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Structure and function of the ubiquitin-proteasome system in platelets

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Abstract

Platelets are small anucleate blood cells with a life span of 7 to 10 days. They are main regulators of hemostasis. Balanced platelet activity is crucial to prevent bleeding or occlusive thrombus formation. Growing evidence supports that platelets also participate in immune reactions, and interaction between platelets and leukocytes contributes to both thrombosis and inflammation. The ubiquitin-proteasome system (UPS) plays a key role in maintaining cellular protein homeostasis by its ability to degrade non-functional self-, foreign, or short-lived regulatory proteins. Platelets express standard and immunoproteasomes. Inhibition of the proteasome impairs platelet production and platelet function. Platelets also express major histocompatibility complex (MHC) class I molecules. Peptide fragments released by proteasomes can bind to MHC class I, which makes it also likely that platelets can activate epitope specific cytotoxic T lymphocytes (CTLs). In this review, we focus on current knowledge on the significance of the proteasome for the functions of platelets as critical regulators of hemostasis as well as modulators of the immune response.

KEYWORDS
antigen presentation, MHC class I, platelet activation, proteasome endopeptidase complex, ubiquitin

1 | INTRODUCTION

Platelets are non-nucleated blood cells with a small diameter of 2 to 5 µm. After release from megakaryocytes, platelets circulate in the bloodstream for 7 to 10 days.[1] With 150 000-400 000 platelets/µL, platelets outnumber, by more than an order of magnitude, leukocytes. To meet the requirements for platelet activation, aggregation, and hemostasis, platelets express numerous different transmembrane receptors. Amongst these are the fibrinogen receptor (αIIbβ3 or GPIIb/IIIa), the thrombin (protease activated receptors, PAR) receptors PAR1 and 4, the adenosine diphosphate (ADP) receptors P2Y1 and P2Y12, the thromboxane receptor, the collagen receptors (GP Ia/IIa and GPVI), and C-type lectin receptors. Platelets contain different storage vesicles such as α-granules, dense granules, and lysosomes, which contain a large number of different biological active compounds, including clotting factors, chemokines, interleukins and defensins, ADP, and serotonin.[2] After interaction with their physiological or pathogen-derived agonists, the receptors and their linked

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signaling pathways are activated further promoting granule secretion and platelet aggregation.[3,4]

During the last decade the role of platelets was extended through identification of regulatory functions in innate and adaptive immunity.[5] Platelets interact with cells of the innate (monocytes, neutrophils, and dendritic cells) and adaptive immune system (T and B lymphocytes).[6] Upon activation platelets secrete approximately 300 different proteins mainly derived from α-granules.[7] Many granule-derived mediators have been shown to display immune modulatory functions.[7] Amongst them are cytokines (IL-1α, IL-1β, and TGFβ1), chemokines (platelet factor 4 [CXCL4]), macrophage inflammatory protein 1-α (CCL3) and RANTES (CC chemokine ligand 5), low-molecular-weight mediators (eg, ADP), and lipid-derived factors such as platelet-activating factor (PAF) and thromboxane A2.[8] In addition, activated platelets express CD40 and CD40L (CD154) and are able to release soluble CD40L. Platelet-derived CD40L was shown to interact with vascular endothelial cells, to promote dendritic cell maturation and T cell activation, as well as B cell differentiation including immunoglobulin class switching.[9-11] Taken together, platelets modulate innate and adaptive immune responses.

Furthermore, platelets are able to execute de novo protein synthesis and to express proteasomes, multicatalytic protease complexes that are responsible for protein degradation, and regulation of signal transduction processes.[12,13] A prominent example is the regulation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway originally described in nucleated cells but surprisingly observed in platelets as well. Here activation is accomplished by ubiquitination of the NF-κB-inhibitor IκBα and its subsequent degradation by the proteasome complex (Figure 1).[14-16] The proteasome is also relevant for modulating the signaling pathways of αIIbβ3, GPVI, and CLEC-2 (Figure 1) and might play a pivotal role in additional signaling pathways in platelets.[17-22] In this review, we summarize the known fundamental regulatory mechanisms and functional aspects of the proteasome in human platelets.

Essentials

- Platelets express all constituents of the proteasome and the immunoproteasome.
- Platelets express the machinery for ubiquitination of proteins.
- Components of the antigen processing and presentation pathway (APP) are expressed in platelets.
- The platelet proteasome links platelets and immune regulation.

2 | PROTEIN DEGRADATION BY PROTEASOME COMPLEXES

The structure and function of the approximately 2.5 MDa large ubiquitin-proteasome complex (Figure 2) has been described in detail in various cell types apart from platelets.[23,24] In eukaryotic cells it is localized in the cytoplasm and nucleus. One of the most important functions of the proteasome is to maintain cellular protein homeostasis by degrading misfolded, short-lived, and abnormal proteins.[24-26] In addition, the ubiquitin-proteasome system is involved in regulation of signal transduction cascades, eg, induction of apoptotic processes and inflammation, control of cell division, survival and cell cycle, as well as in the supply of antigenic peptides for major histocompatibility complex (MHC) class I antigen presentation.[27-29] The transfer of ubiquitin moieties, internally linked via Lysin 48 (K48) residues, to protein substrates has been shown to be crucial for proteasomal substrate recognition and degradation.[28,30] Because there are different nomenclatures for the proteasome subunits established, we summarize the most frequently used names in the mammalian system, which are also identified in platelets (Table 1).

The mammalian 26S proteasome consists of a central, catalytically active 20S subcomplex (750 kDa) and one or two regulatory 19S complexes (900 kDa each), which are attached to one or both ends of the 20S core particle[23,31] (Figure 2). The 19S complex is responsible for recognition and unfolding of ubiquitinated proteins and has deubiquitinating activity.[32] Subsequently, protein substrates are translocated to the 20S complex, which has the shape of a hollow cylinder and is composed of four rings, two outer α-rings and two inner β-rings. These rings contain seven structurally similar α- and β-subunits each. Three β-type subunits, β1/delta, β2/Z, and β5/MB1, are catalytically active.[24] They display caspase-like, trypsin-like, and chymotrypsin-like activity, cleaving peptide bonds post-acidic, -basic, and –hydrophobic amino acids, respectively.[23] The α-subunits of the 20S complex form an almost completely closed gate under non-stimulated conditions, controlling protein access into the inner catalytic chamber.[33] Upon activation of the proteasome, the gate is opened and ubiquitinated proteins are degraded.

Another layer of complexity of proteasome functions is added, because the composition of proteasome complexes can be dynamically adjusted to varying cellular requirements, eg, cellular stress caused by infection. Cytokines such as type I/II interferons (IFNs), or bacterial lipopolysaccharide (LPS) induce the expression of the so called immunosubunits β1i(induced)/LMP2 (low molecular mass protein), β2i/MECL-1 (multicatalytic endopeptidase complex like-1) and β5i/LMP7 in cells expressing the corresponding receptor profile. The immunosubunits display structural homologies to the standard proteasome (SP) subunits. Proteasome isoforms containing these immunosubunits are called immunoproteasome (IP) (Figure 2),[34-41] which are constitutively expressed in cells of myeloid or lymphoid origin.[42] Immunoproteasome subunits are preferentially incorporated into newly synthesized proteasome complexes by a selective assembly mechanism.[23,42] Compared to the standard proteasome, immunoproteasomes display an

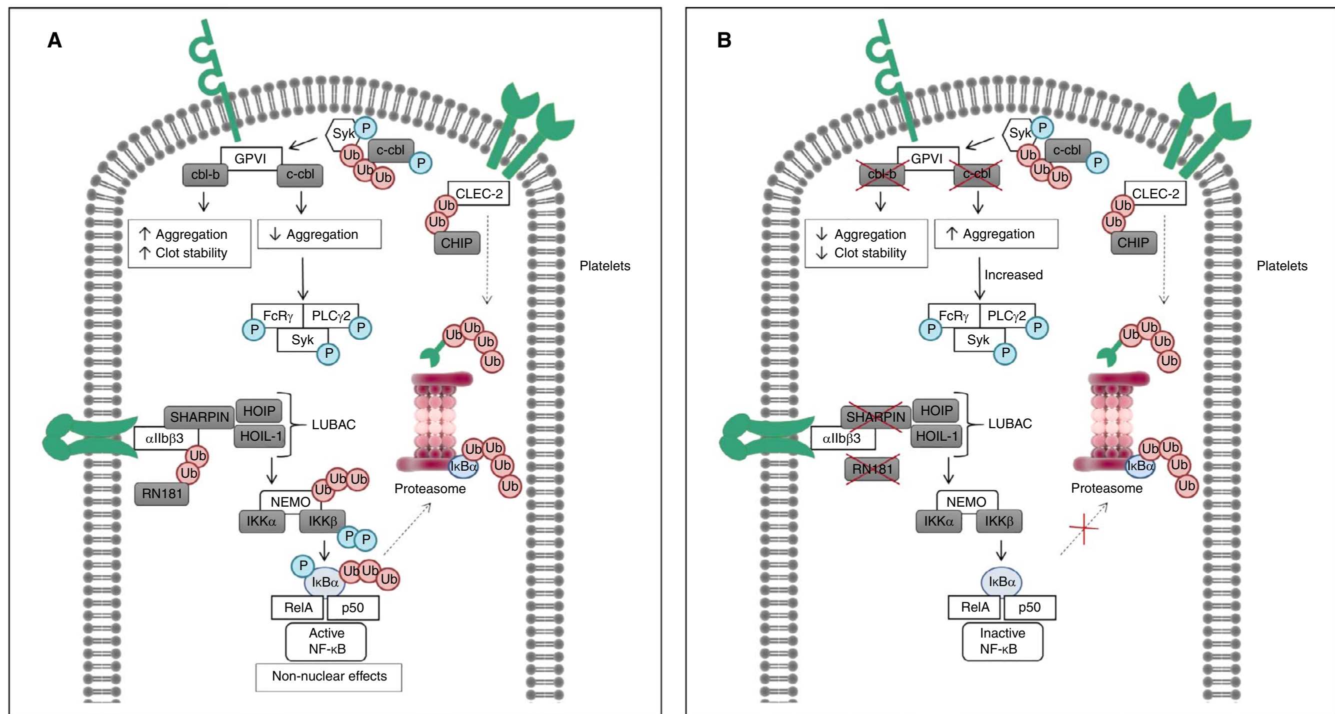

FIGURE 1 NF-κB-pathway and function of E3 ubiquitin ligases in platelets. The scheme highlights the known functions of E3 ubiquitin ligases in platelet activation pathways. A, Cbl-b regulates the GPVI pathway by increasing aggregation and clot stability. In contrast, c-Cbl displays the opposite function by reducing platelet aggregation after GPVI activation. In addition, c-Cbl can ubiquitinate the phosphorylated tyrosine kinase Syk, which leads to increased Syk activity and GPVI signalling. The E3 ligase CHIP is responsible for ubiquitination of CLEC-2, which causes its proteasomal degradation. The integrin-associated protein SHARPIN interacts with αIIbβ3 and induces ubiquitination of NF-κB proteins via the linear ubiquitin chain assembly complex (LUBAC) and the E3 ligases HOIP and HOIL-1. In addition to this mechanism, E3 ligase RN181 interacts with αIIbβ3 and K48-polyubiquitinated IκBα can be degraded by the proteasome. The activated NF-κB can induce non-nuclear effects in platelets. B, Cbl-b possesses a positive modulatory role upon activation of the GPVI signalling pathway, because depletion results in decreased aggregation and reduced clot stability. In contrast, c-Cbl displays negative modulation, because depletion results in increased aggregation and increased phosphorylation of downstream effectors. SHARPIN knockdown leads to reduced ubiquitination of NF-κB proteins and the active IκBα inhibits the activation of NF-κB. Cbl, casitas B-lineage lymphoma; CHIP, C terminus of Hsc70-interacting protein; CLEC-2, C-type lectin-like receptor 2; FcRγ, Fc receptor γ-chain; GPVI, glycoprotein VI; HOIP and HOIL-1, heme-oxidized IRP2 ubiquitin ligases; IκBα, inhibitor of NF-κB; LUBAC, linear ubiquitin chain assembly complex; NF-κB, nuclear factor 'kappa-light-chain-enhancer' of activated B cells; PLCγ2; phospholipase C gamma 2; RN: RING finger protein; SHARPIN, SHANK-associated RH domain interactor; Syk, spleen tyrosine kinase

enhanced capacity to degrade protein substrates to protect cells against protein stress.[43-45] Due to their increased substrate degradation capacity immunoproteasomes are also highly relevant for the generation of MHC class I peptides. Mice deficient for all three immunoproteasome subunits show substantial differences (~50%) in their MHC class I peptide repertoire compared to the “peptidome” of wild type mice.[46]

In addition to the standard proteasome and the immunoproteasome, a third group of proteasome complexes has been identified in various tissues, the intermediate proteasome types. Intermediate proteasomes express only the immunosubunits β5i/LMP7 and β1i/LMP2 or β5i/LMP7. Thereby their catalytic properties differ from that of standard or immunoproteasomes.[38] In conclusion standard-, immuno-, and intermediate proteasome complexes differ in their 20S unit. In addition, IFNs induce the expression of another 20S proteasome regulator, the PA28α/β complex (PA, proteasome activator). PA28 has been associated with enhanced epitope processing by facilitating the access of substrates to the active sites of the proteasome.[47,48]

Platelets constitutively express standard and immunoproteasome subunits and display each of the three protease activities, caspase-like, trypsin-like, and chymotrypsin-like activity, executed by the catalytically active β subunits.[13,49] Table 1 summarizes the proteasome and immunoproteasome subunits identified in platelets. Furthermore, we identified the expression of all catalytically active standard and immunoproteasome subunits in purified platelets (Figure 3). These data indicate that the proteasome and the immunoproteasome may play a substantial role in megakaryocyte and platelet biology.

3 | IMPACT OF PROTEASOME FUNCTION ON PLATELET PRODUCTION AND ACTIVATION

To characterize the proteasome activity under different conditions, proteasome inhibitors are important tools for in vitro and

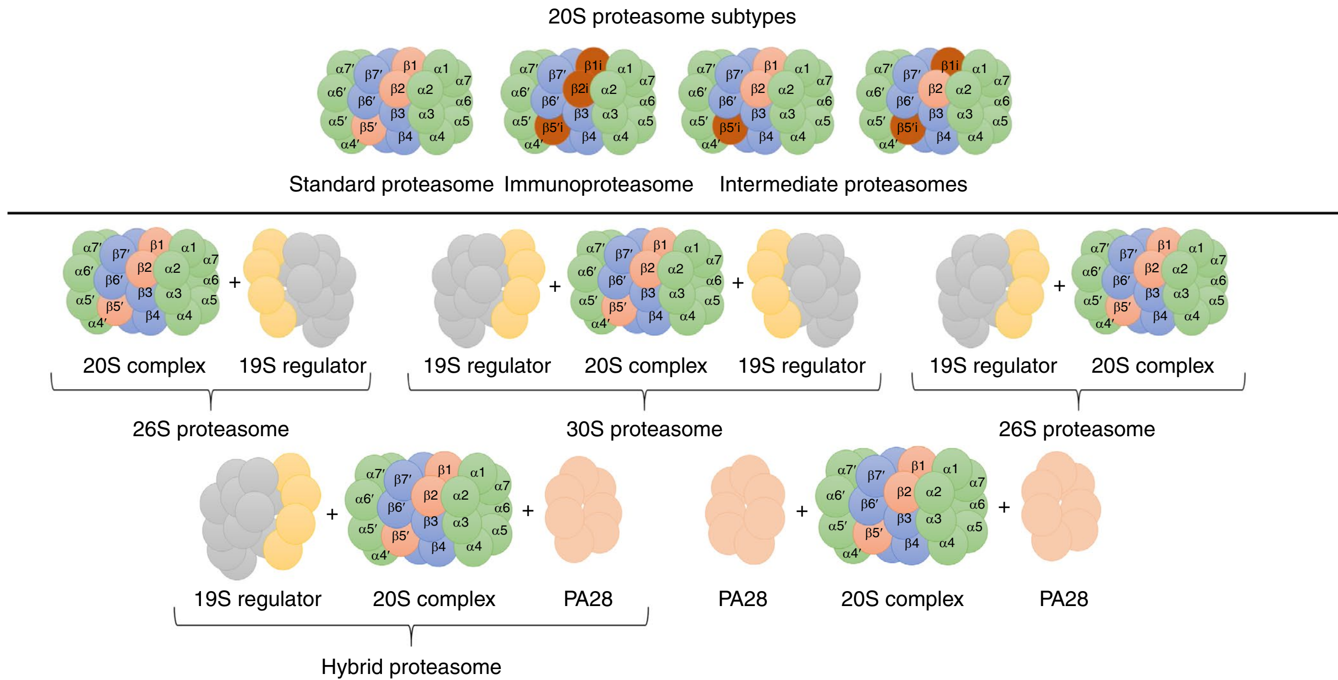

FIGURE 2 Structure of the proteasome complexes and regulators. The proteasome consists of a 20S core complex, which is composed of four rings. These rings contain seven α- and β-subunits each. Three β-type subunits, β1/delta, β2/Z, and β5/MB1, are catalytically active. In immune cells or upon induction with interferons (IFN) or lipopolysaccharide (LPS) immunoproteasome subunits are synthesized and form immunoproteasomes (IPs) with the catalytic active subunits β1i/LMP2, β2i/MECL-1, and β5i/LMP7. In addition to standard and immunoproteasomes, two additional intermediate proteasome types exist, which contain a mixture of standard and immunosubunits. The 20S core complex interacts with regulatory particles. The 30S proteasome consists of two 19S regulatory complexes bound to the 20S, while the 26S proteasome is associated with one 19S complex. In addition, PA28αβ (PA, proteasome activator) is a regulatory particle which can be attached to the 20S complex. The combination of 19S, 20S, and PA28αβ is called hybrid proteasome[15,30]

in vivo experimental settings, eg, bortezomib, which mainly targets the chymotrypsin-like activity of the β5/MB1 and β5i/LMP7 subunits.[50-52]

The platelet proteasome interferes with platelet aggregation. Proteasome inhibitors block human platelet aggregation, whereas agonist induced platelet activation results in enhanced proteasomal peptide hydrolyzing activity.[15,16,53-56] Platelets activated by thrombin receptor-activating peptide (TRAP), ADP, collagen, or thrombin showed a tendency to an increased basal proteasome activity. Proteasome inhibitor treatment resulted in decreased aggregation in response to low concentrations of thrombin (0.025 U/mL) and collagen (1-2 µg/mL). However, as the experimental conditions varied between the studies, it is too early to draw definite conclusions on underlying mechanisms.[15,54-56] In addition to direct interference of the proteasome activity with platelet aggregation, the platelet cytoskeleton proteins Filamin A and Talin-1 are ubiquitinated and subsequently degraded by proteasomes.[55]

Based on the observation that patients treated with proteasome inhibitors often develop thrombocytopenia, proteasome function could be linked to thrombopoiesis. Analyzing mice deficient for the 19S proteasome regulator complex subunit PSMC1 in platelets in vivo and in vitro data displayed a significant reduction of proplatelet formation in proteasome-inhibited megakaryocytes due to hyperactivation of the small GTPase RhoA.[57] Consistent with this finding, in vivo application of the proteasome inhibitor bortezomib causes pronounced reduction in proplatelet formation and platelet count.[58] The reduced platelet number after proteasome inhibition can be further explained by the finding that platelet life span depends on proteasome activity. In platelets exposed to proteasome inhibitors, stabilized expression of the pro-apoptotic protein Bax resulted in a reduced platelet half-life.[59]

Furthermore, some patients suffering from chronic atypical neutrophilic dermatosis with lipodystrophy and elevated temperature (CANDLE) syndrome, a rare human genetic disorder caused by mutations in genes of the ubiquitin-proteasome system (mostly *PSMB8* encoding the β5i/LMP7 subunit), exhibit next to intracellular accumulation of ubiquitinated proteins and elevated type I interferon levels also thrombocytopenia.[60] This indicates an interconnection between the ubiquitin proteasome system and megakaryocytes, platelets, inflammation, and immune response, although no data on platelet studies in patients with CANDLE syndrome are available yet.

4 | UBIQUITINATION AS A POST-TRANSLATIONAL MODIFICATION

Post-translational modification of cellular proteins by ubiquitin is an upstream effect, which is indispensable to mark protein cargoes for proteasomal degradation. We therefore address in the following some aspects of ubiquitination of proteins in platelets.

TABLE 1 Nomenclature of the different proteasome subunits and proteasome regulator complex subunits also identified in platelets

Protein subunit	Gene name	
19S regulator		
ATPase subunits		
Rpt1	PSMC2	
Rpt2	PSMC1	
Rpt3	PSMC4	
Rpt4	PSMC6	
Rpt5	PSMC3	
Rpt6	PSMC5	
non-ATPase subunits		
Rpn1	PSMD2	
Rpn2	PSMD1	
Rpn3	PSMD3	
Rpn4	PSMD9	
Rpn5	PSMD12	
Rpn6	PSMD11	
Rpn7	PSMD6	
Rpn8	PSMD7	
Rpn9	PSMD13	
Rpn10	PSMD4	
Rpn11	PSMD14	
Rpn12	PSMD8	
20S proteasome		
SP		
β1/Delta (δ)	PSMB6	Caspase-like
β2/Z	PSMB7	Trypsin-like
β5/MB1	PSMB5	Chymotrypsin-like
IP		
β1i/LMP2	PSMB9	Caspase-like
β2i/MECL-1	PSMB10	Trypsin-like
β5i/LMP7	PSMB8	Chymotrypsin-like
α1	PSMA6	
α2	PSMA2	
α3	PSMA4	
α4	PSMA7	
α5	PSMA5	
α6	PSMA1	
α7	PSMA3	
β3	PSMB3	
β4	PSMB2	
β6	PSMB1	
β7	PSMB4	
11S regulator		
Pa28α	PSME1	
Pa28β	PSME2	

Note: Details are described in the manuscript.[13,83,84]

Ubiquitination of a protein is a signal to induce its degradation by the proteasome. The 76 amino acid containing ubiquitin (Ub) is a highly conserved protein, which is covalently bound to an internal lysine residue of a substrate protein. This requires the coordinated reaction of three key enzymes, the Ub-activating (E1), Ub-conjugating (E2), and Ub-ligating (E3) enzyme. Platelets constitutively express ubiquitin and the three classes of key enzymes.[55] In addition, it has been shown that the amount of ubiquitinated proteins can be increased by platelet stimulation with, eg, collagen-related peptide (CRP-XL).[17,61] Ubiquitin itself contains seven internal lysine residues (K6, K11, K27, K29, K33, K48, K63) and methionine at position 1, where other ubiquitin moieties can bind resulting in the formation of (branched) poly-Ub chains.[62]

The type of ubiquitin chain impacts the functional characteristics of ubiquitinated proteins. Ubiquitination can mediate protein interactions, modulate different signaling cascades such as NF-κB, and can target protein substrates to the autophagy-lysosome degradation pathway.[62] K48 polyubiquitinated proteins, for example, are preferentially degraded by the proteasome, whereas K63 polyubiquitination contributes to the assembly of protein complexes required for signal transduction to activate NF-κB.[14,63]

Ubiquitination can be reversed by the action of deubiquitinating enzymes, which cleave ubiquitin from the substrate protein or edit ubiquitin chains by the removal of single ubiquitin moieties. Deubiquitinating enzymes have also been detected in platelets and pre-treatment of platelets with deubiquitinase inhibitors results in diminished thrombin-stimulated platelet adhesion and reduced ADP-, collagen-, and thrombin-induced platelet aggregation.[61,64]

The biological relevance of a functional ubiquitin-proteasome system in platelets is demonstrated by patients with *ANKRD26* thrombocytopenia, a rare form of dominantly inherited thrombocytopenia. In *ANKRD26* thrombocytopenia the function of the ubiquitin-proteasome system in platelets is impaired as evidenced by the accumulation of polyubiquitinated proteins.[65] In addition, it has been observed that even platelet activation itself results in an accumulation of ubiquitinated proteins.[61] This might be contradictory to the fact that platelets release their granule proteins upon activation, resulting in a significant loss in intracellular proteins. One possible explanation could be the fact that various proteins required for cytoskeletal rearrangement, eg, Filamin A or Talin-1, are ubiquitinated but not released upon activation.[55]

5 | IDENTIFICATION OF E3 UBIQUITIN LIGASES IN PLATELETS

Because of their exceptional function in transferring ubiquitin to specific protein substrates, E3 ligases are essential regulators of ubiquitination. Up to now more than 600 E3 ligases have been identified in humans.[66] Several classes of E3 ligases have been identified according to their ubiquitin transfer mechanism. The most prominent are the HECT (homologous to the E6-AP carboxyl terminus) domain and RING (really interesting new gene) finger domain containing ligases.[62] By proteomics some non-HECT E3 ligases have been identified in

platelets including C terminus of Hsc70-interacting protein (CHIP), RING finger protein RN181, and casitas B-lineage lymphoma c-Cbl and Cbl-b. These E3s possess important functions in the regulation of platelet activation and signal transduction pathways.[17-20]

C-Cbl was shown to interfere with glycoprotein VI (GPVI)-dependent platelet activation. It is involved in ubiquitination of Syk (important for signaling through GPVI), which leads to a five-fold activation compared to non-ubiquitinated Syk.[21] As a prerequisite, c-Cbl has to be phosphorylated by the Src-family kinases Fyn and Lyn activated downstream of GPVI-mediated signaling.[19] On the contrary c-Cbl possesses an inhibitory function, because c-Cbl-deficient platelets display an enhanced aggregation response to collagen and increased phosphorylation of Fc receptor (FcR) γ-chain, Syk, and phospholipase C gamma 2 (PLCγ2).[19] In contrast, depletion of the E3 ligase Cbl-b resulted in decreased platelet activation, aggregation, and reduced clot stability.[20]

In addition, αIIbβ3 (or GPIIb/IIIA) and C-type lectin-like receptor 2 (CLEC-2) are involved in ubiquitination-dependent modulation of platelet function. The αIIbβ3-associated protein SHARPIN induces ubiquitination and activation of NF-κB proteins via the linear ubiquitin chain assembly complex (LUBAC) and the E3 ligases HOIP and HOIL-1 after platelet stimulation with thrombin or inflammatory agonists.[22] Another E3 ubiquitin ligase, RN181, was shown to interact with platelet integrin αIIbβ3 suggesting a further link between integrin signal transduction and ubiquitination.[17] Finally, the E3 ligase CHIP is a negative regulator of CLEC-2 mediating its K48-ubiquitination and subsequent degradation by the proteasome[18] (summarized in Figure 1).

Thus, platelet activation is regulated by the ubiquitin-proteasome system, partially via NF-κB-activation.[15,16] NF-κB is also a key regulator of inflammation. This indicates that the proteasome and E3 ligase activity might be a central component linking not only platelet formation and function, but also platelet production and inflammation. Considering our current understanding of the role in platelets and thrombo-inflammation, the proteasome might not only regulate the number of platelets but also their phenotype in different disease states, either toward platelets with primary functions in hemostasis or toward platelets with primary functions as immune regulators.[67]

6 | PLATELETS AND THEIR RELEVANCE AS IMMUNE AND IMMUNOMODULATORY CELLS

MHC class I peptide ligands are generated from, eg, pathogen-derived proteins processed by cellular proteasome complexes and

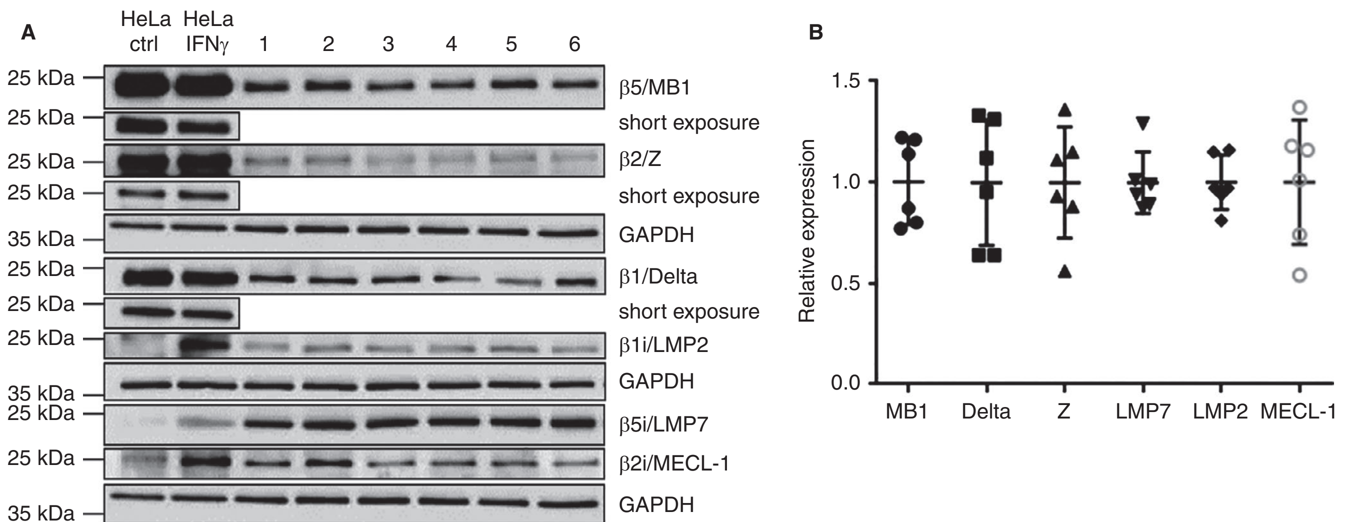

FIGURE 3 Expression of catalytically active proteasome subunits in platelets. A, Platelets isolated from human volunteers (n = 6) were analyzed for their proteasome expression. Blood was obtained from healthy volunteers who met the German criteria for blood donation and after informed consent and ethical committee (University Medicine Greifswald) approval. Whole blood was collected in acid citrate dextrose A (ACD-A, Sigma-Aldrich, United States). Platelet-rich plasma (PRP) was obtained by differential centrifugation (120 × *g*, 20 minutes, room temperature), transferred by careful removal. Five units of apyrase (Sigma-Aldrich) and 111 µL ACD-A were subsequently added per 1 mL PRP. A volume of 5 mL PRP (in a 13 mL polystyrene tube [Sarstedt, Germany]) was centrifuged (650 × *g*, 7 minutes, RT, without break). The resulting pellet was re-suspended in 5 mL washing buffer and after a resting phase of 20 minutes it was centrifuged again (650 × *g*, 7 minutes, RT). Platelet pellets were immediately re-suspended and adjusted to 300 000/µL in Tyrodes buffer containing Ca++ and Mg++. The purity of platelet preparations was analyzed by a Sysmex cell counter and was about 99%. Western blot was performed as described.[82] Briefly, 25 µg protein of cell lysate per lane were separated on SDS-PAGE, transferred to nitrocellulose membranes and were immunoblotted for proteasome subunits β1/delta (laboratory stock), β2/Z (Cell Signaling), β5/MB1 (Cell Signaling), β1i/LMP2 (Santa Cruz), β2i/MECL-1 (Santa Cruz), β5i/LMP7 (Cell Signaling) and GAPDH, loading control (Ab frontier). HeLa cell lysates of unstimulated and stimulated HeLa cells (200 U/mL, 24 hours) were used as control. Membranes were developed with enhanced chemiluminescence (Cell Signaling). B, Quantification was performed using GraphPad Prism 7 (La Jolla, CA, USA). Specific signals were normalized to GAPDH and the average signal intensity was determined for each proteasomal subunit. Relative expression of the different donors is displayed for each protein related to the mean (donors are representative for >20 donors)

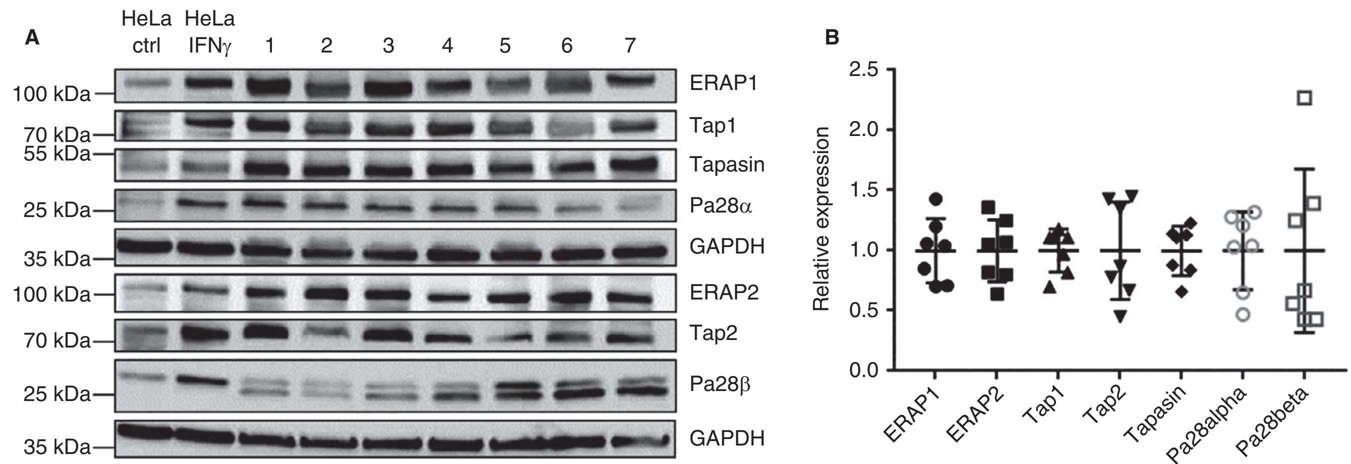

FIGURE 4 Expression of antigen processing and presentation pathway components in platelets. A, Platelets isolated from human volunteers (n = 7) were analyzed for their expression of endoplasmic reticulum (ER) aminopeptidases ERAP1/2, transporter associated with antigen processing Tap1 and Tap2, Tapasin, and PA28α/β proteasome activator. The method of isolation and platelet preparation was performed as described in the Figure 3 legend. Western blot was performed as described.[82] 100 µg protein of cell lysate were blotted and probed with ERAP1 (Santa Cruz), ERAP2 (3F5, R&D Systems), Tap1 (Sigma), Tap2 (laboratory stock), Tapasin (laboratory stock), PA28α (laboratory stock), PA28β (laboratory stock) and GAPDH, loading control (Ab frontier). Membranes were developed with enhanced chemiluminescence (Cell Signaling). B, Quantification was performed using GraphPad Prism 7 (La Jolla, CA, USA), the signals were normalized to GAPDH. The average signal intensity was determined for each protein. The specific signal is shown for each donor at which error bars show the heterogeneity of expression between the donors

additional components of the antigen processing and presentation pathway (APP). They are subsequently loaded on empty MHC class I molecules.[68] Increasing evidence supports the presence of a fully active MHC class I signaling system in platelets. This is based on the observation that almost all components of the APP machinery are present in platelets, albeit at different expression levels (Figures 3 and 4 and Table 1), and by recently published data demonstrating (a) a regulatory function of platelet β2-microglobulin on monocyte differentiation, (b) a protection of tumor cells from NK cell surveillance by transfer of platelet MHCs to tumor cells, and (c) by the presentation of MHC class I complexes loaded with peptides on platelets of normal as well as individuals with immune thrombocytopenia (ITP).[69-73]

Activated platelets form aggregates with T cells, which most likely establish immunological synapses between these two cell types.[74] Although platelets mainly express denatured MHC class I heavy chains on their surface, which are adsorbed from plasma and cannot stimulate cytotoxic T lymphocytes (CTL)-mediated cytotoxicity,[75] especially young platelets express intact MHC class I.[76] In addition, intact MHC molecules including β2-microglobulin are stored in platelet α-granules and can be translocated to the cell surface upon platelet activation.[69] Cytotoxic (CD8+) T lymphocytes are activated as soon as their specific T cell receptors (TCRs) recognize MHC class I molecules loaded with their cognate peptides. In a mouse model of cerebral malaria induced by a modified *Plasmodium berghei* parasite expressing parts of ovalbumin (OVA), platelets presented the ovalbumin SIINFEKL epitope on MHC class I molecules and induced an OVA-specific CD8 + T cell response.[77] Moreover, in viral hepatitis platelets facilitate the intrahepatic accumulation of virus-specific CTLs accompanied by severe liver damage.[78]

Additionally, dengue virus infection leads to an enhanced expression of human leukocyte antigen (HLA) class I molecules on the platelet surface, which could be suppressed by the proteasome inhibitor bortezomib.[79] This observation strongly supports the connection between the proteasome system and MHC class I expression in platelets.

Interestingly, MHC molecules are not only relevant for activation of lymphocytes. They are involved in reverse signaling causing attenuated toll like receptor (TLR)-triggered innate inflammation.[80] Potentially the MHC class I molecules expressed in platelets also exert such functions, which would protect the organism from uncontrolled immune reactions. This hypothesis is provocative but would make sense as usually inhibitory mechanisms outweigh stimulatory mechanisms. This could be a potential additional reason why platelets are present in such a high number, although 20 000 to 50 000 platelets/L would be fully sufficient for hemostasis.

7 | CONCLUDING REMARKS

Despite their prevalent function in hemostasis, there is evidence that platelets play an important and complex role in the activation and continuum of the innate and adaptive immune response.

A few studies analyzing the function of proteasome activity in the context of platelet activation found only limited effects of the proteasome on platelet function.[15,16,54-56] On the other hand, humans affected by rare gene mutations impairing the ubiquitin-proteasome system (CANDLE syndrome, *ANKRD26* thrombocytopenia) or who are treated with proteasome inhibitors (bortezomib) show reduced platelet numbers.[58,60,65] The constitutively expressed standard- and

immunoproteasomes in platelets might have other important functions in platelet biology. Platelets seem to be able to impact the adaptive immune response by proteasomal processing of (foreign) proteins into peptide fragments which are loaded on MHC class I molecules and are subsequently presented as peptide-MHC class I complexes on the platelet surface.[77,81] In particular, immunoproteasomes might be important regulators of the role of platelets in adjusting components of innate and adaptive immunity and further emphasizing the role of platelets as a link between hemostasis and inflammation.

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CONFLICTS OF INTEREST

None of the authors has a conflict of interest to declare with regard to the work presented in the manuscript.

AUTHOR CONTRIBUTIONS

LC drafted the manuscript. US, CC, and AG contributed to writing the manuscript. All authors read and approved the final version.

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