Heterotrimeric G-protein subunit $\text{G}_\alpha_{i2}$ contributes to agonist-sensitive apoptosis and degranulation in murine platelets

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Abstract
$\text{G}_\alpha_{i2}$, a heterotrimeric G-protein subunit, regulates various cell functions including ion channel activity, cell differentiation, proliferation and apoptosis. Platelet-expressed $\text{G}_\alpha_{i2}$ is decisive for the extent of tissue injury following ischemia/reperfusion. However, it is not known whether $\text{G}_\alpha_{i2}$ plays a role in the regulation of platelet apoptosis, which is characterized by caspase activation, cell shrinkage and cell membrane scrambling with phosphatidylserine (PS) translocation to the platelet surface. Stimulators of platelet apoptosis include thrombin and collagen-related peptide (CoRP), which are further known to enhance degranulation and activation of $\alpha_{\text{IIb}}\beta_3$-integrin and caspases. Using FACS analysis, we examined the impact of agonist treatment on activation and apoptosis in platelets drawn from mice lacking $\text{G}_\alpha_{i2}$ and their wild-type (WT) littermates. As a result, treatment with either thrombin (0.01 U/mL) or CoRP (2 µg/mL or 5 µg/mL) significantly upregulated PS-exposure and significantly decreased forward scatter, reflecting cell size, in both genotypes. Exposure to CoRP triggered a significant increase in active caspase 3, ceramide formation, surface P-selectin, and $\alpha_{\text{IIb}}\beta_3$-integrin activation. These molecular alterations were significantly less pronounced in $\text{G}_\alpha_{i2}$-deficient platelets as compared to WT platelets. In conclusion, our data highlight a previously unreported role of $\text{G}_\alpha_{i2}$ signaling in governing platelet activation and apoptosis.

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Introduction

Heterotrimeric G proteins are coupled to plasma membrane receptors and participate in the regulation of a wide range of cellular functions such as migration, differentiation, proliferation, ion channel activity, and apoptosis (Hilger et al. 2018; Squires et al. 2018). In platelets, fundamental elements of the hemostasis machinery, a wide array of cell surface receptors coupled to G proteins mediate their activation (Offermanns 2006). The P2Y12 receptor, an important therapeutic target contributing to purinergic stimulation of platelets, is coupled to heterotrimeric G\(_{a12}\) protein, the predominant G\(_a\) isoform (Gachet 2012). More recently, G\(_{a12}\) protein was reported to play a thrombo-inflammatory role in orchestrating thrombus stabilization during hemostasis and mediating tissue damage during experimental cerebral and myocardial ischemia-reperfusion injury (Devanathan et al. 2015).

Platelets are activated by a wide range of agonists such as subendothelial collagen, thrombin, ADP secreted from stimulated platelets, and by collagen-related peptide (CoRP) (Lang et al. 2013). In the presence of stimulatory signals, platelets degranulate, undergo phospholipid scrambling on their cell membrane, and aggregate to form thrombi that results in vascular blockage (Varga-Szabo et al. 2009; Munzer et al. 2014). Cell membrane scrambling with phosphatidylserine (PS) exposure fosters the assembly of tenase and prothrombinase complexes, and subsequently promoting factor Xa and thrombin generation (Lebois and Josefsson 2016). In platelets, unconstrained apoptosis has been implicated in thrombocytopenia, bleeding disorders, microparticle shedding as well as affecting their quality during storage (Lebois and Josefsson 2016; Quach et al. 2018). PS exposure in apoptotic platelets, triggered by exogenous agents or physiological changes, leads to their clearance from the circulation (Lebois and Josefsson 2016; Quach et al. 2018). G\(_{a12}\) has previously been shown to participate in the apoptotic machinery of different cell types (Lopez-Aranda et al. 2008; Bissinger et al. 2016). Despite the recent evidence linking G\(_{a12}\) protein to the execution of thrombosis, its role in platelet survival has hitherto remained elusive. In the present study, using thrombin and collagen-related peptide (CoRP), an agonist mimicking the effect of contact with collagen and a powerful stimulator of platelet apoptosis (Munzer et al. 2018), we investigated the influence of G\(_{a12}\) on platelet survival and degranulation ex vivo.

Materials and Methods

Mice

Experiments were performed in G\(_{a12}\)-deficient mice (G\(_{a12}\)\(^{-/-}\)) and their wild-type (WT) littermates of 10–12 weeks of age. The mice were generated and initially characterized on a SV129 background (Rudolph et al. 1995). Mice were backcrossed on a C57BL/6 background and kept under specified pathogen-free (SPF) environment in individually ventilated cages (IVC) to prolong life expectancy (Wiege et al. 2013; Devanathan et al. 2015). All animal experiments were conducted according to the German law for the care and use of laboratory animals and were approved by local government authorities (Regierungspräsidium Tübingen according to §4 of 19/12/2011).

Preparation of mouse platelets

Platelets were obtained from 10- to 12-week-old mice of either sex which were treated according to the protocol approved by government authorities. Eight hundred µl blood was obtained into tubes containing 200 µl acid-citrate-dextrose buffer. Platelet rich plasma (PRP) was obtained by centrifugation at 260 g for 5 min. PRP was then centrifuged at 640 g for 5 min to pellet the platelets. Where necessary, apyrase (0.02 U/mL; Sigma-Aldrich) and prostaglandin I\(_2\) (0.5 μmol/L; Calbiochem) were added to the PRP to prevent activation of platelets during isolation. After two washing steps, the pellet of washed platelets was resuspended in modified Tyrode-HEPES buffer (pH 7.4, supplemented with 1 mmol/L CaCl\(_2\)). Where indicated, thrombin (0.01 U/mL, Roche, Basel, Switzerland) or collagen-related peptide (CoRP, 2 µg/mL or 5 µg/mL, kindly provided by R. Farndale, University of Cambridge, Cambridge, UK) were added (Liu et al. 2016).

Cytosolic calcium

For the measurement of the cytosolic Ca\(^{2+}\) concentration, the platelet preparation was washed once in Tyrode buffer.
(pH 7.4), stained with 3 μmol/L Fluo-3AM (Biotium, USA) in the same buffer and incubated at 37°C for 30 min. Following the indicated experimental treatment, relative fluorescence was measured utilizing a BD FACS Calibur (BD Biosciences, Heidelberg, Germany) (Towhid et al. 2011; Liu et al. 2016).

**P-selectin and activated integrin abundance**

Fluorophore-labeled antibodies were utilized for the detection of P-selectin expression (Wug.E9-FITC, Emfret Analytics, Eibelstadt, Germany) and the active form of αIIbβ3 integrin (JON/A-PE, Emfret Analytics, Eibelstadt, Germany). Washed mouse platelets (1x10⁶) were suspended in modified Tyrode buffer (pH 7.4) containing 1 mmol/L CaCl₂ and antibodies (1:10 dilution) and subsequently subjected to the respective treatments and for the indicated time periods at room temperature (RT). The reaction was stopped by addition of PBS and the samples were immediately analyzed by FACS analysis (Liu et al. 2015).

**Phosphatidylserine exposure and forward scatter**

Phosphatidylserine exposure was determined in platelets with and without a 10 min thrombin or CoRP treatment (Liu et al. 2016). To this end, the platelet preparation was centrifuged at 660 g for 5 min followed by washing once with Tyrode buffer (pH 7.4) with 1 mmol/L CaCl₂ staining with 1:20 dilution of Annexin-V FITC (ImmunoTools, Germany) in Tyrode buffer (pH 7.4) in the presence of 2 mmol/L CaCl₂ and incubation at 37°C for 30 min. Annexin-V binding reflecting surface exposure of phosphatidylserine was evaluated by FACS analysis. In parallel,

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**Table 1.** Analyzed parameters in WT and Gα<sub>i2</sub>-deficient mice

### A: Annexin-V-binding and forward scatter in WT female and male mice

| Parameter | Resting | Thrombin | CoRP |
|-----------|---------|----------|------|
| Annexin-V-binding [%] | 0.89 ± 0.02 | 17.07 ± 2.50 | 19.22 ± 0.87 |
| Forward Scatter [Geomean; arb. units] | 14.95 ± 1.00 | 10.43 ± 0.95 | 8.15 ± 0.18 |

### B: Annexin-V-binding and forward scatter in Gα<sub>i2</sub>-deficient female and Gα<sub>i2</sub>-deficient male mice

| Parameter | Resting | Thrombin | CoRP |
|-----------|---------|----------|------|
| Annexin-V-binding [%] | 1.13 ± 0.17 | 13.65 ± 0.66 | 8.75 ± 2.84 |
| Forward Scatter [Geomean; arb. units] | 15.94 ± 0.53 | 12.04 ± 0.87 | 11.10 ± 0.86 |

### C: P-selectin, activated integrin αIIbβ3, caspase-3-positive cells and ceramide abundance in WT female and WT male mice

| Parameter | Resting | CoRP |
|-----------|---------|------|
| P-selectin [MFI] | 5.29 ± 0.43 | 28.01 ± 5.63 |
| Activated integrin αIIbβ3 [MFI] | 5.59 ± 0.27 | 96.61 ± 15.64 |
| Caspase-3-positive cells [%] | 4.65 ± 0.60 | 21.91 ± 1.65 |
| Ceramide abundance [MFI] | 19.38 ± 2.28 | 25.48 ± 0.28 |

### D: P-selectin, activated integrin αIIbβ3, caspase-3-positive cells and ceramide abundance in Gα<sub>i2</sub>-deficient female and Gα<sub>i2</sub>-deficient male mice

| Parameter | Resting | CoRP |
|-----------|---------|------|
| P-selectin [MFI] | 5.28 ± 0.34 | 22.98 ± 4.65 |
| Activated integrin αIIbβ3 [MFI] | 5.30 ± 0.13 | 59.79 ± 16.38 |
| Caspase-3-positive cells [%] | 4.10 ± 0.13 | 8.75 ± 1.52 |
| Ceramide abundance [MFI] | 19.25 ± 1.44 | 20.13 ± 2.33 |

Annexin-V-binding, forward scatter, P-selectin abundance, activated integrin αIIbβ3, caspase-3-positive cells, and ceramide abundance in female and male WT and Gα<sub>i2</sub>-deficient mice after thrombin and/or CoRP stimulation.
the forward scatter (FSC) of the platelets was determined by FACS analysis, as a measure of platelet size (Liu et al. 2016).

**Caspase-3 activity**

Caspase 3 activity was determined utilizing a CaspGlow Fluorescein Active Caspase-3 Staining kit from BioVision (CA, USA) according to the manufacturer’s instruction. Fluorescence intensity was measured at an excitation wavelength of 488 nm and an emission wavelength of 530 by FACS analysis.

**Ceramide abundance**

Ceramide abundance at platelet cell surface was measured by a monoclonal antibody-assay. For this purpose, platelets were stained at 37°C for 1 h at a concentration of 1 μg/mL anti-ceramide antibody (clone MID 15B4, Enzo Life Science GmbH, Lorrach, Germany) in Tyrode buffer at a dilution of 1:10. Platelets were washed once with Tyrode buffer. Subsequently, platelets were stained for 30 min with polyclonal fluorescein isothiocyanate (FITC) conjugated goat anti-mouse IgG and IgM specific antibody (BD Pharmingen, Hamburg, Germany) at a concentration of 1:50 in Tyrode buffer. Washing of the platelets was performed in order to remove unbound secondary antibody. Finally, the samples were analyzed by flow cytometry at an excitation wavelength of 488 nm and an emission wavelength of 530 nm, as has previously been shown (Gatidis et al. 2010).

**Statistical analysis**

Data are shown as means ± SD; n represents the number of independent experiments. All data were tested for...
significance using ANOVA. \( P < 0.05 \) was considered statistically significant.

**Results**

This study addressed the impact of \( \text{G}_{\alpha 12} \) on activation and apoptosis of murine platelets. For this purpose, experiments were performed in mice lacking \( \text{G}_{\alpha 12} \) and corresponding wild type mice. The platelets were analyzed with or without prior exposure to thrombin (0.01 U/mL) or collagen-related peptide (CoRP, 2 \( \mu \)g/mL and 5 \( \mu \)g/mL). Individual values (mean ± SD) of female and male WT and \( \text{G}_{\alpha 12} \)-deficient mice of annexin-V-binding, platelet volume, caspase-3-positive cells, ceramide abundance, P-selectin abundance and activated integrin \( \alpha_{IIb} \beta_{3} \) are displayed in Table 1A–D. No significant differences were observed between male and female WT or \( \text{G}_{\alpha 12} \)-deficient mice.

Phosphatidylserine exposure, a hallmark of platelet apoptosis, was quantified by flow cytometry analysis using annexin-V-binding as described previously (Cao et al. 2017). As illustrated in Figure 1A and D, the percentage of PS-positive platelets in untreated resting \( \text{G}_{\alpha 12} \)-deficient mice of annexin-V-binding, platelet volume, caspase-3-positive cells, ceramide abundance, P-selectin abundance and activated integrin \( \alpha_{IIb} \beta_{3} \) are significantly different in resting platelets of either genotype but was significantly reduced following thrombin and CoRP treatment, an effect significantly less pronounced in \( \text{G}_{\alpha 12} \)-deficient platelets than in WT platelets (Fig. 1F–H).

We then sought to elucidate the mechanism(s) regulating \( \text{G}_{\alpha 12} \)-mediated apoptosis. To this end, we analyzed intracellular \( \text{Ca}^{2+} \) levels \( [\text{Ca}^{2+}]_{i} \) in murine platelets of either genotype using Fluor AM fluorescence as described previously (Cao et al. 2017). As a result, prior to CoRP treatment, \( [\text{Ca}^{2+}]_{i} \) in resting platelets was similar in \( \text{G}_{\alpha 12} \)-deficient platelets (22.73 ± 0.69, a.u.; \( n = 10 \)) and in WT platelets (20.95 ± 0.50, a.u.; \( n = 10 \)). Treatment with CoRP was followed by a profound and significant increase of \( [\text{Ca}^{2+}]_{i} \) in both \( \text{G}_{\alpha 12} \)-deficient (109.3 ± 1.46, a.u., \( n = 10 \)) and WT platelets (105.9 ± 0.69, a.u.; \( n = 10 \)).

Figure 2. \( \text{G}_{\alpha 12} \) participates in the regulation of platelet caspase 3 activation and ceramide formation. (A and B) Original histogram overlays of the caspase 3 activity in platelets isolated from WT mice (blue shadow) and \( \text{G}_{\alpha 12} \)-deficient mice (black line) without (A) and with (B) a 10 min treatment with collagen-related peptide CoRP (5 \( \mu \)g/mL). (C) Arithmetic means ± SD (\( n = 6 \)) of the caspase 3 activity (arbitrary units) in platelets isolated from WT mice (blue bar) and \( \text{G}_{\alpha 12} \)-deficient mice (black bar) prior to (control) and following a 10 min treatment with collagen-related peptide CoRP (5 \( \mu \)g/mL). ** (\( P < 0.001 \)) indicates statistically significant difference from absence of CoRP, *** (\( P < 0.001 \)) indicates statistically significant difference from WT mice. (D and E) Original histogram overlays of the ceramide abundance of platelets isolated from WT mice (blue shadow) and \( \text{G}_{\alpha 12} \)-deficient mice (black line) without (D) and with (E) a 10 min treatment with collagen-related peptide CoRP (5 \( \mu \)g/mL). (F) Arithmetic means ± SD (\( n = 4 \)) of the ceramide abundance of platelets isolated from WT mice (blue bar) and \( \text{G}_{\alpha 12} \)-deficient mice (black bar) prior to (control) and following a 10 min treatment with collagen-related peptide CoRP (5 \( \mu \)g/mL). ** (\( P < 0.01 \)) indicates statistically significant difference from absence of CoRP, ** (\( P < 0.01 \)) indicates statistically significant difference from WT mice.
The increase following CoRP treatment was, however, not significantly different between the two genotypes, thus, ruling out the involvement of Ca²⁺ signaling in Gαi2-mediated platelet apoptosis.

Next, we explored whether other putative mechanisms underpin the regulation of Gαi2-mediated platelet apoptosis. To this end, caspase 3 fluorescence (Cao et al. 2017) at the platelet surface reflecting enhanced degranulation; this effect was significantly reduced in Gαi2-deficient platelets as compared to WT platelets (Fig. 3D and F). In addition, CoRP treatment further stimulated active integrin αIIbβ3 expression (Cao et al. 2017) at the platelet surface, an effect that was again significantly attenuated in Gαi2-deficient platelets (Fig. 3E and F). Thus, Gαi2 is dichotomously involved in both platelet degranulation and apoptosis following agonist exposure.

**Discussion**

Previous studies have shown that the effect of CoRP on degranulation, PS exposure, cell volume, and αIIbβ3 integrin abundance is mediated, at least in part, by increased [Ca²⁺]i (Varga-Szabo et al. 2009; Lang et al. 2013), which
is known to trigger platelet activation and stimulate arterial thrombosis (Lang et al. 2013). Surprisingly, elevation of [Ca\textsuperscript{2+}]\textsubscript{i} was similar in platelets of either genotype suggesting that G\textsubscript{ai2} modifies platelet activation and apoptosis by interfering with mechanisms other than Ca\textsuperscript{2+} entry.

Acid sphingomyelinase degrades membrane sphingomyelin and generates ceramide, which is responsible for cell membrane scrambling and degranulation in a variety of cell types; this lipid signaling is vital in the pathophysiology of several cardiovascular diseases (Lang et al. 2015). Remarkably, both genetic deficiency and pharmacological inhibition of acid sphingomyelinase has been shown to thwart platelet secretion and PS exposure independently of Ca\textsuperscript{2+} signaling, purportedly due to a signaling defect downstream of phospholipase C activation (Munzer et al. 2014). Ceramide metabolism further involves ceramidases which has been reported to ameliorate CoRP-triggered glycoprotein VI-dependent platelet aggregation and thrombus formation (Munzer et al. 2018). Along these lines, it is tempting to speculate that PS-exposure and degranulation of platelets mediated by G\textsubscript{ai2}-sensitive ceramide generation is a possible mechanism linking previous observations of tissue injury and thrombosis after experimental ischemia-reperfusion.

In conclusion, the present study sheds light on a novel function of G\textsubscript{ai2} protein, that is, the regulation of platelet apoptosis, a process, at least in part, mediated by agonist-sensitive ceramide formation. Our observations on G\textsubscript{ai2}-mediated platelet survival may have pathophysiological implications in thrombo-inflammatory conditions. Further investigations are warranted to dissect the relevance of this mechanism in platelet-related disorders in humans.

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**Conflict of Interest**

The authors state that they have no conflict of interest.

**References**

Bissinger, R., E. Lang, M. Ghashghaeinia, Y. Singh, C. Zelenak, B. Fehrenbacher, et al. 2016. Blunted apoptosis of erythrocytes in mice deficient in the heterotrimeric G-protein subunit Galphai2. Sci. Rep. 6:30925.

Cao, H., A. A. M. Bhuyan, A. T. Umbach, R. Bissinger, M. Gawaz, and F. Lang. 2017. Inhibitory effect of afatinib on platelet activation and apoptosis. Cell. Physiol. Biochem. 43:2264–2276.

Devananthan, V., I. Hagedorn, D. Kohler, K. Pexa, D. Cherpokova, P. Kraft, et al. 2015. Platelet Gi protein Galphai2 is an essential mediator of thrombo-inflammatory organ damage in mice. Proc. Natl. Acad. Sci. USA 112:6491–6496.

Gachet, C. 2012. P2Y(12) receptors in platelets and other hematopoietic and non-hematopoietic cells. Purinergic Signal. 8:609–619.

Gatidis, S., O. Borst, M. Foller, and F. Lang. 2010. Effect of osmotic shock and urea on phosphatidylserine scrambling in thrombocyte cell membranes. Am. J. Physiol. Cell Physiol. 299:C111–C118.

Hilger, D., M. Masureel, and B. K. Kobilka. 2018. Structure and dynamics of GPCR signaling complexes. Nat. Struct. Mol. Biol. 25:4–12.

Lang, F., P. Munzer, M. Gawaz, and O. Borst. 2013. Regulation of STIM1/Orai1-dependent Ca\textsuperscript{2+} signalling in platelets. Thromb. Haemost. 110:925–930.

Lang, E., R. Bissinger, E. Gulbins, and F. Lang. 2015. Ceramide in the regulation of eryptosis, the suicidal erythrocyte death. Apoptosis 20:758–767.

Lebois, M., and E. C. Josefsson. 2016. Regulation of platelet lifespan by apoptosis. Platelets 27:497–504.

Liu, G., G. Liu, H. Chen, O. Borst, M. Gawaz, A. Vortkamp, et al. 2015. Involvement of Ca\textsuperscript{2+} activated Cl- channel Ano6 in platelet activation and apoptosis. Cell. Physiol. Biochem. 37:1934–1944.

Liu, G., G. Liu, M. Chatterjee, A. T. Umbach, H. Chen, M. Gawaz, et al. 2016. Influence of gamma-secretase inhibitor 24-Diamino-5-Phenylihiazole DAPT on platelet activation. Cell. Physiol. Biochem. 38:726–736.

Lopez-Aranda, M. F., I. Navarro-Lobato, J. F. Lopez-Tellez, E. Blanco, M. Masmudi-Martin, and Z. U. Khan. 2008. Activation of caspase-3 pathway by expression of sGalphai2 protein in BHK cells. Neurosci. Lett. 439:37–41.

Munzer, P., O. Borst, B. Walker, E. Schmid, M. A. Feijige, J. M. Cosemans, et al. 2014. Acid sphingomyelinase regulates platelet cell membrane scrambling, secretion, and thrombus formation. Arterioscler. Thromb. Vasc. Biol. 34:61–71.

Munzer, P., S. Mittelstadt, S. Geue, M. C. Manke, B. Walker-Allgaier, F. Lang, et al. 2018. Ceramidase critically affects GPVI-dependent platelet activation and thrombus formation. Biochem. Biophys. Res. Commun. 496:792–798.

Offermanns, S. 2006. Activation of platelet function through G protein-coupled receptors. Circ. Res. 99:1293–1304.

Quach, M. E., W. Chen, and R. Li. 2018. Mechanisms of platelet clearance and translation to improve platelet storage. Blood 131:1512–1521.

Rudolph, U., M. J. Finegold, S. S. Rich, G. R. Harriman, Y. Srinivasan, P. Brabet, et al. 1995. Ulcerative colitis and...
Adenocarcinoma of the colon in G alpha i2-deficient mice. Nat. Genet. 10:143–150.
Squires, K. E., C. Montanez-Miranda, R. R. Pandya, M. P. Torres, and J. R. Hepler. 2018. Genetic analysis of rare human variants of regulators of G protein signaling proteins and their role in human physiology and disease. Pharmacol. Rev. 70:446–474.
Towhid, S. T., E. M. Schmidt, E. Schmid, P. Munzer, S. M. Qadri, O. Borst, et al. 2011. Thymoquinone-induced platelet apoptosis. J. Cell. Biochem. 112:3112–3121.

Varga-Szabo, D., A. Braun, and B. Nieswandt. 2009. Calcium signaling in platelets. J. Thromb. Haemost. 7:1057–1066.
Wiege, K., S. R. Ali, B. Gewecke, A. Novakovic, F. M. Konrad, K. Pexa, et al. 2013. Galphai2 is the essential Galphai protein in immune complex-induced lung disease. J. Immunol. 190:324–333.