

Review Article

The plasma membrane redox system in human platelet functions and platelet-leukocyte interactions

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Summary

The plasma membrane electron transport is crucial for blood coagulation and thrombosis, since reactive oxygen species and thiol changes, generated by plasma membrane redox reactions, modulate activation of platelets, as well as their interaction with leukocytes. Several antioxidants are linked to this system; thus,

platelets are also able to counterbalance radical production and to regulate thrombus growth. Aim of this review is to give an update on the plasma membrane redox system in platelets, as well as on its role in platelet functions and leukocyte-platelet cross-talk.

Keywords

Platelets, plasma membrane redox system, NAD(P)H oxidase, ubiquinone, antioxidant vitamins

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Introduction

Reactive oxygen species (ROS), including superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2), are constitutively released from platelets and their production is enhanced by classical agonists, such as thrombin, collagen and immunological stimuli (1–4). As thoroughly described in recent reviews (5, 6), endogenously produced ROS play autocrine and/or paracrine roles in platelet activation, likely those reported for exogenous ROS; platelet-derived ROS can arise from the activity of several cytosolic enzymes, including xanthine oxidase, monoaminoxidase, lipooxygenase and the endothelial isoform of nitric oxide (NO) synthase (eNOS), as well as from mitochondrial membrane-associated respiratory enzymes. An alternative source of ROS is represented by the plasma membrane redox (PMR) system or the plasma membrane electron transport (PMET); it has been known since 1970, although its physiological relevance has only recently been investigated. By this system, intracellular electrons (derived from NADH, NADPH or vitamin C) (7, 8) flow outwards thanks to their sequential transfer to several carriers [the most widely used is ubiquinone (or coenzyme Q; CoQ), although other acceptors (b cytochromes, flavin, vitamin E and membrane proteins) exist] (9), through the activity of enzymes localized in

the plasma membrane (for an overview of the enzymatic systems involved see [10]); once outside, electrons reduce extracellular molecules (especially molecular oxygen), thus modulating the redox state of the microenvironment surrounding cells (11). Partial oxygen reduction leads to generation of O_2^- and H_2O_2 , which regulate normal cellular functions, but, if overproduced, can contribute to pathological diseases, including endothelial dysfunction and cardiovascular diseases (12). The components of the PMR system are summarized in Figure 1.

Almost all studies concerning the PMR system have been focused on the NADPH oxidase family (NOX), whose best characterized member is the phagocytic enzyme NOX2 (11). Also in platelets, the aggregation-induced ROS production has been ascribed to the activity of an enzyme similar to NOX2. Nonetheless, the experimental settings have overestimated the role of NADPH oxidase to the detriment of other mechanisms. This misunderstanding principally relies on the use of inhibitors, which are not specific: diphenylene iodonium is an inhibitor of flavoproteins (13) rather than a specific NADPH oxidase inhibitor and apocynin is a general antioxidant rather than an enzymatic inhibitor (14). Indeed, almost all cells (including platelets) possess, together with NOX, a NADH oxidase (ECTO-NOX), which uses electrons derived from intracellular NADH or CoQ to

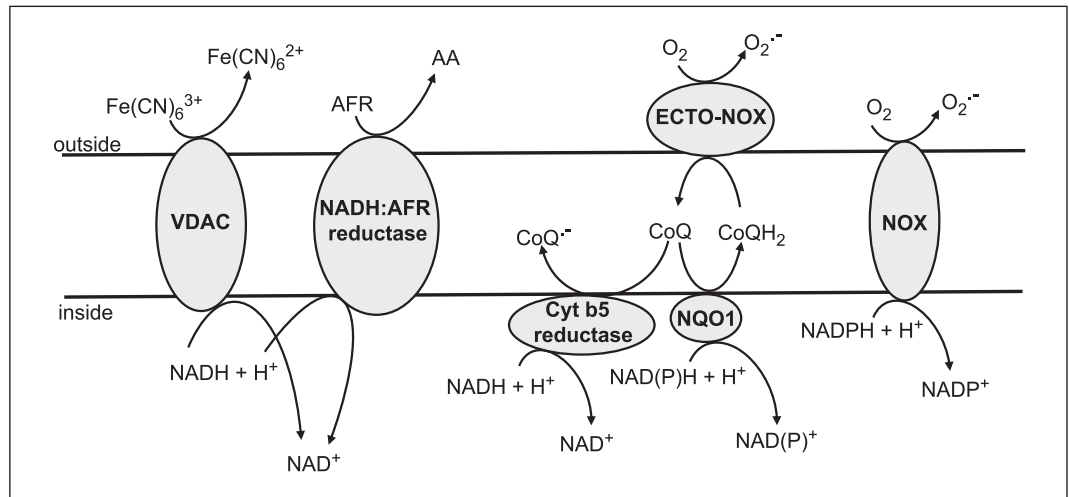
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Figure 1: Key enzymes of the PMR system. Membrane localisation and catalysed reactions for each enzyme are shown. These enzymes are differentially expressed in different cell types (see the text for those present in platelets). AA, ascorbate; AFR, ascorbyl free radical; VDAC, voltage-dependent anion-selective channel or NADH:ferricyanide reductase; NQO1, NAD(P)H:ubiquinone oxidoreductase or DT-diaphorase; NOX, NADPH oxidase; ECTO-NOX, NADH oxidase.



produce superoxide and hydrogen peroxide; thus, this enzyme requires redox carriers different from those used by NOX and strictly depends on the cytoplasmic NADH concentrations.

Aim of this review is to collect all evidences showing the involvement of the PMR system in platelet signalling, as well as its role in platelet-leukocyte cross talk.

The PMRS in platelets

Findings that both resting and stimulated platelets can reduce cell-impermeant tetrazolium salts have indicated the presence of transplasma membrane or surface reducing activities, which can alternatively use NADH or NADPH (4). Several components of the PMR system have been identified so far, whose best characterized elements are different NAD(P)H oxidases and thiol-related enzymes.

Platelets express both catalytic and regulatory subunits of NADPH oxidase (NOX), a multi-subunit enzyme first identified in phagocytic cells (15). The enzyme consists of two membrane-bound subunits (p22phox and gp91phox) catalyzing one-electron transfer from NADPH to molecular oxygen, thus generating O_2^- . Small GTPases (Rac1 or Rac2) and cytosolic factors (p47phox, p67phox, p40phox), which translocate to the plasma membrane after phosphatidylinositol 3-kinase- (PI3K) and protein kinase C (PKC) -dependent phosphorylation, are needed for the catalytic activity (16). Platelets are not true phagocytic cells and they do not kill bacteria (17); so, platelet NADPH oxidase may play a different role related to $\alpha_{IIb}\beta_3$ -integrin activation, ADP release and platelet recruitment (18, 19). Classical agonists activate this gp91phox-dependent enzyme: collagen and thrombin induce the NADPH oxidase-dependent superoxide release in platelets, while $\alpha_{IIb}\beta_3$ inhibitors prevent the aggregation-induced membrane translocation of p67phox and p47phox subunits. Accordingly, platelets from gp91phox-deficient patients produce very small amounts of ROS (20). Platelet NADPH oxidase exerts roles other than those associated with aggregation. Indeed, the angiotensin II pathway activates this enzyme, which is thus related to hypertension-associated overproduction of O_2^- ; in host defence response, platelet NADPH oxidase causes thromboxane

A2 release which, in turn, enhances ROS production and the cytotoxic action of neutrophils (21).

Platelets also possess a NADH oxidase ([1, 22] and our unpublished data), belonging to the protein family named “external NADH oxidases” or “ECTO-NOX” (23). These enzymes are peculiar, as they are not trans-membrane proteins, but instead cell surface-associated oxidases; they show hydroquinone (NADH) oxidase and protein disulfide-thiol interchange activities, that alternate giving rise to oscillations with a period of 24 minutes (24). In platelets, the enzyme may be responsible for agonist-induced H_2O_2 production (2); then, H_2O_2 acts extracellularly or diffuses inside cells, where it functions as a second messenger (25). The ability of the enzyme to cycle between ROS generation and cleavage of disulfide bonds can explain its role in platelet regulation; this requires that surface sulphhydryl groups are redox-sensitive sites, whose oxidation leads to changes in the conformation of cell surface integrins, thus modulating platelet functions (26). This should be relevant in hypoxic conditions, when the protein disulfide-thiol interchange activity predominates, because of the lack of molecular oxygen.

At least two thiol isomerases [protein disulphide isomerase (PDI) and endoplasmic reticulum protein 5 (ERP5)] and a glutathione reductase have been identified on platelet surface (27–30). These enzymes, interacting with integrin receptors, are crucial for rearrangement of membrane disulfide bonds needed for platelet aggregation, secretion and post-aggregation events (26, 27, 31, 32). ERP5 is recruited to the cell surface in response to platelet agonists and becomes physically associated with β_3 integrin (30), while PDI interacts with $\alpha_2\beta_1$ and $\alpha_{IIb}\beta_3$ integrins (32, 33). It is noteworthy the potential interplay among the different enzymes. It has been shown that PDI interacts with NADPH oxidase, thus regulating its activity (34). On the other hand, PDI is a redox-sensitive enzyme, since changes in composition of the glutathione redox buffer are known to modulate the rate of PDI-catalyzed folding of a target protein (35); thus, it is conceivable that glutathione reductase, by maintaining adequate concentrations of external GSH, may regulate PDI activity.

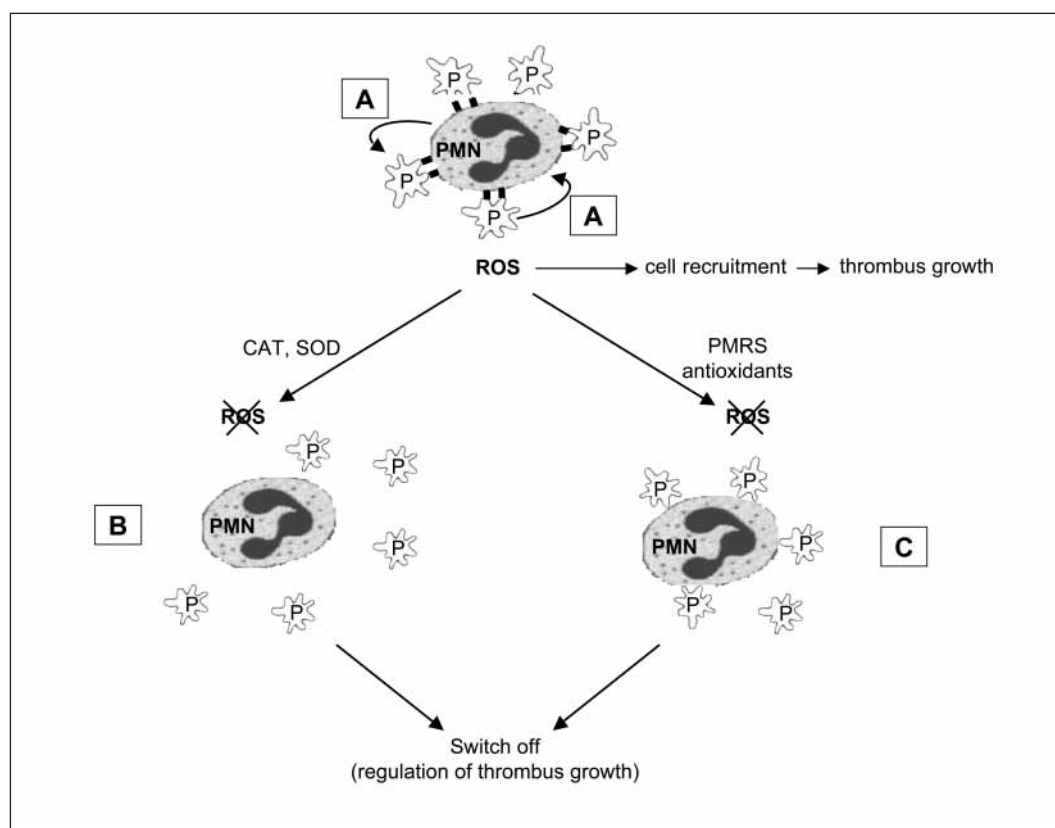


Figure 2: Interaction between platelets and leukocytes during clot formation. A) Platelet-leukocyte conjugates. Tight cell-to-cell contacts activate cells via ROS production. B) Platelets may release antioxidant molecules (catalase, peroxidase...), which scavenge ROS and inhibit cell activation. C) The platelet PMR system serves as a surface antioxidant system (ascorbate, vitamin E, coenzyme Q). CAT, catalase; SOD, superoxide dismutase; PMN, polymorphonucleate cells; P, platelets.

Finally, platelets have a NADH:ascorbate free radical oxidoreductase (our unpublished data), a membrane-bound enzymatic activity not yet unequivocally ascribed to a specific protein. It uses intracellular NADH to reduce extracellular ascorbyl free radical; CoQ can be an electron linker, since its removal decreases the extent of radical reduction. By recycling ascorbate, this oxidoreductase may modulate cell response to extracellular redox changes.

Non-enzymatic scavenger molecules are strictly linked to a functional PMR system; their low molecular mass allows them to remove ROS at sites where larger antioxidant enzymes cannot access. In particular, vitamin C and vitamin E have physiological relevance in platelets. We demonstrated that intracellular ascorbate modulates the redox state of surface sulphhydryl groups, thus regulating aggregation and thrombus strength generated during post-clotting events (36). Moreover, the general antioxidant role of ascorbate regulates those membrane systems sensitive to ROS-mediated signalling, by reverting the NADPH oxidase-mediated effects in chronic smokers (37) or after chronic exposure to organic nitrates (38). Ascorbate may also play a role in thiol-disulfide reactions, since dehydroascorbate can be a substrate for PDI (39). Due to its relevance in platelet physiology, vitamin C uptake is strictly controlled: platelets compensate for fluctuations in ascorbate levels by modulating (at translational level) the expression of the Na⁺-dependent transporter SVCT2 (36). Vitamin E interferes with the PKC signalling pathway and NADPH-oxidase activation, thus regulating platelet adhesion, activation and aggregation (40, 41). It also exerts antioxidant effects, by preventing lipid peroxidation and prostaglandin pro-

duction (42). In agreement with these data, decreased platelet antioxidant content is related to increased platelet aggregation; likewise, platelet hyperactivity can be normalized by adding exogenous antioxidants (43).

The PMR system and platelet/leukocyte cross-talk

As ROS generation in platelets may occur either inside or on the external surface (19, 44, 45), platelet PMR activity may induce autocrine and paracrine effects. In particular, it can be involved in complex interactions among platelets, leukocytes and endothelial cells, occurring during thrombus and clot formation, as well as during inflammation and atherosclerosis (46).

Platelets and leukocytes mutually activate themselves, when contacts are established between platelet surface molecules and leukocyte receptors (47, 48). Adherent platelets and platelet-released molecules enhance leukocyte recruitment and adhesion to endothelial cells, as well as their phagocytic activity (49); in the mean time, activated leukocytes enhance platelet adhesion, aggregation and secretion (50). This cell-to-cell adhesion facilitates platelet-leukocyte crosstalk, but it may also lead to inflammation and may support several pathological events, such as plaque formation in atherosclerosis (51).

Platelet/leukocyte interactions are associated with increased ROS that, when accumulated in the "microenvironment", drive specific cellular responses. Paradoxically, stimulated platelets can also down-regulate leukocyte ROS production, by releasing

“stop signals” (serotonin, adenosine, catalase, superoxide dismutase and peroxidase): it has been shown that platelet supernatants inhibit chemiluminescence (i.e. concentration of ROS) and myeloperoxidase activity of phagocytic cells, thus impairing chemotaxis and cytotoxicity (52, 53). Thus, the dual action of platelets on plasma ROS levels may be required to maintain an adequate blood flow and prevent pathologic thrombosis (54).

Accumulating evidences suggest a pro-thrombotic effect of platelet-derived ROS, associated with the outbreak of myocardial infarct and angina. Chronic activated platelets, cross-reacting with several cell types and overproducing ROS, lead to inflammatory processes, which are involved in the pathogenesis and progression of atherosclerosis and other cardiovascular diseases (6). During activation, platelets release exosomes in plasma, which induce cell death through a NADPH oxidase-dependent pathway; this is an additional mechanism of platelet-induced vascular dysfunction (55). An excess in platelet ROS production has been demonstrated in hypercholesterolemia and platelet-associated NADPH oxidase produces a thrombogenic phenotype associated with elevated cholesterol levels (56). In the same way, hyperhomocysteinemia, which is a risk factor for cardiovascular disease, is a potent inducer of endothelial PMR activity (57); this modulating effect should also be extended to platelets. Recently, increased eosinophil- and neutrophil-platelet interactions and ROS production have been related to inflammatory responses and increased risk of vascular thrombotic events promoted by strenuous exercise (58). Exercise-triggered thrombosis can also be related to changes in the GSH/GSSG ratio: vigorous exercise in not-trained subjects leads to increased GSSG levels, thus contributing to alterations in platelet functions (59).

The pro-oxidant activity of the PMR system can be counterbalanced by endogenous and exogenous antioxidants, which inhibit ROS production and platelet functions. Such an example, polyphenols decrease platelet recruitment, via inhibition of NADPH oxidase activity (60); this should explain the cardioprotective effects of phenol-containing foods. Development and progression of atherosclerosis can be inhibited by antioxidants, including vitamins E and C. In platelet-leukocyte interactions, vitamin C changes NO levels regulating cell responses (61). Vitamin E may affect atherogenesis by inhibiting cell-to-cell adhesion, platelet functions and mural thrombi through inhibition of PKC (62, 63). The effects of antioxidants on platelet-leukocyte aggregation has been reported in cigarette smokers, during strenuous exercise and in haematological pathologies such as paroxysmal nocturnal haemoglobinuria and sickle cell disease, as well as in allergic, immunological, thrombotic and inflammatory vascular diseases (46, 64, 65, 66).

In this context, the PMR system can be viewed as an additional mechanism through which platelets control blood redox state; activation or inhibition of the PMR components by circu-

lating products (hormones, growth factors and other cell activators) (67) can promote the pro/anti-oxidant shift in platelet action. Recent evidences suggest that RNA present in blood may be placed among these circulating regulators (68–70), as it induces conformational changes in several enzymes, thus modulating their activity. By looking at the ECTO-NOX primary structure, we found an RNA recognition motif (RRM) probably diagnostic of an RNA binding protein, since it has been found in eukaryotic proteins known to bind single stranded RNA (71). Motif Scan analysis (72) of the sequence gives an E-value of 6.3×10^{-6} for the RRM match (the E-value is the number of matches with a score equal to or greater than the observed score that is expected to occur by chance); therefore, the observed low value means that the match is likely a true positive. Vascular injury results in RNA and RNase release; it has been proposed that this RNA-RNase balance contributes to vascular homeostasis, since RNA, activating specific coagulation factors, promotes thrombus formation, while RNase is required to stop it (68–70). As RNA appears to be a key factor in thrombus formation, and ECTO-NOX is a putative RNA binding protein, is tempting to speculate a possible regulatory role of this platelet enzyme during the coagulation process.

From these observations, it can be proposed a model (Fig. 2) in which platelet/leukocyte cross-talk and ROS generation are needed for priming the coagulation process; but, moving away from the clot centre, microenvironment changes (lower levels of radicals, less pronounced cell-to-cell contacts and activation), so that the antioxidant action of platelets counterbalances the effects of oxidative stress and restricts the extension of clot growth. Alterations on redox balance dramatically change platelet functions, contributing to the pathogenesis of several cardiovascular diseases, including thrombotic vascular occlusion and thromboembolic complications.

In conclusion, the PMR system is crucial in platelets, offering an additional mechanism to regulate thrombus growth and cell-to-cell interactions. It must be recalled that the PMR system works in platelets as pro-oxidant, but it can also have antioxidant effects; these effects should be temporally and spatially regulated, in order to modulate the entire process of coagulation, as well as immunological and inflammatory events. Further knowledge on the role of the PMR system in platelet functions should promote the development of therapeutic approaches against several diseases, including diabetes, inflammation and thromboembolic pathologies.

Abbreviations

PMR, plasma membrane redox; CoQ, coenzyme Q; ROS, reactive oxygen species; PDI, protein disulphide isomerase; ERP5, endoplasmic reticulum protein; PKC: protein kinase C.

References

- Del Principe D, Mancuso G, Menichelli A, et al. Production of hydrogen peroxide in phagocytosing human platelets: an electron microscopic cytochemical demonstration. *Biol Cell* 1980; 38: 135–140.
- Finazzi-Agrò A, Menichelli A, Persiani M, et al. Hydrogen peroxide release from human blood platelets. *Biochim Biophys Acta* 1982; 718: 21–25.
- Bakdash N, Williams MS. Spatially distinct production of reactive oxygen species regulates platelet activation. *Free Radic Biol Med* 2008; 45: 158–166.
- Del Principe D, Mancuso G, Menichelli A, et al. Oxygen consumption in platelets of newborn infants before and after stimulation by thrombin. *Thromb Haemost* 1976; 35: 712–716.
- Krötzig F, Sohn HY, Pohl U. Reactive oxygen species: players in the platelet game. *Arterioscler Thromb Vasc Biol* 2004; 24: 1988–1996.
- Freedman JE. Oxidative Stress and Platelets. *Arterioscler Thromb Vasc Biol* 2008; 28: 11–16.
- Berridge MV, Tan AS. High-capacity redox control at the plasma membrane of mammalian cells: transmembrane, cell surface, and serum NADH-oxidases. *Antioxid Redox Signal* 2000; 2: 231–242.
- VanDuijn MM, Van der Zee J, Van den Broek PJ. The ascorbate-driven reduction of extracellular ascorbate free radical by the erythrocyte is an electrogenic process. *FEBS Lett* 2001; 491: 67–70.
- Arroyo A, Kagan VE, Tyurin VA, et al. NADH and NADPH-dependent reduction of coenzyme Q at the plasma membrane. *Antioxid Redox Signal* 2000; 2: 251–262.
- Ly JD, Lawen A. Transplasma membrane electron transport: enzymes involved and biological function. *Redox Rep* 2003; 8: 3–21.
- Geiszt M, Leto TL. The Nox family of NAD(P)H oxidases: host defense and beyond. *J Biol Chem* 2004; 279: 51715–51718.
- Cave AC, Brewer AC, Narayanapanicker A, et al. NADPH oxidases in cardiovascular health and disease. *Antioxid Redox Signal* 2006; 8: 691–728.
- O'Donnell VB, Smith GC, Jones OT. Involvement of phenyl radicals in iodonium inhibition of flavoenzymes. *Mol Pharmacol* 1994; 46: 778–785.
- Heumüller S, Wind S, Barbosa-Sicard E, et al. Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant. *Hypertension* 2008; 51: 211–217.
- Seno T, Inoue N, Gao D, et al. Involvement of NADH/NADPH oxidase in human platelet ROS production. *Thromb Res* 2001; 103: 399–409.
- Zielinski T, Wachowicz B, Saluk-Juszczak J, et al. The generation of superoxide anion in blood platelets in response to different forms of Proteus mirabilis lipopolysaccharide: effects of staurosporin, wortmannin, and indomethacin. *Thromb Res* 2001; 103: 149–155.
- White JG. Platelets are coverocytes, not phagocytes: uptake of bacteria involves channels of the open canalicular system. *Platelets* 2005; 16: 121–131.
- Krötzig F, Sohn HY, Gloe T, et al. NAD(P)H oxidase-dependent platelet superoxide anion release increases platelet recruitment. *Blood* 2002; 100: 917–924.
- Begonja AJ, Gambaryan S, Geiger J, et al. Platelet NAD(P)H-oxidase-generated ROS production regulates alphaIIb beta3-integrin activation independent of the NO/cGMP pathway. *Blood* 2005; 106: 2757–2760.
- Carnevale R, Pignatelli P, Lenti L, et al. LDL are oxidatively modified by platelets via GP91(phox) and accumulate in human monocytes. *FASEB J* 2007; 21: 927–934.
- Chlopicki S, Olszanecki R, Janiszewski M, et al. Functional role of NADPH oxidase in activation of platelets. *Antioxid Redox Signal* 2004; 6: 691–698.
- Peter AD, Morrè DJ, Morrè DM. A light-responsive and periodic NADH oxidase activity of the cell surface of tetrahymena and of human buffy coat cells. *Antioxid Redox Signal* 2000; 2: 289–300.
- Morrè DJ, Morrè DM. Cell surface NADH oxidases (ECTO-NOX proteins) with roles in cancer, cellular time-keeping, growth, aging and neurodegenerative diseases. *Free Radic Res* 2003; 37: 795–808.
- Morrè DJ, Chueh PJ, Lawler J, et al. The sulfonylurea-inhibited NADH oxidase activity of HeLa cell plasma membranes has properties of a protein disulfide-thiol oxidoreductase with protein disulfide-thiol interchange activity. *J Bioenerg Biomembr* 1998; 30: 477–487.
- Irani K, Pham Y, Coleman LD, et al. Priming of platelet $\alpha_{IIb}\beta_3$ by oxidants is associated with tyrosine phosphorylation of β_3 . *Arterioscler Thromb Vasc Biol* 1998; 18: 1698–1706.
- Lahav J, Jurk K, Hess O, et al. Sustained integrin ligation involves extracellular free sulfhydryls and enzymatically catalyzed disulfide exchange. *Blood* 2002; 100: 2472–2478.
- Essex DW, Li M, Feinman RD, et al. Platelet surface glutathione reductase-like activity. *Blood* 2004; 104: 1383–1385.
- Chen K, Lin Y, Detwiler TC. Protein disulfide isomerase activity is released by activated platelets. *Blood* 1992; 79: 2226–2228.
- Essex DW, Chen K, Swiatkowska M. Localization of protein disulfide isomerase to the external surface of the platelet plasma membrane. *Blood* 1995; 86: 2168–2173.
- Jordan PA, Stevens JM, Hubbard GP, et al. A role for the thiol isomerase protein ERP5 in platelet function. *Blood* 2005; 105: 1500–1507.
- Lahav J, Gofer-Dadosh N, Luboshitz J, et al. Protein disulfide isomerase mediates integrin-dependent adhesion. *FEBS Lett* 2000; 475: 89–92.
- Lahav J, Wijnen EM, Hess O, et al. Enzymatically catalyzed disulfide exchange is required for platelet adhesion to collagen via integrin alpha2beta1. *Blood* 2003; 102: 2085–2092.
- Manickam N, Sun X, Li M, et al. Protein disulfide isomerase in platelet function. *Br J Haematol* 2008; 140: 223–229.
- Janiszewski M, Lopes LR, Carmo AO, et al. Regulation of NAD(P)H oxidase by associated protein disulfide isomerase in vascular smooth muscle cells. *J Biol Chem* 2005; 280: 40813–40819.
- Lyles MM, Gilbert HF. Catalysis of the oxidative folding of ribonuclease A by protein disulfide isomerase: dependence of the rate on the composition of the redox buffer. *Biochemistry* 1991; 30: 613–619.
- Savini I, Catani MV, Arnone R, et al. Translational control of the ascorbic acid transporter SVCT2 in human platelets. *Free Radic Biol Med* 2007; 42: 608–616.
- Takajo Y, Ikeda H, Haramaki N, et al. Augmented oxidative stress of platelets in chronic smokers. Mechanisms of impaired platelet-derived nitric oxide bioactivity and augmented platelet aggregability. *J Am Coll Cardiol* 2001; 38: 1320–1327.
- McVeigh GE, Hamilton P, Wilson M, et al. Platelet nitric oxide and superoxide release during the development of nitrate tolerance: effect of supplemental ascorbate. *Circulation* 2002; 106: 208–213.
- Wells WW, Xu DP, Yang YF, et al. Mammalian thioltransferase (glutaredoxin) and protein disulfide isomerase have dehydroascorbate reductase activity. *J Biol Chem* 1990; 265: 15361–15364.
- Freedman JE, Farhat JH, Loscalzo J, et al. alpha-tocopherol inhibits aggregation of human platelets by a protein kinase C-dependent mechanism. *Circulation* 1996; 94: 2434–2440.
- Liu M, Wallmon A, Olsson-Mortlock C, et al. Mixed tocopherols inhibit platelet aggregation in humans: potential mechanisms. *Am J Clin Nutr* 2003; 77: 700–706.
- Taccone-Gallucci M, Lubrano R, Del Principe D, et al. Platelet lipid peroxidation in haemodialysis patients: effects of vitamin E supplementation. *Nephrol Dial Transplant* 1989; 4: 975–978.
- Blache D. Involvement of hydrogen and lipid peroxides in acute tobacco smoking-induced platelet hyperactivity. *Am J Physiol* 1995; 268: H679–H685.
- Del Principe D, Menichelli A, De Matteis W, et al. Hydrogen peroxide is an intermediate in the platelet activation cascade triggered by collagen, but not by thrombin. *Thromb Res* 1991; 62: 365–375.
- Rosado JA, Nunez AM, Lopez JJ, et al. Intracellular Ca²⁺ homeostasis and aggregation in platelets are impaired by ethanol through the generation of H₂O₂ and oxidation of sulfhydryl groups. *Arch Biochem Biophys* 2006; 452: 9–16.
- May AE, Seizer P, Gawaz M. Platelets: inflammatory firebugs of vascular walls. *Arterioscler Thromb Vasc Biol* 2008; 28: s5–s10.
- Cerletti C, Evangelista V, de Gaetano G. P-selectin-beta 2-integrin cross-talk: a molecular mechanism for polymorphonuclear leukocyte recruitment at the site of vascular damage. *Thromb Haemost* 1999; 82: 787–793.
- Williams LA, Martin-Padura I, Dejana E, et al. Identification and characterisation of human Junctional Adhesion Molecule (JAM). *Mol Immunol* 1999; 36: 1175–1188.
- Lösche W, Dressel M, Krause S, et al. Contact-induced modulation of neutrophil elastase secretion and phagocytic activity by platelets. *Blood Coagul Fibrinolysis* 1996; 7: 210–213.
- Li N, Hu H, Lindqvist M, et al. Platelet-leukocyte cross talk in whole blood. *Arterioscler Thromb Vasc Biol* 2000; 20: 2702–2708.
- Langer HF, Gawaz M. Platelet-vessel wall interactions in atherosclerotic disease. *Thromb Haemost* 2008; 99: 480–486.
- Del Principe D, Menichelli A, Di Giulio S, et al. Stimulated platelets release factor(s) affecting the in vitro response of human polymorphonuclear cells. *J Leukoc Biol* 1990; 48: 7–14.
- Jancinová V, Drábková K, Nosál' R, et al. Inhibition of FMLP-stimulated neutrophil chemiluminescence by blood platelets increased in the presence of the serotonin-liberating drug chloroquine. *Thromb Res* 2003; 109: 293–298.
- Del Principe D, Frega G, Palumbo G. Platelet-leukocyte interactions: platelets possess an antioxidant armamentarium. *Thromb Haemost* 2003; 90: 759–760.
- Janiszewski M, Carmo AO, Pedro MA, et al. Platelet-derived exosomes of septic individuals possess proapoptotic NAD(P)H oxidase activity: a novel vascular redox pathway. *Crit Care Med* 2004; 32: 818–825.
- Stokes KY, Russell JM, Jennings MH, et al. Platelet-associated NAD(P)H oxidase contributes to the thrombogenic phenotype induced by hypercholesterolemia. *Free Radic Biol Med* 2007; 43: 22–30.
- Rodríguez-Alonso J, Montañez R, Rodríguez-Caso L, et al. Homocysteine is a potent modulator of plasma membrane electron transport systems. *J Bioenerg Biomembr* 2008; 40: 45–51.
- Wang JS, Lin HY, Cheng ML, et al. Chronic intermittent hypoxia modulates eosinophil- and neutrophil-platelet aggregation and inflammatory cytokine secretion.

- tion caused by strenuous exercise in men. *J Appl Physiol* 2007; 103: 305–314.
59. Pittaluga M, Parisi P, Sabatini S, et al. Cellular and biochemical parameters of exercise-induced oxidative stress: relationship with training levels. *Free Radic Res* 2006; 40: 607–614.
60. Pignatelli P, Di Santo S, Buchetti B, et al. Polyphenols enhance platelet nitric oxide by inhibiting protein kinase C-dependent NADPH oxidase activation: effect on platelet recruitment. *FASEB* 2006; 20: 1082–1089.
61. Raghavan SA, Sharma P, Dikshit M. Role of ascorbic acid in the modulation of inhibition of platelet aggregation by polymorphonuclear leukocytes. *Thromb Res* 2003; 110: 117–126.
62. Mabile L, Bruckdorfer KR, Rice-Evans C. Moderate supplementation with natural alpha-tocopherol decreases platelet aggregation and low-density lipoprotein oxidation. *Atherosclerosis* 1999; 147: 177–185.
63. Murohara T, Ikeda H, Otsuka Y, et al. Inhibition of platelet adherence to mononuclear cells by alpha-tocopherol: role of P-selectin. *Circulation* 2004; 110: 141–148.
64. Wang JS, Lin HY, Cheng ML, et al. Chronic intermittent hypoxia modulates eosinophil- and neutrophil-platelet aggregation and inflammatory cytokine secretion caused by strenuous exercise in men. *J Appl Physiol* 2007; 103: 305–314.
65. Amer J, Zelig O, Fibach E. Oxidative status of red blood cells, neutrophils, and platelets in paroxysmal nocturnal hemoglobinuria. *Exp Hematol* 2008; 36: 369–377.
66. Amer J, Ghoti H, Rachmilewitz E, et al. Red blood cells, platelets and polymorphonuclear neutrophils of patients with sickle cell disease exhibit oxidative stress that can be ameliorated by antioxidants. *Br J Haematol* 2006; 132: 108–113.
67. Morr  DJ, Rodriguez-Aguilera JC, Navas P, et al. Redox modulation of the response of NADH oxidase activity of rat liver plasma membranes to cyclic AMP plus ATP. *Mol Cell Biochem* 1997; 173: 71–77.
68. Preissner KT. From Molecules to Medicine: New Horizons in Vascular Biology and Thrombosis (Part II). *Thromb Haemost* 2008; 99: 465.
69. Kannemeier C, Shibamiya A, Nakazawa F, et al. Extracellular RNA constitutes a natural procoagulant cofactor in blood coagulation. *PNAS* 2007; 104: 6388–6393.
70. Nakazawa F, Kannemeier C, Shibamiya A, et al. Extracellular RNA is a natural cofactor for the (auto-)activation of factor VII-activating protease (FSAP). *Biochem J* 2005; 385: 831–838.
71. Bandziulis RJ, Swanson MS, Dreyfuss G. RNA-binding proteins as developmental regulators. *Genes Dev* 1989; 3: 431–437.
72. Pagni M, Ioannidis V, Cerutti L, et al. MyHits: a new interactive resource for protein annotation and domain identification. *Nucleic Acids Res* 2004; 32 (Web Server issue): W332–335.