhibition of platelet activation by the GPVI-specific agonist (collagen-related peptide) and ADP (an agonist of GPCR) (Table 1 and Figure 1).

12.1 PECAM-1

PECAM-1 (or CD31, molecular weight 130 kDa), the adhesion molecule, bears a pivotal role in the negative regulation of platelet aggregation by inhibiting the platelet activation. PECAM-1 is expressed on the cell surface of hematopoietic and immune cells, which include platelets, neutrophils, monocytes, megakaryocytes, natural killer cells, some T cells, and on endothelial cells, particularly at the borders of the adjacent cells. PECAM-1 is a member of the Ig superfamily (like GPVI receptor), consists of (a) six extracellular Ig domains, (b) transmembrane domain, and (c) cytoplasmic tail. The cytoplasmic domain contains an ITIM, which becomes phosphorylated upon stimulation by homophilic interactions and/or clustering. PECAM-1 is found to ease the recruitment of tyrosine, serine/threonine, sometimes possibly the lipid phosphatases, and consequent kinase-dependent signaling inhibition to inhibit the platelet activation by attenuating the thrombus formation and thrombin-mediated platelet activation (by means of negative regulation) involving GPVI and GPIb (Table 1) [66]. PECAM-1 is an efficient signaling molecule in platelet and is capable of exhibiting both outside-in and inside-out signaling. PECAM-1 is also implicated in numerous other biological functions including apoptosis, platelet aggregation, thrombosis, and angiogenesis.

12.2 G6b-B

Among the novel plasma membrane proteins (identified via proteomics study), the immunoglobulin superfamily member G6b is one of them. It consists of 241 amino acids (26 kDa). It is found to undergo extensive alternate splicing. In stimulated platelets, G6b-B undergoes tyrosine phosphorylation in association with the Src homology-2 (SH2) domain-containing phosphatase (SHP-1). It suggests its importance to play a novel role in limiting platelet activation. Only the G6b-B is found to have both (a) a transmembrane region and (b) two ITIM. This ITIM supports binding to the two SHPs, i.e., SHP1 and SHP2. Heparan sulfate has recently been found as a ligand for G6b-B receptors (Table 1). The second ITIM, with a slightly different sequence (TXYYYV), is located around 20 amino acids downstream of the first ITIM of G6b-B [67]. In addition, G6b-B is encoded by a gene that is variously called G6b, C6orf25, or MPIG6B. It is a platelet and megakaryocyte-specific receptor.

12.3 VPAC1

VPAC1 is the vasoactive intestinal peptide (VIP)/pituitary adenylate cyclase-activating peptide (PACAP) receptor 1. The PACAP is a neuropeptide of the VIP, a member of secretin/glucagon superfamily. The PACAP receptor (vasoactive
intestinal peptide/pituitary adenylate cyclase-activating peptide receptor 1) or VPAC1 in platelets is coupled to adenyl cyclase activation. The VPAC1 and VPAC2 are found to couple with the G-protein (Gs) resulting in the stimulation of cell adenyl cyclase. The VPAC1, together with the VPAC2 receptor subtype, mediates a large array of VIP or pituitary adenylate cyclase activating peptide actions on different physiological functions including exocrine secretions, release of hormones, relaxation of muscles, metabolism, growth control of fetuses, and embryonic brain development. Patients with severe mental retardation have found to have a bleeding tendency with mild thrombocytopenia. Increased basal cAMP level in platelets is providing a basis for the reduced platelet aggregation. Megakaryocyte-specific transgenic overexpression of PACAP consequently increased the PACAP release from platelets to reduce the platelet activation and thereby prolongation of the bleeding time. In the management of arterial thrombosis and arterial bleeding, the therapeutic potential of PACAP is now considering clinical practices as inhibitor of thrombus formation [68].

13. Toll-like receptors (TLRs)

These are transmembrane proteins consisting of a lipoprotein receptor-related protein (LRP) extracellular domain, a transmembrane region, and a Toll-IL-1R domain, present on the surface of platelets. These exist in smaller amounts than GPIb-IX-V complex. Human platelet TLRs are similar to Toll receptor in Drosophila with four types (TLR-1, TLR-2, TLR-4, and TLR-6). However, platelets and megakaryocytes express mRNA and/or protein for different TLRs (e.g., TLR-1, TLR-2, TLR-3, TLR-4, TLR-6, TLR-7, TLR-8, and TLR-9) that detect and bind viral components and nucleic acids at the cell surface [69]. These TLRs (Table 1) play an important role in innate immunity by their ability to identify the products of bacteria, viruses, protozoa, and fungi, and important for their clearance (Table 2). After identification of these products, TLRs activate intracellular signaling pathways to

| Receptors   | Viruses                                                                 |
|-------------|------------------------------------------------------------------------|
| αvβ3        | Hantaviruses, coxsackieviruses A9 and A16, human adenovirus type 2, echovirus 9, and human parechovirus |
| αIIbβ3      | Human parechovirus                                                     |
| α 2β1       | Echovirus 1 and rotavirus                                              |
| DC-SIGN     | Lentivirus, HIV, and Ebola virus                                       |
| Axl         | Lassa fever virus (LASV)                                               |
| Tyro3; CCR-3 and -4; CXCR-1, -2, and -4; CLEC-2; DC-SIGN             | HIV                                                                   |
| GP-VI       | HCV                                                                   |
| CR2         | EBV                                                                   |
| α2β1        | Rotavirus                                                             |
| α2β3 (GPIIbβ3a) | Adenovirus               |

CLEC-2: C-type lectin-like type II transmembrane receptor; CR: complement receptor; CCR: C-C chemokine receptor; CXCR: C-X-C chemokine receptor; DC-SIGN: dendritic cell-specific intercellular adhesion molecule-3-grabbing; non-integrin; GP-VI: glycoprotein VI. Other details of platelet receptors are same as presented in Table 1. (This table is adopted from Seyoum et al. [69].)

Table 2. Different classes of platelet receptors for viruses.
induce an inflammatory response [70]. TLR-4 promotes platelet-neutrophil interaction and also causes activation of the neutrophils [71]. However, the involvement of FcγRIIA and serotonin may not be ignored [72].

14. Serotonin receptors

Serotonin (5-hydroxytryptamin) receptor of 2A type or 5-HT2A and 5-HT3A has also been found on platelet membrane [73, 74]. Platelet activation has been found to release stored serotonin from dense granules by amplifying release reaction and thereby promoting platelet aggregation. However, serotonin itself does not cause platelet aggregation but enhances platelet aggregation, induced by its other agonists (e.g., ADP and thrombin) [75]. It has been depicted that the interaction of 5-HT2A with serotonin initiates calcium signaling. The 5-HT3 receptor has been found to increase immunoreactivity with the platelet activation (with ADP and thrombin receptor activating peptide, TRAP). Serotonin is also known to cause vasoconstriction of the blood vessels with damaged endothelium and promotes thrombus formation. It has the ability to attach to a large number of substrates including fibrinogen, vWF, thrombospondin, and fibronectin [76]. The release of serotonin precedes the neutrophil contribution to shock, consistent with the reported role of serotonin in neutrophil activation (Table 1 and Figure 1). The vasodilation was also present in the absence of neutrophils, suggesting that the platelets orchestrate neutrophil activation and endothelial cell functions through serotonin and this event can occur independently critically with the FcγRIIA and serotonin [72].

15. Leucine-rich repeat receptors (LRRs)

The LRR is a protein structural motif with a repetition of 20–30 amino acids which are rich in leucine, a hydrophobic amino acid. It is involved in the formation of protein-protein interactions. This LRR includes GPIb-IX-V complex (see Section 2) and TLRs (see Section 13) of platelets. Though these receptors have their own class, they also have similarities or common factors between them (Table 1).

16. Complement receptors

Several types of complement receptors (CRs) are also expressed by the platelets (e.g., CR2, CR3, CR4, C3aR, C5aR, gC1qR, and cC1qR) [77]. These complements act as receptor for pathogens (Table 2) and implement multiple functions with both direct and indirect antimicrobial host defense (including cell lysis, opsonization, and chemotaxis) [69].

17. DC-SIGN

Recently, it has been found that the platelet granules can express the dendritic cell-specific ICAM3-grabbing non-integrin (DC-SIGN) receptor (Figure 1 and Table 2), like other cells which are used for the interaction with HIV-1 [78]. Platelet surface DC-SIGN receptors or enhancement of the receptor FcγII play an important role in binding of platelets with Dengue virus (DENV). DC-SIGN and heparin
Platelets

sulfate proteoglycan are receptors for DENV [69]. DENV infection leads to thrombocytopenia by increasing phagocytosis of DENV-induced platelets apoptosis by macrophages via a phosphatidylserine-recognizing pathway [69].

This detailed description of different platelet receptors will help us to understand the molecular changes of platelet receptors in different conditions.

18. Molecular changes of platelet receptors in different conditions

18.1 During platelet storage

In the cases of thrombocytopenia and bleeding complications, transfusion of platelet concentrates (PCs) is one of the most important therapeutic approaches and its management formula. Regardless of preparation techniques, stored platelets gradually experience inevitable deleterious changes called as platelet storage lesion (PSL). It may lead to a progressive structural and functional damage of platelet adhesion receptors from the time of platelets isolation till the transfusion to a recipient. It is induced mainly by either reversible or irreversible increases in the basal levels of platelet activation, and with the irreversible changes in platelet morphology and function the most important phase of PSL is associated with. These changes of platelet are significantly initiated with platelet α granules release (as mentioned in Sections 11 and 12) and its conversion from pro-aggregatory status to pro-inflammatory phenotypes which is identified by the P-selectin and CD40L expression [86]. The platelet activation increases the intracellular Ca²⁺ levels and acts as the main modulator of signaling events. This results in platelet receptor ectodomain shedding and membrane loss due to microparticulation. Both of these phenomena (ectodomain shedding and membrane loss) can cause progressive loss of platelet adhesive receptors during platelet storage [4, 87] and may affect its proper function required for therapeutic uses. During the storage of platelet concentrates (PCs), the GPIbα expression levels have been found to be decreased [77, 78]. The continuous shedding can lead to a significant decrease in GPIbα expression in 5-day-stored platelets [86]. More old platelets with the lower GPIbα expression may show less functionality after transfusion. The metalloproteinase-dependent loss of surface GPIbα plays a role in the clearance of aged platelets from the circulation. In older PCs, these receptors with higher levels of shedding could also be associated with a rapid clearance of transfused platelets leading to reduced platelet recovery and survival. In addition to this, the GPVI receptor expression also showed to be modulated during storage. A negative correlation between GPVI expression and shedding was also observed, a finding that verifies the main role of ectodomain shedding in the modulation of GPVI expression. There is a direct correlation between the shedding levels of P-selectin and GPVI during platelet storage. It suggests that this adhesion receptor is a valid marker of PSL [87]. The levels of soluble GPVI have been correlated with soluble P-selectin in patients with acute coronary syndrome and/or with acute ischemic stroke [88]. Considerable shedding of GPVI and its association with decreasing expression of this receptor on the surface of stored platelets can affect GPVI-dependent platelet function during storage. There is a direct correlation between this observed decreased adhesive capacity with the increasing levels of GPVI shedding during storage [4].

This enucleated blood component (platelet) may have a chance to interact with or interacts definitely under different pathophysiological conditions. Now, we will focus on those pathophysiological changes in relation to the platelet receptors.
18.2 During different pathophysiology

18.2.1 Inflammation

Platelets do not only play a role in thrombus formation but are also important in atherogenesis, and deficiency of an individual receptor or ligand has been found to be compensated by other receptors [89–92]. The inhibition of platelet adhesion in atherosclerosis-prone mice impedes the development of atherogenesis in it (these models of study) [93]. Platelet adhesion receptors discussed above in the context of thrombosis also support interactions between platelets, endothelial cells, and leukocytes involved in other vascular processes [94, 95]. Activated platelets with a potential mechanism adhered (by GPIbα binding) to the vessel wall with the support of leukocyte adhesion (with integrin, αMβ2). The GPIbα-binding site of the GPIb-IX-V complex similarly involves in the insertion of αM I domain of the αMβ2 to the vWF, homologous to the vWF-A1 domain [8]. Another mechanism involved here is the interaction between the platelet P-selectin and leukocyte PSGL-1 binding, as mentioned previously [9], and finally, a link between platelets and endothelial cells forms by the fibrinogen or vWF via the engagement of integrins on both platelets (such as αIIbβ3) and endothelium (such as αβ3) [96]. Understanding of the involvement of the networking of these platelet-specific receptors in thrombosis pathophysiology in vivo is yet to confirm.

18.2.2 Bernard-Soulier syndrome

The Bernard-Soulier syndrome (BSS) is the defect in the three GPIb encoding genes (as mentioned earlier), which gives rise to a serious bleeding diathesis, accompanied by anomalies in platelet morphologies, especially giant platelets. It is a rare hereditary thrombocytopathy, first described in 1948 by Jean Bernard and Jean-Pierre Soulier, two French hematologists, in a young male patient who had severe mucocutaneous bleeding, prolonged bleeding time with normal platelet count, and abnormally large platelets (macrothrombocytopenia) [90]. In view of these defects, the disorder was named as “Dystrophie thrombocytaire-hémorragique congénitale” (hemorrhagic thrombocytic dystrophy) [97]. In most cases, bleeding symptoms manifest rapidly after birth or during early childhood. Clinical manifestations usually include purpura, epistaxis, gingival bleeding and menorrhagia (menstrual periods with abnormally heavy or prolonged bleeding), and more rarely gastrointestinal bleeding and hematuria (presence of blood in a person’s urine). Severe bleeding episodes are associated with trauma and surgical procedures (e.g., tonsillectomy, appendectomy, splenectomy, during dental extractions), gastric ulcers, and menses. However, indeed the severity and frequency of bleeding vary between individuals [21]. Ultrastructural studies of affected platelets show a dilated open canalicul system, prominent dense tubular system, and vacuolization. It is rare with a reported prevalence of 1 in 1,000,000. Patients have a prolonged bleeding time, thrombocytopenia, and larger platelets than the normal individual due to defective thrombopoiesis in GPIb defective megakaryocytes [98]. Few mutations were reported that cause a gain-of-function in the GPIbα chain, leading to the so-called platelet-type GPIB [99], showing a phenotype similar to that of certain subtypes of von Willebrand disease [100]. The syndrome, as an autosomal recessive trait, is found to be transmitted with an underlying defect of deficiency or dysfunction of the GPIb-V-IX complex, required for normal primary hemostasis. The GPIb-V-IX complex binds to vWF, allowing platelet adhesion and platelet plug formation at sites of vascular injury (as mentioned in Section 2.1) (Table 1) [21].
18.3 Aging and aging-related pathophysiological conditions

During aging, the platelet receptor expression levels have also been investigated and found that the platelet responsiveness to collagen decreases with age with an aging-dependent decrease in GPVI-dependent platelet activation [101]. Adhesion receptor levels on nucleated thrombocytes have also been found to decrease during aging in correlation with the decrease in participation in thrombus formation. The younger thrombocytes contain more adhesive receptors with a higher propensity to form the thrombi than the aging counterpart [102]. The mechanism of platelet shedding in regulation of aging-related changes in adhesion receptor levels has not been demonstrated yet [103]. Aging with the different biochemical alterations due to alteration in cellular microenvironments positively associates with the downregulation of different receptors, its consequent signaling pathways, biochemical cascade mechanisms, formation of abnormal proteins, its related neurodegeneration, immunosuppression, susceptibility to viral infection (e.g., COVID-19), etc. and negatively associates with the survival from the diseased condition.

18.3.1 Thromboxane alterations

Thromboxane (A$_2$ and B$_2$) is produced from arachidonic acid through endoperoxidase by the cyclooxygenase and thromboxane synthase enzyme activity, respectively [104]. It plays a pivotal role in platelet aggregation in vivo. It has been found that thromboxane production is enhanced in diabetic subjects resulting in platelet aggregation, which further provides a higher risk of cardiovascular disease [104]. A number of risk factors have been proposed to elevate CVD risk in type 1 diabetes mellitus (T1DM) patients, including hyperglycemia, dyslipidemia, inflammation, oxidative stress, and genes among others. A significant contributing factor to the diabetic prothrombotic state is the aberrant regulation of antiplatelet-activating mechanisms that normally maintain high levels of inhibitory cAMP to prevent aggregation. Molecules directly affecting platelet cAMP production are the arachidonic acid metabolites TXA$_2$ and prostacyclin (PGI2). TXA$_2$ is produced in the platelets themselves and is a positive-feedback mediator of platelet activation, while PGI2 is produced in endothelial cells and is an inhibitor of platelet aggregation. The platelet activator TXA$_2$ is synthesized by cyclooxygenase 1 (COX-1) in the platelets; the inhibition of COX-1 (with aspirin), which is irreversible and semi-selective, could improve platelet reactivity of T1DM subjects [104].

18.3.2 Cardiovascular diseases

The numbers of platelet GP receptors are found to enhance in cardiovascular patients and diabetic subjects. The HbA1c is the useful marker to detect the diabetes in individuals. The CD40L on platelets has been found to be correlated with this HbA1c concentration, as an upregulation of the CD40-CD40L system has been observed in diabetes mellitus [56] along with an increase in GPIa/IIa, GPIIb/IIIa, P-selectin (CD62), vWF, and CD63 [105]. Patients with ischemic heart disease and depression concomitantly may have increased risk of thrombosis due to abnormal platelet activation. Moreover, high vWF may increase the chance of cardiovascular disease. Elevated plasma levels of vWF are associated with established cardiovascular risk factors such as age, smoking, cholesterol, diabetes mellitus, and hypertension [105, 106]. Moreover, raised levels of vWF are predictive of stroke and vascular events among patients with atrial fibrillation [106].
18.3.3 Neurodegeneration

Platelet dysfunctions associated with aging can be linked to molecular alterations affecting several cellular systems that include cytoskeleton rearrangements, signal transduction, vesicular trafficking, and protein degradation. Aging in platelets and their age-dependent dysfunctions are of interest when evaluating the contribution of aging to the onset of aging-dependent pathologies, such as those affecting the nervous system linked to neurodegenerative diseases including Alzheimer’s disease (AD), Parkinson’s disease (PD), multiple sclerosis (MS), Huntington’s disease (HD), and amyotrophic lateral sclerosis (ALS) [107, 108].

It is well understood from the above information that the platelets have two important types of granules, dense granules and alpha granules. The dense granules are loaded with proaggregatory factors (e.g., serotonin, calcium, and ADP), and during platelet activation, these granules release their content to the open canalicular system to be expelled out by the platelet, whereas the alpha granules have many hemostatic proteins (e.g., platelet-derived growth factor, vWF, and fibrinogen) [108]. Aging-related variations in the expression of specific platelet receptors and platelet activators have been reported and exemplified by a decrease in the number of receptors for PGI2 potent inhibitor of platelet function and high levels of TXA2 activator of platelet function in older individuals [109]. Even though the most important function of platelets is to prevent bleeding, they also play an important function in pathological conditions including neurological and neurodegenerative diseases (e.g., PD, schizophrenia, and AD). It is also important that platelets show high expression of several proteins associated with the development of AD, such as the APP amyloid precursor protein (APP) and tau protein. Additionally, platelets express enzymes involved in protein modifications such as glycogen synthase kinase 3β (GSK-3β), α- , β-, and γ-secretases. Platelets have been compared with neurons because they have many biochemical similarities [108], as they have the storage and release capacity of neurotransmitters from platelets such as serotonin, glutamate, and dopamine [110, 111] and the expression of neuron-related proteins such as NMDA receptors [112]. Together, this makes it interesting to consider the contribution of platelets to the hallmarks of neurodegeneration.

It is very imperative to note that the brains of cerebral amyloid angiopathy (CAA) patients (a disorder characterized by deposits of Aβ40 in cerebral arteries and capillaries) is estimated its prevalence in 90–98% of AD patients and this AD is present in 30% of individuals without dementia over 60 years old. It is needless to mention here that AD is related with the amyloid-beta (Aβ) pathogenesis. Aβ40 peptide activates and promotes platelet adhesion and aggregation [113] by different receptors such as CD36 and GPIIb, triggering several signal transduction pathways involving p38MAPK and COX1 and synthesis of TXA2, which ultimately increase Ca^{2+} levels, activates calpain, and increases Aβ40 peptide secretion [114]. The thrombin receptor PAR1 could also have a role in the consequent activation of p38 MAPK and cytosolic phospholipase A2 (PLA2), and TXA2 formation. Aβ40 peptides modify platelet shape change and granule release through activation of the small GTPase RhoA and phosphorylation of its downstream effector, myosin light chain kinase, involving cytoskeletal reorganization [113]. In platelets, the production of Aβ40 peptides also regulates the platelets phosphatidyserine exposure which has been found to be involved for further increase in platelet Aβ40 levels. A correlation between increased ROS formation in AD platelets and increased oxidative stress in AD patients has been demonstrated [108].

The coagulation cascade plays the critical role in the development of an inflammatory response in MS. Platelets are trapped in chronic active demyelinating MS lesion. The paralysis and experimental autoimmune encephalomyelitis were found
Platelets to be ameliorated and reduced after inhibiting (the GPIIb/IIIa blocker, abciximab) the main platelets integrin GPIIb/IIIa [115]. A role of thrombin cascade in the development of inflammation in MS has also pointed out [116]. This multifunctional cell (platelets) is activated by different endogenous, physiological agonists, including ADP, collagen, or thrombin, due to the vast number of receptors present on the surface of platelets, as discussed previously. During the vessel wall injury, the circulating platelets are found to (a) immobilize immediately, (b) interact with the vWF binding to collagen and the glycoprotein GPIb-V-IX complex [117], and (c) initiate adhesion of free moving platelets of circulation to the subendothelial extracellular matrix. The information about involvement and changes of platelet receptors in other aging-induced neurodegenerative disorders is yet to be studied.

18.4 Cancer

The knowledge in the last decade on how tumor cells exploit platelets for survival, arrest, and finally extravasation from blood vessels to distant organs has tremendously increased [118]. Platelets protect circulating tumor cells (CTCs) by encing tumor cells in a thrombus (Figure 2), protecting them from cytolysis by natural killer cells [119]. Tumor cells activate platelets for a stable adhesion between platelets and tumor cells by distinct mechanisms, which are the reasons for hypercoagulation and increased risks of thrombosis in cancer patients [107]. Tumor cells release soluble mediators such as ADP, TXA2, or high-mobility group box 1 (HMGB1), which ligates with Toll-like receptor 4 (TLR4) to instigate local platelet activation [120]. The heterogeneous cloaks of platelets and tumor cells protect tumor cells from high shear forces in the blood circulation and from attack by leukocytes by a pseudonormal phenotype, and allow tumor cells to downregulate MHC class I molecules to escape T cell immune surveillance [118]. This finally leads to tumor growth and angiogenesis with a deposition of platelets in tumors by the engagement of the P-selectin-αIIbβ3-talin complex [121]. This P-selectin binding to its ligands can also activate pro-survival kinases, resulting in enhanced tumor growth in mice neuroblastoma cells [122]. Platelets, those bind to colorectal cancer cells by means of P-selectin in the presence of polymorphonuclear leukocytes, were able to activate human microvascular endothelial cells. This in turn is found to express inflammatory proteins, e.g., chemokine CCL5 most abundantly [123]. CCL5 recruited monocytes to the metastatic microenvironment that finally culminated in an augmented number of metastatic foci in the lungs. It suggests that platelets and platelet-derived P-selectin seem to be crucially involved in cancer immunity (Figure 2).

After platelet activation, αIIbβ3 can switch to at least one of the two different active ligand-binding states; both differ in their affinity for fibrinogen, which indicates the contribution of αIIbβ3 to tumor cell platelet interaction and aggregation [118, 124]. Hence, integrin αIIbβ3 is for several reasons an attractive target in hematogenous cancer cell dissemination. Different kinds of drugs which efficiently inhibit integrin αIIbβ3 have been approved for reduction or prevention of thrombotic cardiovascular events. Furthermore, integrin αIIbβ3 is capable of mediating bidirectional signaling [118]. On the one hand, binding of integrin αIIbβ3 to tumor cells can finally culminate in platelet activation, while on the other hand, platelet activation by, e.g., ADP, TXA2, or thrombin, can transfer αIIbβ3 to an active binding state [107]. These inside-out signaling confers the ability to bind several ligands (e.g., melanoma cell expressed αvβ3), which can induce protumorigenic and proangiogenic signals [125].

In different types of cancers, for instance in squamous cell carcinoma, lung and skin cancer, mesotheliomas, and cancer-associated fibroblasts, an
upregulated expression of podoplanin was detected [118, 126], converting an epithelial to mesenchymal transition (EMT) in MDCK cells, by increasing cell migration, and was associated with tumor invasion [127]. In healthy human tissues, podoplanin is expressed in lymphatic endothelial cells, osteocytes, keratinocytes, podocytes, and myofibroblasts among many other cell entities [128]. Podoplanin deficiency leads to form defective lymphatic vessels and causes death due to respiratory failure after birth [129]. Thus, the CLEC-2-podoplanin axis is an interesting target in course of hematogenous metastasis (especially for those patients with podoplanin-positive tumors), though more study is needed. Meanwhile, the tumor cell-induced platelets aggregating effect of the podoplanin CLEC-2 interplay has been revealed in several animal models, and by this way, different antibodies targeting different epitopes are generated [118, 130]. CLEC-2 ligand responsible for pronounced platelet aggregation and a podoplanin recognition domain in CLEC-2 was elucidated [131]. Podoplanin contains three platelet aggregation-stimulating domains in the extracellular section which is crucial for the aggregating function [132]. GPVI cytoplasmic tail is associated through a salt bridge with the Fc receptor γ chain (FcRγ), and upon ligand-mediated GPVI crosslinking and clustering, ITAM motif in FcRγ chains are unmasked and phosphorylated. This phosphorylated ITAM is sequentially found to recruit and activate Syk downstream signaling complex (composed of LAT and SLP76) to activate platelet and spreading of this platelet activation [133]. However, the study on the role of this ITAM-containing receptor in the interaction of platelets with tumor cells is very limited. Indeed, it has been observed that in C57BL/6J mice, deficiency in GPVI leads to decrease in thrombus formation with a prolongation of bleeding time [134]. Furthermore, an IgG-independent FcγRIIa-mediated cooperation between GPVI, GPIb-IX-V, and αIIbβ3 for platelet activation and spreading has been suggested. Studies dealing with the participation of FcγRIIa in tumor metastasis are barely available [135].
18.5 Platelet receptors and viral pathogens

There are different platelet receptors including TLRs, CRs, and DC-SIGN (as described in Sections 13, 16, and 17) that facilitate the direct interaction of platelets with viral pathogens. This interaction through different receptors causes both quantitative and qualitative dysfunctions of platelets associated with viral pathogens (Table 2). Platelets play a role in defending viral pathogen by binding of viral pathogen with platelets, which results not only in clearance of platelets but also clearance of viral pathogens [69].

18.5.1 Coronavirus disease-2019 (COVID-19)

Recent worldwide outbreak of COVID-19 is known to us as a flu-like disease, in which the respiratory illness (like the flu) with symptoms such as cough, fever, and in more severe cases, difficulty in breathing occurs. Early during its initiation (December, 2019) of infection, it was named as novel coronavirus disease-2019 or nCOVID-19. But in the next month (January, 2020), it was named as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was later (February, 2020) designated as coronavirus disease 2019 or in short “COVID-19” by WHO (World Health Organization). Despite of its acute respiratory symptom, it is being reported that in more severe cases, abnormal clotting is a common phenomenon that culminates into the risk of cardiovascular disease (CVD), and this fact is the most crucial one here to discuss with (Figure 3). In severe cases of COVID-19 patients, clots in the small vessels of all organs, not only in the lungs but also in heart, liver, and kidney, have been found [136]. Though the involvement of any platelet receptor has not been found yet, the presence of clot indicates toward the involvement of platelet receptors for sure, as there are different platelet receptors already existing which can bind with different viruses (Table 2). Scientists have

Figure 3.
Schematic presentations of clinical courses of major symptoms and outcomes and duration of viral shedding in the COVID-19 patients. ICU: intensive care unit; ARDS: acute respiratory distress syndrome; SARS-CoV-2: severe acute respiratory syndrome coronavirus 2; and COVID-19: coronavirus disease 2019. (This diagram is adopted from Zhou et al. [136].)
noticed till date about the presence of the biomarker of clot, known as D-dimer in the blood samples of COVID-19 patients with severity [136]. The presence of D-dimer in the circulation has its normal range of <0.5 μg/ml. D-dimers are not normally present in human blood plasma, except when the coagulation system has been activated (Figure 4). The structure of D-dimer is either a 180- or 195-kDa molecule of two D domains or a 340-kDa molecule of two D domains and one E domain of the original fibrinogen molecule [137]. D-dimer levels over 1 μg/ml at the time of admission predicted an almost 18-fold increase in odds of dying before discharge of nCOVID-19 patients seen at two hospitals in Wuhan, China [136]. D-dimer, a fibrin degradation product indicating thrombosis, can exceed 70 or 80 μg/ml, which clearly can indicate the severity of the illness. In that case, the anticoagulation therapy (with the anticoagulation, regardless of the underlying mechanism) may be initiated for severe COVID-19 patients, unless otherwise contraindicated the consequences [136].

19. Conclusion

Platelet receptors, particularly their adhesion receptors, execute an important role in the regulation of circulatory hemostasis. A rapid transition of circulating resting platelets to the activated state, adhesion, and aggregation in thrombus formation happen by the cascades of events. Briefly, these events are: (i) initial contact adhesion with the platelet GPIb-IX-V and GPVI, and collagen or collagen-bound vWF; (ii) activation, spreading, and secretion, involving GPIb-IX-V- and GPVI-dependent signaling pathways; (iii) secretion of agonists (e.g., ADP), the P2Y1/P2Y12 receptors activation, and upregulation of integrin αIIbβ3; and (iv) αIIbβ3-dependent aggregation, involving vWF or fibrinogen (Table 1 and Figure 1). These processes

Figure 4.
Schematic diagram of D-dimer formation as a risk factor for the development of CVD in the COVID-19. Fibrinogen is made up of one E domain and two D domain, whereas D-dimer has the same number of domains but with different structural orientation with cross-linked D domain.
happen very fast, and to imagine their rate of reactions, it may be informed that the initiation of thrombus formation starts within seconds after an injury, and complete thrombus is formed within minutes. Consequent to the aggregation process by recruiting free flowing inactivated platelets, the coagulation process accelerates on the activated platelet surface with the involvement of leukocytes and RBCs (red clot), followed by stabilization of the thrombus with the polymerized fibrin, and finally the αIIbβ3-mediated clot contraction in vivo. The dysfunction of GPIb-IX-V in humans (Bernard-Soulier syndrome) or mice (GPIbα gene knockouts) thrombus formation under high shear conditions is specifically impaired [5, 6]. Recent evidence also reveals a requirement for GPVI in stable thrombus formation at high shear rates, which provides the evidence of functional co-association of GPVI and GPIb-IX-V [6, 25]. The recent discovery of 5-HT receptors (2A and 3) and their role on platelet aggregation added a special attention on the interaction of neurotransmitter with the platelet adhesion receptors (Table 1), which may open a new avenue in geriatrics research. New molecular insights into the role of primary platelet adhesion receptors in vascular biology would be beneficial in pathophysiology study. GPIb-IX-V and GPVI together form a multifunctional adhesive cluster of proteins on the platelet surface that control thrombus formation at high shear and are also central to other vascular processes including inflammation and atherogenesis. Other platelet receptors including GPs, integrins, GPCRs, and Ig superfamily also play a pivotal role to maintain normal physiology. The physiology and pathophysiology of different platelet surface receptors at the molecular level have been described in this chapter. The molecular pathophysiology of different platelet receptors under different pathological conditions has been described. The recent advancement of the knowledge of how platelet is being considered as a biomarker of neurodegeneration and how far it is reliable to consider it (platelet) with central nervous system (CNS) has been pointed out with its updated information. The molecular aspects of inflammation, cancer (Figure 2), and even viral pathogens (Table 2), especially COVID-19 pathology (Figures 3 and 4) in relation to platelet receptors, are also taken under consideration to present in this chapter. This updated information will enlighten and motivate researchers to advance and carry forward their research with platelet at the molecular level of its receptor in different pathophysiological conditions. Finally, it may be stated that all of these information and thoughts may help to find out different biomarkers and treatment for different diseases in the near future.

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Conflict of interest

There is no conflict of interest.
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