Platelets and Megakaryocytes Contain Functional Nuclear Factor-κB


Objective—To investigate the presence and role of NF-κB proteins in megakaryocytes and platelets. The nuclear factor-κB (NF-κB) transcription factor family is well known for its role in eliciting inflammation and promoting cell survival. We discovered that human megakaryocytes and platelets express the majority of NF-κB family members, including the regulatory inhibitor-κB (IκB) and Iκ kinase (IKK) molecules.

Methods and Results—Anucleate platelets exposed to NF-κB inhibitors demonstrated impaired fundamental functions involved in repairing vascular injury and thrombus formation. Specifically, NF-κB inhibition diminished lamellapodia formation, decreased clot retraction times, and reduced thrombus stability. Moreover, inhibition of IκB-α phosphorylation (BAY-11-7082) reverted fully spread platelets back to a spheroid morphology. Addition of recombinant IKK-β or IκB-α protein to BAY inhibitor–treated platelets partially restored platelet spreading in IκB-α inhibited platelets, and addition of active IKK-β increased endogenous IκB-α phosphorylation levels.

Conclusion—These novel findings support a crucial and nonclassical role for the NF-κB family in modulating platelet function and reveal that platelets are sensitive to NF-κB inhibitors. As NF-κB inhibitors are being developed as antiinflammatory and anticancer agents, they may have unintended effects on platelets. On the basis of these data, NF-κB is also identified as a new target to dampen unwanted platelet activation. (Arterioscler Thromb Vasc Biol, 2010; 30:591-598.)

Key Words: megakaryocyte ■ platelet ■ NF-κB inhibitor ■ cytoskeleton

The traditional view of platelet biology has dramatically changed as novel activities of these anucleate cells continue to be discovered. Historically, platelets were viewed solely as mediators of thrombosis and hemostasis, but clearly platelet function has expanded to include key roles in inflammation and immunity.1,2 Platelets are the product of the megakaryocyte. Remarkably, platelets have unexpected features, including the presence of a substantial and diverse transcriptome,3 spliceosomal components for mRNA processing,4 and signal-dependent translation, providing unique mechanisms to rapidly produce new proteins.5,6 Recent characterization of the de novo synthesis of platelet mRNAs7,8 demonstrates the sophistication of platelet signaling and function, underscoring their role as formidable players in regulating coagulant and inflammatory pathways.

Many novel and unexpected proteins have been identified in platelets, including transcription factors.9 We recently demonstrated that platelets contain the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) and its heterodimeric partner retinoid X receptor.9,10 PPARγ ligands attenuate platelet release of proinflammatory and procoagulant mediators, including soluble CD40L and thromboxane A29,10 suggesting a new role for platelets in inflammation.11

Our laboratory further demonstrated that platelet microparticle–released PPARγ was capable of transcellular biological activity.10 In addition, it was previously reported that platelets contain some nuclear factor-κB (NF-κB) family members.6,12-14

The NF-κB protein family regulates both activation and repression of gene transcription involved in complex signaling pathways, including apoptosis, immune responses, and inflammation (see Gilmore15 and references therein). Five Rel/NF-κB DNA-binding subunits (RelA [p65], RelB [p68], c-Rel, p50 [NF-κB1], and p52 [NF-κB2]) form both heterodimeric and homodimeric complexes and are found in the cytoplasm of most nucleated cells bound to IκB proteins that maintain these complexes in an inactive state. In response to specific stimuli, differentially formed NF-κB dimers and
inhibitory proteins are regulated by an I-κ kinase (IKK) complex via classical or alternative pathways to regulate a multitude of genes. Moreover, Rel/NF-κB activation can be blocked by nongenomic mechanisms, such as protein modification or physical association with other proteins. For example, binding of RelA (p65) by PPARγ prevents nuclear translocation and also expedites nuclear export of NF-κB. Although NF-κB regulation has been extensively studied, the prodigious number of physiological processes controlled by these proteins still provides many challenges to understanding the mechanisms involved in NF-κB signaling pathways. On the basis of our prior finding of the transcription factor PPARγ in platelets, we were interested in looking for other transcription factors. Identification of other transcription factors in platelets is important, as these proteins may have important nontranscriptional roles. Herein, we present our findings on the presence and activity of NF-κB family members.

Methods

Blood Collection and Preparation of Washed Platelets

Whole blood was obtained with institutional review board approval, following informed consent, from male and female donors 21 to 65 years of age who were NSAID free for 2 weeks before donation. Blood was collected by venipuncture, and platelets were washed and prepared for spreading as described. Platelet purity was determined to be >99%.

Western Blot for NF-κB Family Members

Western blot analysis of lysates (5 to 10 μg/lane) was performed using mouse monoclonal (p50 [E-10], p52 [C-5], and IKK-β [H-4]) or rabbit polyclonal (p65 [C-20], c-Rel, RelB [C-19]), IκB-α [C-21], IκB-β, IKK-γ, and Bcl-3) antibodies (Santa Cruz Biotechnology, Santa Cruz, Calif) and goat polyclonal glutathione S-transferase (GST) (GE Healthcare, Piscataway, NJ) followed by goat anti-rabbit, goat anti-mouse (Jackson ImmunoResearch Laboratory, West Grove, Pa) or donkey anti-goat (Rockland, Gilbertsville, Pa) horseradish peroxidase secondary antibody. Platelet activation was performed at 37°C for 30 minutes.

NF-κB Transcription Factor Assays

Measurements of p50 and RelA (p65) in platelet lysates were obtained using commercially available, highly specific and sensitive TransAM transcription factor assay kits (Active Motif, Carlsbad, Calif).

Inhibitor and Recombinant Protein Studies

Washed platelets (3×10^9 cells/mL) were incubated at 37°C for 30 minutes with vehicle (dimethyl sulfoxide <0.1%), BAY-11-7082 (1 μmol/L) (Biomol, Plymouth Meeting, Pa), or SC-514 (10 μmol/L) (Calbiochem, San Diego, Calif). Concentrations were predetermined by dose-response assays. Pellets were resuspended in Nonidet P-40 (Sigma) lysis buffer containing protease inhibitor cocktail (Sigma). Spread platelets posttreated with inhibitors were washed and incubated with inhibitors for up to 30 minutes at 37°C. Human recombinant IKK-β (Active Motif) or I-κB-α protein (Santa Cruz) was added to platelets in Tyrode’s buffer (Sigma) containing dimethyl sulfoxide (<0.1%), as a vehicle for uptake. To examine phosphorylation levels following recombinant IKK-β addition, platelets were lysed in buffer containing 25 mmol/L Tris, 150 mmol/L NaCl, 1% (octylphenox) polyethoxethanol (IGEPAL), 1% sodium deoxycholate, 0.1% SDS, 1 mmol/L sodium fluoride, 10 mmol/L sodium pyrophosphate, 1 mmol/L sodium orthovanadate, and protease inhibitor cocktail (Sigma).

Platelet Spreading

Platelet spreading was performed as described. Cells were stained with phalloidin–Alexa Fluor 488 (Invitrogen, Carlsbad, Calif). Platelet spreading was imaged using Nomarski differential interference contrast optics and SPOT RT software (New Hyde Park, NY) with an Olympus BX51 microscope (Melville, NY). Platelets for scanning electron microscopy were imaged using a Zeiss 40VP scanning electron microscope (Thornwood, NY).

Cell Line and Culture Conditions

Meg-01 megakaryocytic cells were purchased from the American Type Culture Collection (Rockville, Md) and cultured as previously described. 

Megakaryocyte Differentiation From Human Cord Blood–Derived CD34+ Cells

Human CD34+ cord blood cells were cultured as described and supplemented with 100 ng/mL recombinant human (rh) thrombopoietin. CD61-fluorescein isothiocyanate–labeled megakaryocytes were identified as described.

Clot Retraction Assay

Human platelet-rich plasma (PRP) was incubated with or without inhibitors (30 minutes at 37°C) followed by the addition of thrombin (1 U/mL). Clot formation and retraction were recorded using a Kodak ZB812 IS digital camera. Clot size was calculated as the ratio of the area of the retracted clot/area of the total PRP volume and recorded as a percentage.

Prothrombin, Thromboplastin, and Euglobulin Clot Lysis Times

Standard clinical laboratory clotting assays were performed to detect deficiencies in certain factors of the extrinsic (prothrombin time [PT]) or intrinsic (partial thromboplastin time [PTT]) pathways and fibrinolysis (euglobulin clot lysis time [ECLT]). The times reported are referenced to a standardized control.

Flow Experiments and Data Acquisition

Glass capillaries were coated with 100 μg/mL collagen (Chromolog) and blocked with 0.5% BSA. Perfusion was performed via a motorized multiple syringe pump (New Era Pump Systems), and images were obtained using an IX-81 motorized inverted microscope (Olympus America Inc.) coupled to a charge-coupled device camera (Hitachi), and connected to a TV monitor through a DVD recorder (Sony Electronics). IPLab deconvolution software (version 4.04, Scanalytics, Inc.) enabled automated data acquisition in all 3 dimensions. Whole blood samples were perfused for 8 minutes through capillaries at a shear rate of 500 per second (flow rate=368 μL/minute), followed by a PBS wash at the same shear rate for approximately 8 minutes, during which time images were photographed.

Statistics

Experiments were repeated from a minimum of 3 individuals except where stated. Results are shown as mean±standard error (SE). Data
IgG, and platelet lysates (10 μg/lane). PHM were activated with PMA (50 ng/mL) and harvested at 4 and 24 hours. Platelets were activated with thrombin (Thr) (0.8 U/mL) or collagen (Col) (10 μg/mL) or left untreated (UT).

were evaluated by 1-way or repeated measures ANOVA. A value <0.05 was considered statistically significant.

Results

Human Platelets and Megakaryocytes Express NF-κB Family Members

Human platelets, primary megakaryocytic (human cord blood–derived CD34+ cells), and Meg-01 cells, a cell line extensively used as a model of human megakaryocytes, were analyzed by Western blot using antibodies specific for NF-κB family members. For each cell type, bands were found corresponding to classic Rel/NF-κB and I-κB subunits (Figure 1). The protein levels of some family members were reduced following agonist activation, as was observed in platelets for RelA. In contrast, RelA, p50, p100, and p52 increased following activation of primary human megakaryocytic cells with a phorbol ester, phorbol 12-myristate 13-acetate (PMA). RelB was present in Meg-01 cells and induced in primary human megakaryocytic cells, but it was only weakly present in platelets. Agonist-stimulated platelets contained higher levels of I-κB-α and Bcl-3, but PMA activation did not substantially change levels of these proteins in primary human megakaryocytic cells. Platelets were previously shown to upregulate Bcl-3 protein on agonist stimulation. In primary human megakaryocytic cells, the levels of inhibitor kinases IKK-β and IKK-γ (NF-κB essential modulator) were altered by PMA activation, whereas there was no change in platelets in response to agonist stimulation. The doublet bands evidenced in the IKK-γ immunoblot (Figure 1, bottom panel) likely represent a phosphorylated form of IKK-γ.

Platelet-Derived RelA (p65) and p50 Proteins Retain DNA-Binding Activity

To detect whether platelet NF-κB can form complexes and bind to its DNA consensus, a TransAM NF-κB activity assay was used to measure protein binding to plate-bound NF-κB DNA oligonucleotides. The subsequent detection of bound proteins with NF-κB-specific antibodies demonstrated DNA-binding activity for RelA (p65) and p50 (Figure 2) and was confirmed by electromobility shift assay (data not shown).
demonstrates the cytoarchitectural changes in the inhibitor-dosed platelets.

Quantification of our data further supports the conclusion that lamella formation is abrogated in BAY-11-7082-treated platelets and reduced following SC-514 treatment (Figure 3D). The effects of SC-514 were even better appreciated when platelet surface area was examined (Figure 3E), demonstrating a significant reduction in the surface area covered compared with untreated platelets. A time course determined that platelet spreading did not recover in inhibitor-treated platelets after 90 minutes (not shown).

**BAY Inhibitor Treatment Converts Fully Spread Platelets Back to a Spherical Shape**

To better understand the involvement of NF-κB proteins in platelet shape change, fully spread platelets were treated with increasing doses of BAY inhibitor. Figure 4A demonstrates that following posttreatment with BAY inhibitor, large circumferential lamellipodia were retracted, and platelets returned to a convoluted spheroid shape. This effect was dose-dependent, as increasing concentrations of BAY produced greater numbers of rounded platelets. Quantification of this process is shown in Figure 4B.

**Recombinant Full-Length Human IKK-β or I-κB-α Partially Restores Lamellipodia Formation Following Inhibition by BAY-11-7082**

To determine whether the effects of BAY-11-7082 were dependent on NF-κB function, the selectivity of this inhibitor was measured. GST-tagged rhlIKK-β or rhl-κB-α was added to platelets pretreated with BAY inhibitor and spread on a fibrinogen. After thorough washing, adherent platelets were imaged or were lysed and removed. A Western blot revealed bands representing the exogenously added rhlIKK-β or rhl-κB-α in lysates treated with recombinant protein only (Figure 5A). An anti-GST antibody identified the same bands in the samples containing the recombinant proteins. All samples contained the endogenous IKK-β and I-κB-α proteins at their expected molecular masses (data not shown; the GST tag adds 27 kDa to the recombinant proteins). As shown by light microscopy for rhl-κB-α and graphically for IKK-β, addition of these recombinant proteins partially restored lamellipodia formation in BAY-treated platelets (Figure 5B and 5C). Untreated or vehicle-treated platelets were not significantly changed following addition of recombinant protein (data not shown). Furthermore, active rhlIKK-β was transferred to platelets to investigate the phosphorylation status of the IKK-β substrate I-κB-α. An antibody that recog-
nizes only the phosphorylated form of I-κB-α indicated that the level of phosphorylation was significantly increased only in platelets that received rhIKK-β. Moreover, fluorescently labeled rhIKK-β was internalized in BAY-treated platelets (data not shown).

Platelets Treated With NF-κB Inhibitors Aggregate Similarly to Untreated Platelets

To investigate whether SC-514 or BAY inhibitors dampen platelet aggregation, PRP was stimulated with ADP or thrombin, with or without inhibitor pretreatment and aggregation measured over time. Interestingly, aggregation was unaffected by either SC-514 or BAY inhibitors over a wide range of inhibitor concentrations (1 to 200 μmol/L) (data not shown).

BAY-11-7082 Significantly Hinders Clot Retraction

Clot retraction is a platelet-dependent process that results in aggregate contraction essential for thrombus consolidation and resistance to shear stress during wound healing. PRP was pretreated with increasing doses of BAY and SC-514 inhibitors followed by activation with thrombin to observe whether these inhibitors interfered with normal clotting. Significant retraction in untreated or vehicle-treated PRP occurred quickly (Figure 6A). The lower doses of BAY inhibitor (<25 μmol/L) were slightly slower and retracted to ≤20% by 45 minutes (data not shown). In contrast, retraction time was even slower for BAY50 and significantly protracted at higher doses of BAY inhibitor (Figure 6A). Clot retraction in SC-514-treated platelets was not as dramatic as observed in the BAY-treated samples, although it too was significantly delayed compared with controls (data not shown).

To test whether inhibitor effects were platelet-specific or whether other aspects of the clotting cascade were also affected by BAY-11-7082, we performed PT, PTT, and
Platelets Treated With BAY-11-7082

Attenuated Under Flow Conditions In Vitro in Platelets Treated With BAY-11-7082

To further investigate platelet adhesion and thrombus formation in a more physiologically relevant setting, a flow adhesion assay was used to study platelet function under shear conditions. As expected, untreated (data not shown) and vehicle-treated platelets formed stable, compact thrombi that remained invariant throughout the buffer wash (Figure 6B, VEH). In contrast, BAY-treated whole blood formed loose aggregates, and the thrombi that did form were highly unstable. Figure 6B tracks the release of 1 embolus in BAY-treated platelets to illustrate the dynamic process that occurred. In all BAY-treated samples, emboli and cells were continually flowing through the buffer. This effect was greatest at the highest concentration of BAY inhibitor and was reduced as inhibitor dosage decreased. By the end of the buffer wash, inhibitor-treated samples contained large areas that were reduced to a cell monolayer compared with controls (data not shown).

Discussion

Evidence is provided herein for a new pathway of platelet regulation by NF-κB. Our studies demonstrate that primary human megakaryocytic cells, the Meg-01 cell line, and platelets contain nearly all known NF-κB family members, including c-Rel, p105/p50, p100/p52, I-κ-Bs, and IKKs. Moreover, expression levels and modification of specific NF-κB proteins were dependent on cell type and sensitive to agonist stimulation. For example, RelA (p65) and RelB were highly expressed in unstimulated Meg-01 cells. In contrast, normal platelets expressed low levels of RelB, and human primary megakaryocytic cells required agonist stimulation to produce detectable amounts. We also observed activation-specific changes in the levels of some proteins (eg, RelA and p50) and phosphorylated protein (IKK-γ) in both megakaryocytes and platelets. mRNA transcripts for several NF-κB family members have been reported in human platelets, including I-κ-B-α.3,22,23 These observations support the concept that NF-κB proteins are important in platelet function.

Platelets are known to contain other transcription factors, such as PPARγ and its binding partner, retinoid X receptor.9,10,24 Both proteins regulate platelet function by non-genomic mechanisms, including retinoid X receptor interaction with Gq24 and PPARγ ligand dampening of platelet activation.9,10 In addition, PPARγ and retinoid X receptor are released in platelet microparticles and elicit a PPARγ ligand-dependent transcellular response in a monocytic cell line.10 Additional studies are in progress to determine whether NF-κB subunits, like PPARγ, are released during platelet activation.

A role for NF-κB in platelet physiology is also supported by data from the Bloodomics Consortium that identified a transcript in platelets encoding a novel gene, COMMD7 (copper metabolism gene MURR1 domain containing 7), that correlated with both platelet function and coronary artery disease risk. COMMD7 is a member of the COMM domain protein family involved in the negative regulation of NF-κB activity (A.H. Goodall and W.H. Ouwehand, unpublished data, 2009). A Western blot probed with an anti-COMMD7 antibody revealed the presence of the CommD7 protein in platelet lysates (data not shown).

In further support of NF-κB function in platelets, we demonstrate that inhibitors of NF-κB have profound effects on platelet signaling pathways involved in shape change and spreading, modulation of clot retraction, and thrombus stability under flow conditions. The Rho GTPase, Rac1, which is ubiquitously expressed in all mammalian cells, stimulates actin polymerization and lamellae formation in platelets.17 Here, we show that NF-κB inhibition attenuates lamellae formation, an essential function during vascular wound healing. Intriguingly, the Rho GTPase family was previously reported to be specifically involved in regulating NF-κB activity in nucleated cells, including a role in cytoskeletal organization of critical cellular functions.25,26 A significant finding herein was that BAY treatment of fully spread platelets reversed lamellipodia formation, reinstating platelets to a spherical morphology. These new data support a dynamic role for NF-κB in the regulation of platelet cytoskeletal architecture.

Our data also show that NF-κB inhibition significantly hampered clot retraction, although platelets were still able to aggregate as measured by aggregometry at low levels of shear. Our aggregation data are in contrast to the findings of Malaver et al, who showed that BAY-11-7082 inhibited platelet aggregation.13 These differences may be attributable to the concentration of the platelet agonist used in PRP (0.8 U/mL thrombin versus 0.05 U/mL thrombin11). We found by our investigative methods that thrombin addition stimulated clot formation in PRP, but platelets failed to form a tightly condensed clot in a dose-dependent manner. Furthermore, flow adhesion assays indicated that NF-κB-inhibited platelets failed to form stable thrombi on a collagen matrix at physiological levels of shear stress. Although platelets were recruited to the matrix, the aggregates that did form were less compact than untreated or vehicle-treated platelets, and emboli were copiously released. It was previously demonstrated that inhibition of signaling events that support platelet activation following integrin activation and platelet aggregation are necessary for clot retraction and for promoting thrombus growth and stability. Our data support a role for NF-κB inhibitors interfering with outside-in signaling via the fibrinogen-
binding integrin αIβ1. Initial fibrinogen binding mediates aggregation (inside-out signaling), which subsequently triggers outside-in responses that stabilize aggregates and initiates platelet spreading, vesicle secretion, and clot retraction. These observations support a central role for platelet NF-κB during vascular injury and suggest that dysregulation of NF-κB function may contribute to vascular disease processes.

Further investigation revealed that blood treated with NF-κB inhibitors coagulated normally, as evidenced by normal PT, PTT, and ECLT, indicating that inhibitor effects were platelet specific. Interestingly, Ono et al recently demonstrated a fibrin-independent platelet contractile mechanism essential for the initial stages of hemostasis and in promoting thrombus contraction. It will be interesting to discover whether NF-κB plays a role in this regulation.

Of particular importance to our hypothesis was the finding that inhibition of IKK-β activity was attenuated by the addition of active recombinant IKK-β protein. Our data show that the effects on platelet function following BAY inhibitor treatment were overcome by addition of active recombinant IKK-β protein. With that in mind, we also investigated a specific target of IKK-β phosphorylation, I-κB-α, and discovered that rhIKK-β addition increased I-κB-α phosphorylation levels. Moreover, addition of exogenous rhI-κB-α protein restored platelet spreading, supporting the idea that BAY-11-7082 is targeting NF-κB-driven mechanisms.

We very recently initiated studies to comprehensively determine where NF-κB proteins reside (eg, granules, cytoskeleton, adhesion contacts, mitochondria) and to identify the types of NF-κB protein interactions in both untreated and BAY inhibitor–treated platelets. Our immunofluorescent pilot data indicated that both IKK-β and I-κB-α are centrally located in the unspread platelet. Interestingly, in spreading platelets, IKK-β remains centralized, whereas I-κB-α begins to appear throughout the lamellipodial region (data not shown).

Although the underlying mechanism(s) of NF-κB function in platelets requires further analysis, our overall findings provide a strong rationale that NF-κB proteins have an important role in human platelet physiology. The expanding versatility of platelet regulation leads us to envision possible mechanisms. First, our data and those of others indicate that RelA and p50 retain the ability to bind DNA. Although platelets lack nuclear DNA, it is plausible that a novel mechanism of mitochondrial gene regulation may function in platelets. It has been reported that the NF-κB subunits RelA and p50, as well as I-κB-α, play an important role in regulating mitochondrial mRNAs in other cell types. Second, it is conceivable that NF-κB proteins regulate posttranscriptional gene expression in platelets by directly binding RNA in a signal-dependent manner. In fact, we have demonstrated a novel function for glucocorticoid receptor whereby this transcription factor binds directly to monocye chemoattractant protein-1 mRNA, decreasing its stability in arterial smooth muscle cells.

Our final point is that platelet Rel/NF-κB/I-κB proteins could elicit effects through direct protein-protein interactions. For example, PPARγ exerts some of its antiinflammatory effects by directly binding nuclear-localized NF-κB complexes to facilitate nuclear export and sequestration of NF-κB in the cytoplasm. Also consistent with our data is the fact that Bcl-3, an I-κB-α family member, specifically interacts with an Src-family tyrosine kinase, Fyn, and is involved in cytoskeletal regulation and inhibition of clot retraction in platelets. Interestingly, Fyn is an upstream regulator of the Rho family of GTPases and associates with αIβ1 to regulate platelet adhesion and spreading.

Our data support the idea that transcription factors such as NF-κB have important roles in platelet physiology. It is well documented that dysregulation of NF-κB function contributes to the development of many human diseases, including chronic inflammatory diseases and cancer. Approaches to specifically inhibit the NF-κB pathway are under active development as therapeutic interventions. Thus, it is important to now recognize that platelets and megakaryocytes contain nearly all NF-κB family members and that these cells could be affected by NF-κB inhibitory drugs. This new understanding of an NF-κB-platelet connection reveals a novel target, namely NF-κB, for dampening platelet activation. The impact of influencing NF-κB function in platelets and megakaryocytes could lead to a host of new therapeutics for platelets.

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Disclosures
None.

References
synthesis of major platelet proteins in human blood platelets. Eur J

Zimmerman GA. Signal-dependent translation of a regulatory protein,
Bcl-3, in activated human platelets. Proc Natl Acad Sci U S A. 1998;95:
5556–5561.

7. Campa M, Frigerio M, Toschi V, Brambilla M, Rossi F, Cottell DC,
Madera P, Parolari A, Bonzi R, De Vincenti O, Tremoli E. Platelet
activation induces cell-surface immunoreactive tissue factor expression,
which is modulated differently by antplatelet drugs. Arterioscler Thromb

8. Lindemann S, Tolley ND, Dixon DA, McIntyre TM, Prescott SM, Zim-
merman GA, Weyrich AS. Activated platelets mediate inflammatory
485–490.

Human bone marrow megakaryocytes and platelets express
PPAR-gamma, and PPAR-gamma agonists blunt platelet release of

Francis CW, Taubman MB, Phipps RP. Peroxisome proliferator-activated
receptor gamma and retinoid X receptor transcription factors are released
from activated human platelets and shed in microparticles. Thromb Haemost.

11. Wagner DD, Burger PC. Platelets in inflammation and thrombosis. Arterioscler


13. Maltby A, Romaniuk MA, D’Atri LP, Pozner RG, Negrotto S, Benzadon
G, Krasilnikov A, Blesch N, Leamy A, Malyutina S, Benigni A, Gingras A,
Roberts MS. 15-deoxy-Delta12,14-PGJ2 enhances platelet production from

PPAR-gamma and RelA. Inflammation by regulating nuclear-cytoplasmic shuttling of

15. Montaner S, Perona R, Saniger L, Lacal JC. Multiple signalling pathways
lead to the activation of the nuclear factor kappaB by the Rho family of

Y, Yamagishi K, Huaraca M, Sklar J, Izumo S. Nongenomic signaling of the retinoid
X receptor through binding and inhibiting Gq in human platelets. Blood.

SM, Jackson SP. Identification of a fibrin-independent platelet contractile
mechanism regulating primary hemostasis and thrombus growth. Blood.
2008;112:90–99.

B inhibitory subunit, interacts with ANT, the mitochondrial ATP/ADP translocator.

19. Cogswell PC, Kashatus DF, Keifer JA, Guttridge DC, Reuther JY,
Bristow C, Roy S, Nicholson DW, Baldwin AS Jr. NF-kappa B and I
kappa B alpha are found in the mitochondria: evidence for regulation of
mitochondrial gene expression by NF-kappa B. J Biol Chem. 2003;278:
2963–2968.

20. Whelan L, Liu B, Bllaxa JC, Taubman MB. A novel role for the
glucocorticoid receptor in the regulation of monocyte chemotactant

TW, Baeuerle PA, Verma IM. The proto-oncogene bcl-3 encodes an I kappa

22. Samayavardenha LA, Kapur R, Craig AW, Bhatia V, Luchette FA.
Involvement of P60c-src kinase with
IIb platelet spreading. J Cell
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