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Phosphoinositide 3-kinase inhibition reverses platelet aggregation triggered by the combination of the neutrophil proteinases elastase and cathepsin G without impairing $\alpha_{IIb}\beta_3$ integrin activation

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Abstract Neutrophil elastase (NE) upregulates the fibrinogen binding activity of the platelet integrin $\alpha_{IIb}\beta_3$ through proteolysis of the α_{IIb} subunit. This cleavage allows a strong potentiation of platelet aggregation induced by low concentrations of cathepsin G (CG), another neutrophil serine proteinase. During this activation process, we observed a strong fibrinogen binding and aggregation-dependent phosphatidylinositol 3,4-*bis*-phosphate (PtdIns(3,4)P_2) accumulation. PtdIns(3,4)P_2 has been suggested to play a role in the stabilization of platelet aggregation, possibly through the control of a maintained $\alpha_{IIb}\beta_3$ integrin activation. Here we show that inhibition of phosphoinositide 3-kinase (PI 3-K) by very low concentrations of wortmannin or LY294002 transformed the irreversible platelet aggregation induced by a combination of NE and low concentrations of CG into a reversible aggregation. However, although inhibition of PI 3-K was very efficient in inducing platelet disaggregation, it did not modify the level of $\alpha_{IIb}\beta_3$ activation as assessed by binding of an activation-dependent antibody. These results indicate that PI 3-K activity can control the irreversibility of platelet aggregation even under conditions where $\alpha_{IIb}\beta_3$ integrin remains activated.

Key words: Platelet activation; Neutrophil elastase; Cathepsin G; D3-phosphoinositide; Phosphoinositide 3-kinase; $\alpha_{IIb}\beta_3$

1. Introduction

We have previously characterized the molecular mechanisms underlying the synergistic activation of platelets by the secretable neutrophil serine proteinases elastase (NE) and cathepsin G (CG), which have been established as major mediators for the activation of platelets by neutrophils [1]. At concentrations ≥500 nM, CG is a potent platelet agonist, and triggers cell responses similar to those observed with thrombin [2]. As thrombin, CG is a serine proteinase which can potently stimulate platelets through structurally related proteinase-activated receptors (PAR) [3,4]. It was recently demonstrated that activation by CG is specifically mediated by the PAR4 receptor in human platelets [5], while thrombin can activate both PAR1 and PAR4 receptors [6]. At low concentrations (< 200 nM), CG induces a weak aggregation which is strongly enhanced by NE, although NE by itself does not activate human platelets [7]. This cooperation between the two proteinases requires a peculiar NE-dependent upregulation of the fibrinogen binding capacity of $\alpha_{IIb}\beta_3$ integrin, the fibrinogen receptor which supports platelet aggregation, through proteolysis of the carboxyl-terminus of the α_{IIb} subunit heavy chain. Thus, while low CG initiates mandatory intracellular signalling, insufficient by itself to promote a strong and stable aggregation, NE triggers a spatial reorientation of the extracellular domains of $\alpha_{IIb}\beta_3$ integrin allowing an increased binding of fibrinogen. Since more fibrinogen binds and cross-links adjacent platelets, post-occupancy 'outside-in' signalling events through $\alpha_{IIb}\beta_3$ may amplify cell activation initiated by low CG and finally maximize the recruitment of platelets into stable aggregates, apparently similar to those produced by higher concentrations of CG or other strong platelet agonists [8].

Considering the singular role of the post-$\alpha_{IIb}\beta_3$ occupancy signalling in the robust aggregation produced by the combination of NE and low CG, this model may be useful to better understand the close relationship between $\alpha_{IIb}\beta_3$ engagement and the phosphatidylinositol 3,4-*bis*-phosphate (PtdIns(3,4)P_2) accumulation described in several studies [9–13]. Indeed, PtdIns(3,4)P_2 accumulates in an aggregation-dependent manner and a large part of its synthesis requires the engagement of $\alpha_{IIb}\beta_3$ integrin. Moreover, a phosphoinositide 3-kinase (PI 3-K) and its product PtdIns(3,4)P_2 have been shown to play an important role in the stabilization of the irreversible platelet aggregation induced by the thrombin receptor PAR1 activating peptide (TRAP) [14,15]. PI 3-K inhibition by wortmannin or LY294002 does not impair granule secretion but leads to a reversible platelet aggregation. Upon addition of TRAP, the synthesis of phosphatidylinositol 3,4,5-*tris*-phosphate (PtdIns(3,4,5)P_3), another product of PI 3-K activation, is rapid and transient whereas PtdIns(3,4)P_2 accumulates slowly. By adding PI 3-K inhibitors 2 min after platelet stimulation

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Abbreviations: CG, cathepsin G; MFI, median of fluorescence intensity; NE, neutrophil elastase; PAR, proteinase-activated receptor; PI 3-K, phosphoinositide 3-kinase; PtdIns(3,4)P_2, phosphatidylinositol 3,4-*bis*-phosphate; PtdIns(3,4,5)P_3, phosphatidylinositol 3,4,5-*tris*-phosphate; tCG, threshold concentration of CG; TRAP, thrombin receptor PAR1 activating peptide

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by TRAP [15], when aggregation and PtdIns(3,4)P_2 production are at their maximum and PtdIns(3,4,5)P_3 is returned to its basal level, it was further shown that PtdIns(3,4)P_2 rapidly disappears and desaggregation occurs. As PI 3-K inhibition leads to a reversion of $\alpha_{IIb}\beta_3$ from its activated conformation into a resting state in TRAP-stimulated platelets [14], it was suggested that PtdIns(3,4)P_2 plays a role in maintaining $\alpha_{IIb}\beta_3$ in an activated state, which in turn allows stable aggregates to form. Supporting the role of PtdIns(3,4)P_2 in the stabilization of aggregation, ADP, added to a suspension of washed platelets in the presence of exogenous fibrinogen, is not able to induce a sustained synthesis of PtdIns(3,4)P_2 and is only able to induce a reversible platelet aggregation [16]. This spontaneous disaggregation is accompanied by the reversal of the $\alpha_{IIb}\beta_3$ integrin from its activated conformation, as indicated by the release of platelet-bound fibrinogen [17].

Using NE and CG separately or in combination, the present study identifies an original experimental model in which PI 3-K was strictly required for the irreversibility of platelet aggregation without affecting the sustained $\alpha_{IIb}\beta_3$ integrin activation. Since PtdIns(3,4)P_2 but not PtdIns(3,4,5)P_3 markedly accumulated upon activation by the combination of NE and low concentrations of CG, these observations unravel a new mechanism in the formation of stable aggregates depending directly on PtdIns(3,4)P_2 synthesis downstream of $\alpha_{IIb}\beta_3$.

2. Materials and methods

2.1. Materials

The mAb PAC-1 (IgM) [18] and AP-2 (IgG_1) [19] were provided by the University Cell Center of Pennsylvania (Philadelphia, PA, USA) and by Dr. T.J. Kunicki (Scripps Research Institute, La Jolla, CA, USA), respectively. Non-specific murine monoclonal IgG_1 or IgM were obtained from DAKO (Glostrup, Denmark). [^{32}P]Orthophosphate was from Amersham International (Little Chalfont, UK). TLC plates were from Merck (Darmstadt, Germany). The TRAP peptide was purchased from Bachem (Bubendorf, Switzerland). Human thrombin was from Hoffman-La Roche (Basel, Switzerland). Human leucocyte CG and NE were purified using a two-step chromatographic procedure as previously described [7]. Fibrinogen was purchased from Kabi (Stockholm, Sweden). The PI 3-K inhibitors LY294002 and wortmannin were from Biomol Research Laboratories (Plymouth Meeting, PA, USA). Eglin C was provided by Dr. H.P. Schnebli (Ciba-Geigy Research, Basel, Switzerland). All other reagents were obtained as indicated in [8].

2.2. Isolation and labelling of platelets

Human blood platelets were prepared essentially as described previously [8]. For inositol lipid analysis, platelets were labelled with 0.5 mCi/ml of [^{32}P]orthophosphate during 60 min in a phosphate-free Tyrode's buffer (137 mM NaCl; 2.68 mM KCl; 11.9 mM $NaHCO_3$; 0.42 mM NaH_2PO_4; 2 mM $CaCl_2$; 1 mM $MgCl_2$; 5.5 mM glucose; 5 mM HEPES), pH 6.5, at 37°C. ^{32}P-labelled platelets were then washed once in the same buffer and finally resuspended at a final concentration of 4×10^8 platelets/ml in Tyrode's buffer, pH 7.4, supplemented with 0.35% (w/v) BSA. The entire procedure was performed at 37°C and isolated platelets were maintained at this temperature until use.

2.3. Aggregation measurements

Platelet aggregation in 0.5 ml aliquots was monitored at 37°C using a Dual aggrometer (Chrono-Log corp., Havertown, PA, USA) under constant stirring (1 100 rpm). When required, samples were preincubated with various concentrations of LY294002 or wortmannin for 5 and 15 min, respectively, or in the presence of inhibitor vehicle alone (0.2% (v/v) dimethylsulfoxide). Fibrinogen (0.7 mg/ml) was added 2 min before stimulation, which was initiated by addition of 550 nM CG, or a threshold concentration of CG (tCG), or the combination of tCG preceded for 10 s by 400 nM NE. For each tested platelet suspension, tCG was determined as the concentration of enzyme resulting in platelet shape change followed by a 10–15% increase in light transmission within 3 min of stirring. This concentration was always in the range 110–160 nM.

2.4. Lipid extraction and analysis

Aggregations were stopped at given time points by addition of chloroform/methanol (1/1, v/v) containing 0.4 N HCl and lipids were immediately extracted following the modified procedure of Bligh and Dyer [20]. For [^{32}P]PtdIns(3,4)P_2 and [^{32}P]PtdIns(3,4,5)P_3 quantification, lipids were first resolved by TLC using chloroform/acetone/methanol/acetic acid/water (80/30/26/24/14, v/v). The spots corresponding to PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 were then scraped off, deacylated by 20% methylamine and analyzed by HPLC on a Whatman Partisphere strong ion exchange column, as previously described [21].

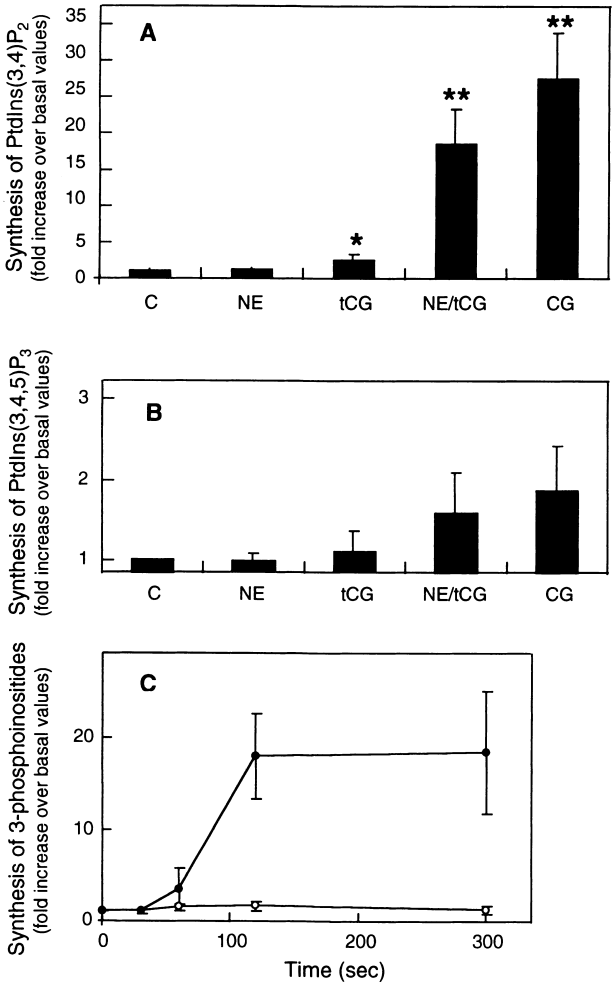

Fig. 1. Synthesis of 3-phosphoinositides in platelets exposed to NE and/or CG. A, B: Suspensions of ^{32}P-labelled platelets, stirred at 37°C, were preincubated for 2 min with 0.7 mg/ml fibrinogen before stimulation for 2 min with 400 nM NE, tCG (110–160 nM) alone or in combination with NE (NE/tCG), or 550 nM CG. Control platelets (C) were incubated non-activated. Cell lipids were extracted and [^{32}P]PtdIns(3,4)P_2 and [^{32}P]PtdIns(3,4,5)P_3 quantified as indicated in Section 2. C: Kinetics of [^{32}P]PtdIns(3,4)P_2 (filled circles) and [^{32}P]PtdIns(3,4,5)P_3 (open circles) accumulation were determined in platelets stimulated by the combination of NE and tCG. Results, expressed as the fold increase of the lipid accumulation relative to the control non-activated platelets, are the mean ± S.E.M. of three to five experiments conducted with cells from different donors. $^*P<0.05$; $^{**}P<0.01$ (Student's t-test for paired samples).

2.5. Flow cytometry analysis

Platelet samples were treated as for aggregation analysis except that exogenous fibrinogen was omitted as this natural ligand of the activated $\alpha_{IIb}\beta_3$ integrin may competitively inhibit the binding of the mAb PAC-1 [18]. In any case, once the agonist has been added (NE, tCG, or NE/tCG as indicated above, or 0.5 U/ml human thrombin, or 7.5 μM TRAP), the stirring was allowed for only 5 s in order to homogenize the milieu, then samples were incubated for 3 min at 37°C undisturbed in order to prevent platelet aggregate formation. The reaction was stopped by the addition of 40 μg/ml (5 μM) eglin C, an inhibitor of CG and NE [22] and 2 mM phenylmethylsulfonyl fluoride. Platelets were then immediately fixed with 1% (v/v) formaldehyde for 30 min at room temperature. Following the fixation, samples were diluted 10-fold in Tyrode's medium containing 0.35% BSA and then incubated for 30 min at 4°C with saturating concentrations of purified PAC-1 (2 μg/ml), AP-2 (1 μg/ml), or with murine IgG_1 or IgM as control isotypes. Subsequent incubations, including that with the corresponding secondary fluorescein isothiocyanate-labelled antibody, and cytometric analysis using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA) were as previously described [8]. The median of fluorescence intensity (MFI) of the cell population was used to measure the PAC-1 or AP-2 epitope expression on the $\alpha_{IIb}\beta_3$ integrin, the MFI obtained with control isotype immunoglobulins being subtracted in all cases.

3. Results

Exposure of platelets to 400 nM NE did not induce PI 3-K activation, as measured through PtdIns(3,4)P_2 and PtdIns(3,4,5)P_3 production (Fig. 1A,B). Stimulation of platelets with threshold concentrations of CG (tCG) induced a slight but significant production of PtdIns(3,4)P_2 but no significant synthesis of PtdIns(3,4,5)P_3 (Fig. 1A,B). Conversely, addition of tCG following NE treatment clearly led to a strong production of PtdIns(3,4)P_2 (Fig. 1A) whereas the synthesis of PtdIns(3,4,5)P_3 remained modest at any time point investigated (Fig. 1C). PtdIns(3,4)P_2 started to accumulate significantly 1 min following addition of tCG (Fig. 1C), which appears rather late if compared to the kinetics previously observed in TRAP-stimulated platelets [15]. This production of PtdIns(3,4)P_2 was strictly dependent upon the presence of exogenous fibrinogen and stirring of platelets, i.e. the aggregation of platelets (data not shown). Finally, a fully activating concentration of CG induced an important accumulation of PtdIns(3,4)P_2 (Fig. 1A) comparable to that induced by thrombin (not shown). No significant increase in PtdIns3P was observed under our experimental conditions.

As previously established [7,8], platelet suspensions stirred for 3 min with 400 nM NE did not show any functional responses (not shown). CG in the range 110–160 nM (tCG) induced a limited but significant reversible platelet aggregation, with 10–15% increase in light transmission at the maximum of aggregate formation (Fig. 2A,B). Addition of NE 10 s before tCG resulted in a potent aggregation, similar to that induced by an optimal concentration of CG (550 nM, Fig. 2C) or 0.5 IU/ml thrombin (not shown). However, this aggregation required addition of exogenous fibrinogen since granule exocytosis, and thus, surface expression of endogenous pro-aggregatory adhesive proteins [23], induced by the combination of NE and tCG is limited [8]. To test whether PI 3-K would be involved in the mechanisms supporting the aggregation induced by the combination of NE and tCG, we used the two unrelated inhibitors of this lipid kinase, LY294002 and wortmannin, at low concentrations. As shown in Fig. 2, LY 294002 (≤5 μM) or wortmannin (≤10 nM) had no significant effect on the pattern of CG-induced platelet aggregation, either at a threshold or a high concentration of the proteinase. Conversely, challenge of platelets with the combination of NE and tCG, in the presence of PI 3-K inhibitors, resulted in a marked and rapid reversibility of the aggregation process without significant changes in the initial velocity and maximal intensity of aggregation (Fig. 2A,B).

These results led us to investigate the activation state of $\alpha_{IIb}\beta_3$ by using PAC-1, an IgM monoclonal antibody acting as a fibrinogen-like ligand for activated $\alpha_{IIb}\beta_3$ [18], and flow cytometry analysis. As previously observed, when using

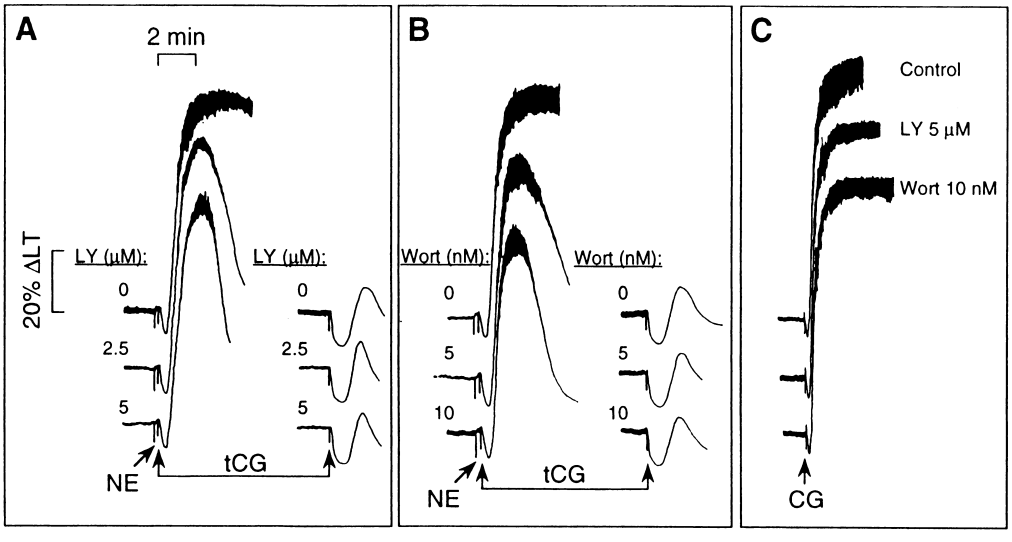

Fig. 2. Effects of low concentrations of PI 3-K inhibitors on the irreversible platelet aggregation induced by the combination of NE and CG. Platelet aggregation was measured as described in Section 2. A, B: Platelet suspensions were preincubated with various concentrations of the two PI 3-K inhibitors, LY294002 (LY, A) or wortmannin (Wort, B), as indicated. They were then stimulated, in the presence of 0.7 mg/ml fibrinogen, with a tCG of 150 nM, either alone or preceded for 10 s by 400 nM NE. C: Aggregation was induced by a fully activating concentration of CG (550 nM) in the absence or presence of 5 μM LY294002 or 10 nM wortmannin. Tracings show the time course of the variation in light transmission (ΔLT) in the cell suspensions, and are representative of at least three experiments performed with platelets from different individuals.

TRAP (7.5 μM) as the platelet agonist [14], we found that the reversion of aggregation induced by wortmannin treatment was accompanied by an inhibition of the sustained activation of $\alpha_{IIb}\beta_3$ (45.4 ± 12.5% of the control, $n = 3$) (Fig. 3A). This inhibition was not due to an impaired translocation of the intracellular pool of $\alpha_{IIb}\beta_3$ to the plasma membrane following TRAP activation [14], as measured with AP-2, an $\alpha_{IIb}\beta_3$ complex-specific antibody reacting with both the resting and active forms of the receptor (Fig. 3B). It is noteworthy that PI 3-K inhibition did not change significantly the platelet aggregation induced by 0.5 IU/ml thrombin (not shown) and did not affect the activation of $\alpha_{IIb}\beta_3$ induced by this concentration of thrombin (Fig. 3A). When initiated by NE or tCG taken separately or, more interestingly, in combination, the level of $\alpha_{IIb}\beta_3$ activation and surface expression remained unaffected upon treatment with 50 nM wortmannin (Fig. 3A,B), a concentration that fully inhibited D3-phosphoinositide synthesis and reversed platelet aggregation (not shown).

These data suggest a new mechanism through which PI 3-K and its product PtdIns(3,4)P_2 stabilize platelet aggregates downstream of $\alpha_{IIb}\beta_3$.

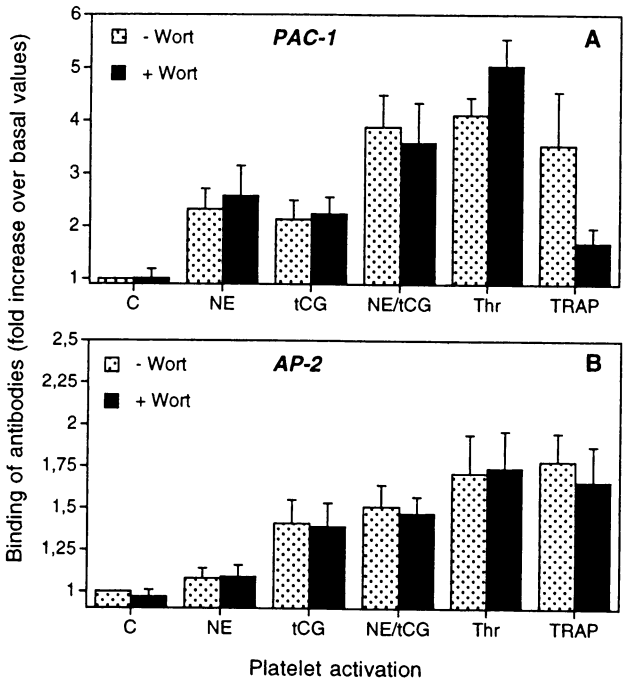

Fig. 3. Effects of wortmannin on the redistribution and activation of the $\alpha_{IIb}\beta_3$ integrin induced by the combination of NE and tCG. Unstirred platelet suspensions were preincubated with 50 nM wortmannin (+Wort) or with vehicle alone (0.2% DMSO, −Wort) for 15 min at 37°C in the absence of exogenous fibrinogen. Cell activation was initiated by adding 400 nM NE, tCG (150–180 nM) alone or in combination with NE (NE/tCG), 0.5 IU/ml thrombin, or 7.5 μM TRAP, and followed for 3 min. Proteinases were blocked with eglin C and PMSF. Binding of PAC-1 (A, activation of $\alpha_{IIb}\beta_3$) and AP-2 (B, redistribution of $\alpha_{IIb}\beta_3$) antibodies to platelets was then measured by flow cytometry as described in Section 2. Results, expressed as the fold increase of the MFI relative to the control non-activated platelets (C), are the mean ± S.E.M. of three or four experiments conducted with cells from different donors.

4. Discussion

The aim of the present study was to investigate the putative role of the PI 3-K-dependent signalling pathway in the synergistic activation of human platelets by the neutrophil proteinases NE and CG. The specific proteolytic activation of the $\alpha_{IIb}\beta_3$ integrin and subsequent binding of fibrinogen induced by NE alone fail to initiate a significant platelet activation and aggregation [8] and did not induce PI 3-K activation. Thus, the peculiar upregulation of the fibrinogen receptor by a direct extracellular proteolytic processing of one of its subunits is not sufficient per se to induce any of the signalling pathways investigated until now. This is different from what is observed when activation of $\alpha_{IIb}\beta_3$ is promoted by the binding of activating antibodies to the integrin [12,14,24] which is an useful model to investigate biochemical events downstream of $\alpha_{IIb}\beta_3$. With this model, Banfic et al. [12] have proposed a new mechanism leading to PtdIns(3,4)P_2 synthesis independently of PtdIns(3,4,5)P_3 production. This pathway involves the HsC2-PI 3-K generating PtdIns3P, subsequently metabolized into PtdIns(3,4)P_2 by a PtdIns(3)P 4-kinase [13].

When $\alpha_{IIb}\beta_3$ activation is the result of the proteolysis by NE combined with an 'inside-out' signalling initiated by a low concentration of CG, a potent accumulation of PtdIns(3,4)P_2 was observed, with only a minute increase in PtdIns(3,4,5)P_3. Since the potentiation between NE and CG occurred without marked granule exocytosis [8], the PtdIns(3,4)P_2 synthesis required the presence of exogenous fibrinogen and was relatively delayed, starting only 1 min after addition of the stimuli. Although we could not observe any increase of PtdIns(3)P, these results support the involvement of HsC2-PI 3-K since no significant formation of PtdIns(3,4,5)P_3 could be observed.

Interestingly, we found that the irreversible platelet aggregation occurring upon stimulation by the combination of NE and CG was strictly dependent on PI 3-K activation and PtdIns(3,4)P_2 production. Indeed, low concentrations of wortmannin or LY294002 (5 nM and 2.5 μM, respectively) resulted in the formation of unstable aggregates. As a comparison, higher doses of wortmannin (≥50 nM) are required to reverse the platelet aggregation induced by TRAP [14,21]. According to the primary agonist used and the signal transduction pathway implicated, several mechanisms may control the stabilization of platelet aggregation. The PI 3-K-dependent pathway appears to be mandatory for a number of agonists (C.T, M.P and B.P., unpublished observations) but not all since platelet aggregation induced by high concentrations of thrombin is unaffected by PI 3-K inhibition. One of the PI 3-K-dependent mechanisms that might control the stability of aggregation is the maintenance of a sustained high affinity of $\alpha_{IIb}\beta_3$ towards fibrinogen. Indeed, in agreement with Kovacsovics et al. [14], we also measured a decrease in PAC-1 binding to $\alpha_{IIb}\beta_3$ when TRAP-stimulated platelets are pretreated by wortmannin without changes in the surface expression of this integrin. However, our results suggest that PtdIns(3,4)P_2 might actually stabilize aggregates by another mechanism since, although platelet aggregation induced by the combination of NE and CG became reversible, PAC-1 binding was unaffected by PI 3-K inhibition. Thus, blocking the PI 3-K pathway may result in platelet disaggregation without inhibition of the major platelet aggregation receptors.

Our previous studies have shown that wortmannin-induced disaggregation of TRAP-stimulated platelets parallels a reorganization of the cytoskeleton, with a rapid destabilization of F-actin-associated signalling complexes and a specific release of the contractile protein myosin [15]. Whether specific

changes in the cytoskeleton dynamic induced by PI 3-K inhibition are responsible for the disaggregation observed here remains to be established. We propose that the present model of $\alpha_{IIb}\beta_3$ activation might be particularly useful to identify direct effectors of PtdIns(3,4)P_2 involved in the strengthening of platelet aggregation and by this way, new potential pharmacological targets in order to modulate irreversible aggregation.

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References

[1] Pidard, D., Si-Tahar, M. and Chignard, M. (2000) in: Platelets, Thrombosis and the Vessel Wall (Berndt, M.C., Ed.), pp. 189–207, Harwood Academic, Amsterdam.
[2] Si-Tahar, M., Renesto, P., Fallet, H., Rendu, F. and Chignard, M. (1996) Biochem. J. 313, 401–408.
[3] Vu, T.K.H., Hung, D.T., Wheaton, V.I. and Coughlin, S.R. (1991) Cell 64, 1057–1068.
[4] Böhm, S.K., McConalogue, K., Kong, W. and Bunnett, N.W. (1998) News Physiol. Sci. 13, 231–240.
[5] Sambrano, G.R., Huang, W., Faruqi, T., Mahrus, S., Craik, C. and Coughlin, S.R. (2000) J. Biol. Chem. 275, 6819–6823.
[6] Kahn, M.L., Nakanishi-Matsui, M., Shapiro, M.J., Ishihara, H. and Coughlin, S.R. (1999) J. Clin. Invest. 103, 879–887.
[7] Renesto, P. and Chignard, M. (1993) Blood 82, 139–144.
[8] Si-Tahar, M., Pidard, D., Balloy, V., Moniatte, M., Kieffer, N., Van Dorsselaer, A. and Chignard, M. (1997) J. Biol. Chem. 272, 11636–11647.
[9] Sultan, C., Plantavid, M., Bachelot, C., Grondin, P., Breton, M., Mauco, G., Lévy-Tolédano, S., Caen, J. and Chap, H. (1991) J. Biol. Chem. 266, 23554–23557.
[10] Guinebault, C., Payrastre, B., Racaud-Sultan, C., Mazarguil, H., Breton, M., Mauco, G., Plantavid, M. and Chap, H. (1995) J. Cell Biol. 129, 831–842.
[11] Rittenhouse, S.E. (1996) Blood 88, 4401–4414.
[12] Banfic, H., Tang, X., Batty, I.H., Downes, C.P., Chen, C. and Rittenhouse, S.E. (1998) J. Biol. Chem. 273, 13–16.
[13] Zhang, J., Banfic, H., Straforini, F., Tosi, L., Volinia, S. and Rittenhouse, S.E. (1998) J. Biol. Chem. 273, 14081–14084.
[14] Kovacsovics, T.J., Bachelot, C., Toker, A., Vlahos, C.J., Duckworth, B., Cantley, L.C. and Hartwig, J.H. (1995) J. Biol. Chem. 270, 11358–11366.
[15] Trumel, C., Payrastre, B., Plantavid, M., Hechler, B., Viala, C., Presek, P., Martinson, E., Cazenave, J.-P., Chap, H. and Gachet, C. (1999) Blood 94, 1–10.
[16] Gachet, C., Payrastre, B., Guinebault, C., Trumel, C., Ohlman, P., Mauco, G., Cazenave, J.-P., Plantavid, M. and Chap, H. (1997) J. Biol. Chem. 272, 4850–4854.
[17] Mustard, J.F., Packham, M.A., Kinlough-Rathbone, R.L., Perry, D.W. and Regoeczi, E. Fibrinogen and ADP-induced platelet aggregation, (1978) Blood 52, 453–466.
[18] Taub, R., Gould, R.J., Garsky, V.M., Ciccarone, T.M., Hoxie, J., Friedman, P.A. and Shattil, S.J. (1989) J. Biol. Chem. 264, 259–265.
[19] Pidard, D., Montgomery, R.R., Bennett, J.S. and Kunicki, T.J. (1983) J. Biol. Chem. 258, 12582–12586.
[20] Sultan, C., Breton, M., Mauco, G., Grondin, P., Plantavid, M. and Chap, H. (1990) Biochem. J. 269, 831–834.
[21] Gratacap, M.P., Payrastre, B., Viala, C., Mauco, G., Plantavid, M. and Chap, H. (1998) J. Biol. Chem. 273, 24314–24321.
[22] Renesto, P., Ferrer-Lopez, P. and Chignard, M. (1990) Lab. Invest. 62, 409–416.
[23] Peerschke, E.I.B. (1992) Blood 79, 948–953.
[24] Frelinger, A.L., Du, X.P., Plow, E.F. and Ginsberg, M.H. (1991) J. Biol. Chem. 266, 17106–17111.