HEMOSTASIS

The platelet proteasome and immunoproteasome are stable in buffy-coat derived platelet concentrates for up to 7 days

Lisa Colberg¹,² | Clemens Cammann² | Jan Wesche¹ | Eylin Topfstedt² | Ulrike Seifert² | Andreas Greinacher¹

¹Institut für Immunologie und Transfusionsmedizin, Universitätsmedizin Greifswald, Greifswald, Germany
²Friedrich Loeffler-Institut für Medizinische Mikrobiologie-Virologie, Universitätsmedizin Greifswald, Greifswald, Germany

Abstract

**Objectives:** Characterization of the proteasome and its stability in buffy-coat derived platelet concentrates (PCs) during storage.

**Background:** The proteasome plays a key role in cell homeostasis by processing misfolded or abnormal proteins and regulating the levels and activities of a high number of proteins contributing to cell cycle, survival, and proliferation. Controversial data exist, whether inhibition of the proteasome affects platelet function. Little is known about function, expression, and stability of the proteasome in PCs during storage, and the potential role of the platelet proteasome in storage lesions.

**Study design and methods:** PCs were produced by the buffy-coat method in additive solution and stored at room temperature under agitation. Platelet aggregation was monitored by light transmission aggregometry. Proteasome complexes were assessed by immunoprecipitation and immunoblotting, and proteasome activity was measured using fluorogenic substrates specific for the three different proteolytic activities over 7 days of storage.

**Results:** Proteasome inhibition led to a decreased platelet aggregation response after activation with collagen, ADP, TRAP-6, and thrombin. There were no changes in the expression of the catalytic active subunits as well as the proteasome activity during storage of PCs, comparing baseline and day 7.

**Discussion:** Platelet proteasome function is relevant for platelet aggregation in response to various agonists. The constitutive and stable expression of the active standard- and immunoproteasome in platelets makes it unlikely that loss of proteasome function is a relevant cause of storage lesions.

**KEYWORDS**

platelet aggregation, platelet concentrate, proteasome system, storage lesions

**Abbreviations:** GP, glycoprotein; IP, immunoprecipitation; LMP, low molecular weight protein; MECL-1, multicatalytic endopeptidase complex like-1; MHC, major histocompatibility complex; PC, platelet concentrate; Ub, ubiquitin.

Ulrike Seifert and Andreas Greinacher equally contributed to this study.

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. Transfusion published by Wiley Periodicals LLC on behalf of AABB.
1 | INTRODUCTION

Platelets are small blood cells without a nucleus. After release from their precursor megakaryocytes they circulate in the bloodstream for 7–10 days.1 Platelets express numerous transmembrane receptors, different storage vesicles such as α-granules, dense granules, and lysosomes.2 Upon activation, platelets adhere, spread, secrete and aggregate.3,4

A relatively new finding is the expression of proteasome complexes in platelets. The proteasome, a 2.5 MDa multicatalytic protease complex, plays a key role for cell homeostasis by processing misfolded, short lived, or abnormal proteins.5,6 It is responsible for protein degradation, regulation of signal transduction processes, and generation of major histocompatibility complex class I epitopes.7,8 Several forms of proteasomes have been described such as the standard proteasome and the immunoproteasome.9,10 The catalytic component is the central 20S complex consisting of regulatory α-subunits and catalytically active β1 (displaying caspase-like activity), β2 (trypsin-like activity), and β5 (chymotrypsin-like activity) subunits as well as inactive β-subunits.5,6,11-13 The immunoproteasome can be expressed after activation of cells as part of immune reactions due to the release of interferons. This leads to the expression and incorporation of the immunoproteasome subunits β1i/LMP2, β2i/MECL-1, and β5i/LMP7. The immunoproteasome is constitutively expressed in immune cells.9,14,15 Proteins become accessible to the proteasome once they are labeled with ubiquitin (Ub) moieties. Ub is a highly conserved protein, which is covalently bound to an internal lysine residue of a substrate protein.

Platelets express the catalytically active components of the standard- and immunoproteasome.16-18 The proteasome seems to be relevant for platelet function, as proteasomal activity does influence platelet aggregation and thrombus formation in vitro and in vivo.19 To which extent inhibition of proteasome function influences platelet aggregation is currently under debate.19-23

Platelet concentrates (PCs) are an essential blood product.24 However, during storage of PCs, the functional capacity of platelets decreases due to the so-called storage lesions.25-32 These lesions arise from platelet activation, metabolic changes, and aging of platelets in vitro. Microvesicles and major apoptosis-like lesions are key features of storage lesions.33,34 Receptors are desensitized, like the ADP receptor, by secreted ADP, or they are cleaved by proteases like calpain and ADAM 10 and 17.35 This leads to cleavage of P-selectin, CD40 ligand, and surface glycoproteins (GPs) (GPIb, GPV, GPVI).36-38 In addition, phosphatidylserine (PS), normally present only on the inner plasma membrane is expressed on the outside.39 With increasing storage time, platelets lose their discoid shape and develop pseudopods.26,29

We addressed the issue, whether the proteasome expression and function are stable in PCs during storage. As we observed an impaired response of platelets to collagen when we inhibited the platelet proteasome with bortezomib or MG 132, increasingly impaired proteasome function during platelet storage could be a possible reason for storage lesions in platelets.

Therefore, we investigated the stability of the proteasome in PCs and platelet functionality after proteasome inhibition.

2 | MATERIAL AND METHODS

2.1 | Material

Collagen (Nycomed, Linz, Austria), ADP, thrombin (Roche, Basel, Switzerland), TRAP-6 (Hart Biologicals, Queens Meadow, Hartlepool), MG132 (Sigma Aldrich, St. Louis), bortezomib (Selleckchem, Houston, Texas).

2.2 | Isolation of washed platelets for aggregometry

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. Whole blood was collected in acid-citrate-dextrose solution (ACD-A, Sigma Aldrich). Platelet-rich plasma (PRP) was obtained by differential centrifugation (120×g, 20 min, room temperature).

Washing of platelets: 5 units of apyrase (Sigma-Aldrich) and 111 μl of ACD-A were added per 1 ml of PRP. A volume of 5 ml PRP (in a 13 ml polystyrene tube (Sarstedt, Germany) was centrifuged (650 g, 7 min, RT). Platelet pellets were immediately re-suspended in Tyrodes buffer,2 0 min, room temperature). The resulting pellet was re-suspended in 5 ml washing buffer (16 mg/ml NaCl, 4 mg/ml KCl, 20 mg/ml NaHCO3, 1 mg/ml Na2PO4, 0.35% BSA, 0.1% glucose, 2.5 U/ml apyrase, 1 U/ml hirudin, pH 6.3) and after a resting phase of 20 min it was centrifuged again (650×g, 7 min, RT). Platelet pellets were immediately re-suspended and adjusted to 300,000/μl in Tyrodes buffer (16 mg/ml NaCl, 4 mg/ml KCl, 20 mg/ml NaHCO3, 1 mg/ml Na2PO4, 0.35% BSA, 0.1% glucose, 2.14 mM MgCl2, 2 mM CaCl2, pH 7.2). The purity of platelet preparations was about 99% analyzed by a Sysmex cell counter.
2.3 | Light transmission aggregometry

For light transmission aggregometry, 180 μl of washed platelets (300,000 per μl) were incubated with 20 μl of agonist in aggregometer cuvettes. Lag time and maximal aggregation were detected using an APACT 4004 aggregometer (LaBiTec, Ahrensburg, Germany). For proteasome inhibition, platelets were incubated with specific inhibitors (bortezomib 50 and 100 nM, MG132 10 μM) for 1 h at 37°C before aggregometry experiments. Light transmission experiments were performed with PRP and washed platelets. Agonists were used in following concentrations: 0.5–1 μg/ml collagen, 5 μM ADP, 10 μM TRAP-6, and 0.05 U/ml thrombin.

2.4 | Preparation and storage of PCs

PCs were produced by the buffy-coat method from whole-blood donations of voluntary donors fulfilling the criteria of the German guidelines of hemotherapy and blood transfusion. Four buffy coats of the same blood group were pooled with 250 ml of PLT storage solution containing 30% plasma (PAS-E, identical to PAS-IIIM, MacoPharma, Tourcoing, France), rested for 30 min and centrifuged (700×g, 4 min, 22°C; Roto Silenta/630 RS, Hettich, Tuttlach, Germany). The PCs were in-process leukoreduced (Autostop BC filters, Pall, New York), and stored in ELX bags (nominal size, 1.5 L, Pall), under agititation at 22 ± 2°C. Twenty milliliter aliquots of the PC were obtained by sterile docking.

2.5 | Immunoblotting

Pellets of washed and stored platelets from PCs were lysed in buffer containing 50 mM Tris (pH 8.0), 1 mM EDTA, 50 mM NaCl, 1% NP40, 0.1% SDS, and protease inhibitor Complete (Roche, Basel, Switzerland). Proteins were quantified via BSA assay according to the manufacturer. Immunoblotting was performed as described.40 Briefly, 25 μg protein of cell lysate were separated on 15% SDS-PAGE, transferred to nitrocellulose membranes, and analyzed for catalytically active proteasome subunits β1i/delta, β2i/Z, β5/MB1 (all Cell Signaling, Cambridge, United States), β1i/LMP2, β2i/MECL-1 and β7/HsN3 (non-catalytic) (all Santa Cruz, Dallas), catalytic active β5i/LMP7 (Abcam, Cambridge), and for poly-ubiquitinated proteins FK1.1 (Enzo Life Sciences, New York). GAPDH (Cell Signaling, Cambridge) was used as internal standard. Membranes were developed with enhanced chemiluminescence on Fusion Fx7 system, Fusion version 15.17, PEQlab (GE Healthcare, Chicago).

2.6 | Proteasome activity assays

Pellets of washed and stored platelets were lysed in buffer containing 10 mM Tris (pH 7.0), 25 mM KCl, 10 mM NaCl, 1.1 mM MgCl2, 1 mM DTT, 10% glycerol, and protease inhibitor Complete. Proteins were quantified by BSA assay according to manufacturer protocol. Cell lysates were subsequently used to determine the platelet proteasome activity. Chymotrypsin-like activity was assessed using 0.1 mM fluorescently tagged substrate Suc-LLVY-AMC, caspase-like activity with Z-LLL-AMC, and trypsin-like activity with Bz-VGR-AMC (all Bachem, Bubendorf, Switzerland). The free AMC fluorescence was quantified in a fluorometer using a 380/460 nm filterset (Fluorescence spectrometer Infinite M200 Pro, software i-control1.7; TECAN, Männedorf, Switzerland). Increase of fluorescence was measured over time. Proteasome turnover was determined by calculating the rise of the linear slope in the beginning of the measurement (0–30 min, substrate turnover [ΔRFU/min]). Control activity was set 100% and other activities were related to the control activity (proteasome activity [%]).

2.7 | Immunoprecipitation

Cell lysis was performed as described in the section immunoblotting. Immunoprecipitation (IP) was performed with monoclonal MCP21 antibody that binds to the non-catalytic subunit alpha 2 of the proteasome41 and 200 μg protein cell lysate overnight. For pulldown of proteasomes, magnetic beads with coupled MCP21 antibody were used and washed three times with cell lysis buffer. Beads were heated to 95°C for 5 min and supernatant containing whole proteasome complexes with the catalytic active subunits β1, 2, and 5 was transferred to SDS-PAGE. Subsequently, immunoblotting against catalytic active β5i/LMP7 subunit and non-catalytic β7/HsN3 proteasome subunit was performed as described above.

2.8 | Ethics

All donors gave written informed consent. The study was approved by the Ethics Committee of the University Medicine Greifswald (BB133/18).

2.9 | Statistics

Aggregation results were analyzed with GraphPad Prism 7. Control activity was set on 100% and proteasome inhibition results were related to control. ****p < .0001, ***p < .001, **p < .01, *p < .1.
3 | RESULTS

3.1 | Proteasome inhibition significantly decreases collagen-induced platelet aggregation

In fresh washed platelets, the IC$_{50}$ for bortezomib (mainly targets the chymotrypsin-like activity of the $\beta 5/MB1$ and $\beta 5i/LMP7$ subunits$^{42,43}$) was approx. 50 nM (Figure 1B); the IC$_{50}$ for MG132 (inhibits 20S proteasome activity and effectively blocks the proteolytic activity of the 26S proteasome complex$^{44}$) was between 5 and 10 μM (Figure 1A). In further analyses, we used 10 μM MG132 and 50 nM as well as 100 nM of bortezomib for proteasome inhibition.

MG132 and bortezomib significantly decreased platelet aggregation induced by collagen (0.5–1 μg/ml) in whole-blood-derived washed platelets, with more...
pronounced inhibition using higher bortezomib concentrations (Figure 1C,D). Inhibition of the proteasome was verified by measuring the proteasomal activity. As expected, the chymotrypsin-like activity was inhibited by both inhibitors. Both inhibitors also inhibited the caspase-like activity, while the trypsin-like activity showed a compensatory effect with slightly increased activity (Figure 1E).

3.2 | Proteasome inhibition significantly decreases collagen, ADP, TRAP-6, and thrombin-induced platelet aggregation in PRP

Consistent with our results using washed platelets, also platelet aggregation in PRP was inhibited by proteasome inhibitors. Collagen-, TRAP-, and thrombin-induced aggregation were inhibited by proteasome inhibition. ADP-induced platelet aggregation was less inhibited (Figure 2A). Platelet proteasome activities were also inhibited in PRP (Figure 2B).

3.3 | Platelets in PCs contain a functional proteasome system

As shown before, the expression of all catalytically active subunits including the standard subunits β1/delta, β2/Z, β5/MB1 as well as the immunoproteasome subunits β1i/LMP2, β2i/MECL-1, and β5i/LMP7 were identified in platelets freshly isolated from citrated whole blood. Platelets in PCs contain a proteasome system highly comparable to freshly washed platelets. All catalytically active

![Figure 2](https://example.com/figure2.png)

**FIGURE 2** Proteasome inhibition in platelet-rich plasma reduces agonist-induced aggregation. The proteasome of PRP obtained from fresh whole blood by differential centrifugation was inhibited by bortezomib (50; 100 nM) and MG132 (10 µM) for 1 h at 37°C followed by aggregation induced by collagen (1.0 µg/ml), ADP (5 µM), TRAP-6 (10 µM), and thrombin (0.05 U/ml). Notably, 180 µl of platelets (300,000 per µl) were transferred to aggregometry cuvettes and 20 µl agonist was added 30 s after starting measurement. Lag time and maximum aggregation were recorded for 420 s using an APACT 4004 aggregometer. Impact of inhibitor treatment for the agonists is compared with the untreated control (collagen n = 3; ADP n = 5; TRAP-6 n = 3; thrombin n = 3; mean ± SD) (A). To control proteasome inhibition chymotrypsin-like activity using the substrate Suc-LLVY-AMC, caspase-like activity with Z-LLE-AMC and trypsin-like activity with Bz-VGR-AMC were assessed. Substrate turnover of control activity was set on 100% and activity measurements with inhibitor-treated platelets were related to control activity, collagen n = 3; ADP n = 5; TRAP-6 n = 3; thrombin n = 3; mean ± SD (B)
3.4 Proteasome expression and activity in PCs remains stable over 7 days of storage

We observed a homogenous expression of all catalytically active subunits of the standard and immunoproteasome in PCs during 7 days of storage (Figure 3B). Furthermore, we showed by IP that incorporation of the proteasomal subunits β5i/LMP7 and β7 were comparable at day 0 and day 7 (Figure 3C), strongly indicating that the proteasome and the immunoproteasome in PCs are correctly assembled during the entire storage time. The amount of incorporated β5i/LMP7 and β7 did not change between day 0 and 7. This we confirmed by measuring the proteasome activity in platelets of PCs using fluorogenic substrates. All three proteasome activities, chymotrypsin-like, caspase-like, and trypsin-like activity were preserved until day 7 of storage (Figure 3D). Based

**FIGURE 3** Storage of platelet concentrates has no impact on proteasome subunit expression and proteasome activity. Platelets from platelet concentrates were lysed and analyzed by immunoblot for their proteasome subunits β1/δ, β2/Z, β5/MB1, β1i/LMP2, β2i/MECL-1, and β5i/LMP7 were identified with a similar expression level as in platelets freshly isolated from citrated whole blood (Figure 3A).
on these results we cannot exclude the presence of free circulating subunits, which, however, most likely have no impact on function of the proteasome.

To get an indication of changes of the platelet proteome upon long-term proteasome inhibition, we determined ubiquitination of the platelet protein pool after exposure of PCs to bortezomib. As shown in Figure 4B, no significant changes of the ubiquitinome occurred in platelets over 24 hours, a time frame where the proteasome activity is still inhibited (Figure 4A).

4 | DISCUSSION

Our study shows that the proteasome and immunoproteasome system is constitutively expressed in platelets and that platelet aggregation induced by several agonists is reduced by proteasome inhibition. We also proved inhibition of the proteasome in parallel by measuring the chymotrypsin-, caspase-, and trypsin-like proteasome activities. To exclude unspecific effects of the proteasome inhibitor bortezomib, we used a second proteasome inhibitor, MG132. As the second inhibitor showed comparable results, it is very unlikely that the observed inhibition of platelet aggregation is an off-target effect. Off-target effects can be caused by too high concentrations of proteasome inhibitors, which lead, for example, to inhibition of other proteolytic pathways or result in cell toxic effects. Previous studies analyzing proteasome inhibition on platelet aggregation show inconsistent results. Some of these studies are difficult to interpret because non-physiologically high concentrations of proteasome inhibitors had been used. In addition, concentration of the aggregation stimulant turned out to be crucial for the effect of proteasome inhibition on platelet aggregation. Our study clearly shows that collagen-, ADP-, thrombin-, and TRAP-induced platelet aggregation is reduced by proteasome inhibition. When lower collagen (up to 2.5 μg/ml) or thrombin (0.025 U/ml) concentrations were used in other studies, the obtained data were consistent with our results displaying inhibition of platelet aggregation also in the presence of lower concentrations of proteasome inhibitors. The same effect has been observed after ADP activation with 5 μM but at non-physiological high concentration of bortezomib of up to 40 μM. However, at high concentrations of agonists, for example, 10 and 3 μg/ml collagen, platelet aggregation was not inhibited by bortezomib. Studies using very high concentrations of bortezomib need to be interpreted with caution. Potentially off-target effects of bortezomib explain some of the findings. A recent study from Klingler et al. investigated long-term proteasome inhibition on platelet functionality and showed an increase in platelet aggregation with threshold agonist concentrations. However, they used whole blood and considerably lower (5 nM) but also higher concentrations (1 μM) of bortezomib compared with our study. They also used higher ADP concentrations (10 μM), which led to unchanged platelet aggregation similar to a few other studies. This is in line with our findings, where
inhibition of the proteasome had the least effect on the platelet aggregation in response to ADP.

To assess whether the stability of the platelet proteasome is altered in the presence of proteasome inhibitors, we measured ubiquitination of the platelet protein content and detected no significant differences in the presence of bortezomib (Figure 4B). This indicates that proteasome inhibition does not alter the platelet proteasome and that potentially alternative degradation routes (e.g. endosomal degradation or autophagy) might be activated in platelets upon proteasome inhibition as compensatory mechanisms. This is in line with a recent study of Guo et al., which shows protein processing in platelets in the presence of proteasome inhibitors.51

Our study implicates that loss of function of the proteasome is most likely not a major cause for platelet storage lesions in PCs.37 Production and storage of PCs did not alter expression or activity of the platelet proteasome over the entire storage period of 7 days. We can exclude relevant contaminations of leukocytes as a source of error as all PCs were in-process leukoreduced by filtration.30 The remaining leukocyte numbers are too low to alter protein analysis by western blot.

Our study has limitations. We assessed buffy-coat derived PCs. However, it is likely that these results can be transferred to other platelet products like apheresis PCs or PCs produced by the PRP method.

An interesting and new finding of our study is that in platelets the proteasome stability, especially of the immunoproteasome, is much higher than in all other cells analyzed up to now. The standard proteasome has a half-life of 5 days as observed in HeLa cells or immunoproteasome-deficient T2 cells,52,53 and the immunoproteasome has a significantly shorter half-life of only 27 h in immunoproteasome-reconstituted T2 cells.52 As platelets are able to synthesize proteins, this raises the question, whether the long half-life of the proteasome in platelets is caused by new synthesis of proteasome subunits. This is especially relevant in the view of pathogen inactivation procedures, which crosslink RNA and might therefore inhibit platelet de novo protein synthesis.54

In conclusion, sustained expression and activity of platelet proteasome complexes indicate an important function of the Ub-proteasome system in platelets. Proteasome function plays a crucial role for platelet aggregation but seems to be not involved in storage lesions. The next important question is to clarify why the proteasome in platelets is much longer stable than in all other cells investigated up to now.

ACKNOWLEDGMENTS
Funded by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation) -Projektnummern 374031971-Trr240 and Individual Research Grant “Structure and Function of the Proteasome System in Platelets” GR2232/8_1 and SE 885/2-1.

CONFLICT OF INTEREST
The authors have disclosed no conflicts of interest.

AUTHOR CONTRIBUTIONS
L.C., C.C., J.W. designed and organized the experiments. L.C. and E.T. conducted the experiments. L.C., U.S. and A.G. wrote the paper. U.S. and A.G. supervised the study. All authors contributed to the article and approved the submitted version.

ORCID
Jan Wesche https://orcid.org/0000-0001-5025-7355
Ulrike Seifert https://orcid.org/0000-0003-1037-3487
Andreas Greinacher https://orcid.org/0000-0001-8343-7336

REFERENCES
1. Periayah MH, Halim AS, Mat Saad AZ. Mechanism action of platelets and crucial blood coagulation pathways in hemostasis. Int J Hematol Oncol Stem Cell Res. 2017;11:319–27.
2. Rendu F, Brohard-Bohn B. The platelet release reaction: granules’ constituents, secretion and functions. Platelets. 2001;12:261–73.
3. Binsker U, Palankar R, Wesche J, Kohler TP, Prucha J, Burchhardt G, et al. Secreted immunomodulatory proteins of Staphylococcus aureus activate platelets and induce platelet aggregation. Thromb Haemost. 2018;118:745–57.
4. Li Z, Delaney MK, O’Brien KA, Du X. Signaling during platelet adhesion and activation. Arterioscler Thromb Vasc Biol. 2010;30:2341–9.
5. Coux O, Tanaka K, Goldberg AL. Structure and functions of the 20S and 26S proteasomes. Annu Rev Biochem. 1996;65:801–47.
6. Tanaka K, Mizushima T, Saeki Y. The proteasome: molecular machinery and pathophysiological roles. Biol Chem. 2012;393:217–34.
7. Kloetzel PM. Antigen processing by the proteasome. Nat Rev Mol Cell Biol. 2001;2:179–87.
8. Ciechanover A. The ubiquitin-proteasome proteolytic pathway. Cell. 1994;79:13–21.
9. Vigneron N, Van den Eynde BJ. Proteasome subtypes and regulators in the processing of antigenic peptides presented by class I molecules of the major histocompatibility complex. Biomolecules. 2014;4:994–1025.
10. Kniepert A, Groettrup M. The unique functions of tissue-specific proteasomes. Trends Biochem Sci. 2014;39:17–24.
11. Gu ZC, Enenkel C. Proteasome assembly. Cell Mol Life Sci. 2014;71:4729–45.
12. Groll M, Ditzel L, Lowe J, Stock D, Bochler M, Bartunik HD, et al. Structure of 20S proteasome from yeast at 2.4 A resolution. Nature. 1997;386:463–71.
13. Lupas A, Zwickl P, Wenzel T, Seemuller E, Baumeister W. Structure and function of the 20S proteasome and of its regulatory complexes. Cold Spring Harb Symp Quant Biol. 1995;60:515–24.

14. Murata S, Takahama Y, Kasahara M, Tanaka K. The immunoproteasome and thymoproteasome: functions, evolution and human disease. Nat Immunol. 2018;19:923–31.

15. Angeles A, Fung G, Luo H. Immune and non-immune functions of the immunoproteasome. Front Biosci (Landmark Ed). 2012;17:1904–16.

16. Klockenbusch C, Walsh GM, Brown LM, Hoffman MD, Ignatchenko V, Kislinger T, et al. Global proteome analysis identifies active immunoproteasome subunits in human platelets. Mol Cell Proteomics. 2014;13:3308–19.

17. Kraemer BF, Weyrich AS, Lindemann S. Protein degradation systems in platelets. Thromb Haemost. 2013;110:920–4.

18. Colberg L, Cammann C, Greinacher A, Seifert U. Structure and function of the ubiquitin-proteasome system in platelets. J Thromb Haemost. 2020;18:771–80.

19. Gupta N, Li W, Willard B, Silverstein RL, McIntyre TM. Proteasome proteolysis supports stimulated platelet function and thrombosis. Arterioscler Thromb Vasc Biol. 2014;34:160–8.

20. Avcu F, Ural AU, Cetin T, Nevruz O. Effects of bortezomib on platelet aggregation and ATP release in human platelets, in vitro. Thromb Res. 2008;121:567–71.

21. Koessler J, Etzel J, Weber K, Boeck M, Kobzar A. Evaluation of dose-dependent effects of the proteasome inhibitor bortezomib in human platelets. Eur J Pharmacol. 2016;791:99–104.

22. Grundler K, Rotter R, Tilley S, Pircher J, Czermak T, Yakac M, et al. The proteasome regulates collagen-induced platelet aggregation via nuclear-factor-kappa-B (NFkB) activation. Thromb Res. 2016;148:15–22.

23. Karim ZA, Vemana HP, Khasawneh FT. MALT1-ubiquitination triggers non-genomic NF-kappaB/IKK signaling upon platelet activation. PLoS One. 2015;10:e0119363.

24. Lee VS, Tarassenko L, Bellhouse BJ. Platelet transfusion therapy: platelet concentrate preparation and storage. J Lab Clin Med. 1988;111:371–83.

25. Isola H, Ravanat C, Rudwill F, Pongerard A, Haas D, Eckly A, et al. Removal of citrate from PAS-III additive solutions improves functional and biochemical characteristics ofuffy-coat platelet concentrates stored for 7 days, with or without INTERCEPT pathogen reduction. Transfusion. 2021;61:919–30.

26. Thon JN, Schubert P, Devine DV. Platelet storage lesion: a new understanding from a proteomic perspective. Transfus Med Rev. 2008;22:268–79.

27. Devine DV, Serrano K. The platelet storage lesion. Clin Lab Med. 2010;30:475–87.

28. Schubert P, Devine DV. Towards targeting platelet storage lesion-related signaling pathways. Blood Transfus. 2010;8(Suppl 3):s69–72.

29. Seghatchian J, Krailadsiri P. The platelet storage lesion. Transfus Med Rev. 1997;11:130–44.

30. Seghatchian J. Platelet storage lesion: an update on the impact of various leukoreduction processes on the biological response modifiers. Transfus Apher Sci. 2006;34:125–30.

31. Bennett JS. Shedding new light on the platelet storage lesion. Arterioscler Thromb Vasc Biol. 2016;36:1715–6.

32. Árnason NÁ, Sigurjónsson ÓE. New strategies to understand platelet storage lesion. ISBT Sci Ser. 2017;12:496–500.

33. Perrotta PL, Perrotta CL, Snyder EL. Apoptotic activity in stored human platelets. Transfusion. 2003;43:526–35.

34. Rinder HM, Smith BR. In vitro evaluation of stored platelets: is there hope for predicting posttransfusion platelet survival and function? Transfusion. 2003;43:2–6.

35. Baurand A, Eckly A, Bari N, Leon C, Heckler B, Cazenave JP, et al. Desensitization of the platelet aggregation response to ADP: differential down-regulation of the P2Y1 and P2yc receptors. Thromb Haemost. 2000;84:484–91.

36. Bergmeier W, Burger PC, Piipath CL, Hoffmeister KM, Hartwig JH, Nieswandt B, et al. Metalloproteinase inhibitors improve the recovery and hemostatic function of in vitro-aged or -injured mouse platelets. Blood. 2003;102:4229–35.

37. Canault M, Duerschmied D, Brill A, Stefanini L, Schatzberg D, Cifuni SM, et al. p38 mitogen-activated protein kinase activation during platelet storage: consequences for platelet recovery and hemostatic function in vivo. Blood. 2010;115:1835–42.

38. Gardiner EE, Karunakaran D, Shen Y, Arthur JF, Andrews RK, Berndt MC. Controlled shedding of platelet glycoprotein (GP)VI and GPIb-IX-V by ADAM family metalloproteinases. J Thromb Haemost. 2007;5:1530–7.

39. Dasgupta SK, Argaiz ER, Mercado JE, Maul HO, Garza J, Enriquez AB, et al. Platelet senescence and phosphatidylserine exposure. Transfusion. 2010;50:2167–75.

40. Elstein F, Lange N, Urban S, Seifert U, Kruger E, Kloetzl PM. Maturation of human dendritic cells is accompanied by functional remodelling of the ubiquitin-proteasome system. Int J Biochem Cell Biol. 2009;41:1205–15.

41. Kaitofo MB, Koch C, Uerkvitz W, Hendii KB. Monoclonal antibodies to the human multicatalytic protease (proteasome). Hybridoma. 1992;11:507–17.

42. Accardi F, Toscani D, Bolzoni M, Dalla Palma B, Aversa F, Giuliani N. Mechanism of action of Bortezomib and the new proteasome inhibitors on myeloma cells and the bone microenvironment: impact on myeloma-induced alterations of bone remodeling. Biomed Res Int. 2015;2015:172458.

43. Goldberg AL. Development of proteasome inhibitors as research tools and cancer drugs. J Cell Biol. 2012;199:583–8.

44. Guo N, Peng Z. MG132, a proteasome inhibitor, induces apoptosis in tumor cells. Asia Pac J Clin Oncol. 2013;9:6–11.

45. Dasgupta S, Castro LM, Dulman R, Yang C, Schmidt M, Ferro ES, et al. Proteasome inhibitors alter levels of intracellular peptides in HEK293T and SH-SY5Y cells. PLoS One. 2014;9:e103604.

46. Thibaudeau TA, Smith DM. A practical review of proteasome pharmacology. Pharmacol Rev. 2019;71:170–97.

47. Gupta A, Chandra T, Kumar A. Retracted: platelet storage lesion: current proteomics approach. Platelets. 2011;22:117–34.

48. Pellom ST Jr, Dudimah DF, Thounaojam MC, Sayers TJ, Shanker A. Modulatory effects of bortezomib on host immune cell functions. Immunotherapy. 2013;7:1011–22.
49. Koessler J, Schuepferling A, Klingler P, Koessler A, Weber K, Boeck M, et al. The role of proteasome activity for activating and inhibitory signalling in human platelets. Cell Signal. 2019;62:109351.

50. Klingler P, Niklaus M, Koessler J, Weber K, Koessler A, Boeck M, et al. Influence of long-term proteasome inhibition on platelet responsiveness mediated by bortezomib. Vascul Pharmacol. 2021;138:106830.

51. Guo L, Shen S, Rowley JW, Tolley ND, Jia W, Manne BK, et al. Platelet MHC class I mediates CD8⁺ T cell suppression during sepsis. Blood. 2021.

52. Heink S, Ludwig D, Kloetzel PM, Kruger E. IFN-gamma-induced immune adaptation of the proteasome system is an accelerated and transient response. Proc Natl Acad Sci U S A. 2005;102:9241–6.

53. Wei ML, Cresswell P. HLA-A2 molecules in an antigen-processing mutant cell contain signal sequence-derived peptides. Nature. 1992;356:443–6.

54. Osman A, Hitzler WE, Meyer CU, Landry P, Corduan A, Laffont B, et al. Effects of pathogen reduction systems on platelet microRNAs, mRNAs, activation, and function. Platelets. 2015;26:154–63.

55. Hendil KB, Uerkvitz W. The human multicatalytic proteinase: affinity purification using a monoclonal antibody. J Biochem Biophys Methods. 1991;22:159–65.

How to cite this article: Colberg L, Cammann C, Wesche J, Topfstedt E, Seifert U, Greinacher A. The platelet proteasome and immunoproteasome are stable in buffy-coat derived platelet concentrates for up to 7 days. Transfusion. 2021;61:2746–55. https://doi.org/10.1111/trf.16605