Platelets are small anucleate blood cells derived from megakaryocytes. In addition to their pivotal roles in hemostasis, platelets are the smallest, yet most abundant, immune cells and regulate inflammation, immunity, and disease progression. Although platelets lack DNA, and thus no functional transcriptional activities, they are nonetheless rich sources of RNAs, possess an intact spliceosome, and are thus capable of synthesizing proteins. Previously, it was thought that platelet RNAs and translational machinery were remnants from the megakaryocyte. We now know that the initial description of platelets as “cellular fragments” is an antiquated notion, as mounting evidence suggests otherwise. Therefore, it is reasonable to hypothesize that platelet transcription factors are not vestigial remnants from megakaryocytes, but have important, if only partly understood functions. Proteins play multiple cellular roles to minimize energy expenditure for maximum cellular function; thus, the same can be expected for transcription factors. In fact, numerous transcription factors have non-genomic roles, both in platelets and in nucleated cells. Our lab and others have discovered the presence and non-genomic roles of transcription factors in platelets, such as the nuclear factor kappa β (NFκB) family of proteins and peroxisome proliferator-activated receptor gamma (PPARγ). In addition to numerous roles in regulating platelet activation, functional transcription factors can be transferred to vascular and immune cells through platelet microparticles. This method of transcellular delivery of key immune molecules may be a vital mechanism by which platelet transcription factors regulate inflammation and immunity. At the very least, platelets are an ideal model cell to dissect out the non-genomic roles of transcription factors in nucleated cells. There is abundant evidence to suggest that transcription factors in platelets play key roles in regulating inflammatory and hemostatic functions.

**Keywords:** platelets, microparticles, transcription factors, NF-kappa B, PPAR gamma, non-genomic, PPAR alpha, steroid receptors

## INTRODUCTION

Platelets are central players in hemostasis and inflammation, contributing to numerous pathophysiologic conditions (1). They are unique from the majority of mammalian cells apart from red blood cells in that they lack a nucleus, and thus have previously been discounted as “cellular fragments.” This antiquated notion has been refuted many times over as platelets are now emerging as cellular mediators of cancer cell metastasis, atherosclerosis, type II diabetes, and even mediate adaptive immune responses (2, 3).

Platelets are metabolically active cells that contain numerous functional organelles, such as endoplasmic reticulum, Golgi apparatus, mitochondrion, and granules that can be released upon activation. Although their lack of a nucleus prevents *de novo* transcription, they can be activated very rapidly to release copious amounts of biological mediators within seconds to minutes of stimulation.

The idea that platelets contain transcription factors is a relatively new concept and has led to the discovery of a large number of transcription factors in platelets (Table 1). This review will discuss the newly described roles of transcription factors in platelets, in addition to proposing uninvestigated potential roles of transcription factors in platelets, as extrapolated from findings in nucleated cells (Table 2).

### NUCLEAR FACTOR KAPPA β

In the Immunology field, nuclear factor kappa β (NFκB) is the most widely recognized transcription factor for its quintessential roles in regulating inflammation and immune responses. Almost any immunologist could rattle off key parts of its signaling pathways in response to toll-like receptor (TLR) signaling, influenza infection, or in cytokine production. Although we are quick to...
identify its essential roles in regulating transcription of inflammatory genes, the non-genomic roles of NFκB are often overlooked. In all fairness, the concept that NFκB has non-genomic roles in nucleated and non-nucleated cells is a relatively new area of study that is still in its early stages (4). NFκB signaling molecules regulate several different stages of the inflammatory response, without ever entering the nucleus. For example, the NFκB regulatory protein, IkappaB kinase β (IKKβ), can alter the function of numerous proteins via phosphorylation in addition to regulating signaling through direct interactions with cellular effector molecules (5).
though the phosphorylation of SNAP-23. Consequently, platelets from IKKβ knockout mice have a reduced ability to release alpha, dense, and lysosomal granules upon thrombin stimulation by approximately 30%. Likewise, treatment of mouse or human platelets with an inhibitor of IKKβ similarly reduced granule secretion upon stimulation. These data suggest that the NFκB signaling molecule, IKKβ, plays an important regulatory role in platelet activation by transducing critical stimulatory signals.

In general, genetic ablation or pharmacological inhibition of IKKβ in mice results in a hyporesponsive phenotype to agonist-induced platelet activation. Inhibition of IKKβ in mice prolonged thrombus formation and increased bleeding times (14). Additionally, a second IKKβ inhibitor, IKK inhibitor VII, recapitulated several of the aforementioned findings in both human and mouse platelets, including preventing P-selectin expression and dampening aggregation (15). Of note, Gambaryan et al. found that IKK inhibitor VII potentiated collagen and thrombin-induced aggregation, rather than having an inhibitory effect (8). They proposed a model in which thrombin and collagen induce a negative feedback loop in platelets that inhibits platelet function through NFκB. Although the work delves into the complex agonist-induced signaling pathways of NFκB in platelets, their data showing potentiation of platelet activation by IKK inhibitor VII is less clear-cut. Treatment of human platelets with IKK inhibitor VII enhanced very low dose (0.001 U/mL) thrombin-induced PAC1 binding by approximately 15%. Additionally, IKK inhibitor VII treatment only mildly potentiated collagen (10 µg/mL) and thrombin (0.01 U/mL)-induced platelet aggregation, with a slight left shift in the aggregation traces compared to control. However, the maximum amplitude of aggregation was indistinguishable between IKK inhibitor VII treatment and control. Although the data demonstrating NFκB signaling post-activation are intriguing, the current consensus is that NFκB primarily plays an important role in positively regulating platelet activation.

As much of the investigations into the non-genomic roles of the transcription factor, NFκB, has involved manipulation of its upstream regulatory kinase, IKK, careful consideration must be taken into account when interpreting the findings of these studies. IKK acts as a kinase that plays a crucial role in regulating NFκB activation, but can also phosphorylate other proteins that may play regulatory roles in platelet activation (5). Thus, inhibition of IKK in platelets may dampen platelet function in a non-canonical fashion, independent of NFκB. On the other hand, many studies have observed changes in p65 phosphorylation in platelets, although the effects of direct inhibition or deletion of p65 has not been investigated to date. It is likely that IKK activation in platelets results in NFκB-dependent and -independent regulation of platelet function. This is supported by the data showing that IKK phosphorylates SNAP-23 (14), leading to granule secretion, but can also activate p65, which can regulate protein kinase A (PKA) (8).

NFκB IN PLATELETS DURING INFLAMMATION

Nuclear factor kappa B in nucleated cells is known to play a crucial role in inflammatory diseases, although its functions in platelets during inflammation are still under active investigation. In one study, IKKβ deficiency increased neointimal formation in low-density lipoprotein receptor (LDLR) knockout mice and exhibited increased leukocyte adherence to the vessel walls after injury (16). Upon further investigation, IKKβ-deficient platelets were unable to shed GPIbα in response to thrombin stimulation. Interestingly, GPIbα shedding in response to ADP or collagen was not affected, suggesting that IKKβ is uniquely involved in thrombin-induced GPIbα shedding. These data are intriguing in light of the fact that many studies evaluating the role of IKKβ in platelets focused on thrombin-induced activation and signaling. However, collagen and ADP-induced aggregation and granule secretion were also dampened by IKKβ deficiency or pharmacological inhibition. Furthermore, no differences in GPVI, GPIX, or αIIbβ3 shedding were found by loss of IKKβ in mouse platelets. These data suggest that although IKKβ plays an important role in the activation of platelets, it may also induce an inhibitory feedback loop, as proposed by Gambaryan et al., perhaps through shedding of GPIbα (8). Sustained levels of GP Ibα on the platelet surface can enhance platelet–leukocyte interactions, and thus, may exacerbate certain conditions.

Platelets are also known to respond to various immunologic stimuli, such as TLR ligands (17). In nucleated cells, bacterial lipopolysaccharide (LPS) signaling through TLR4 is largely through NFκB, leading to the production of proinflammatory cytokines and chemokines. We have recently shown that platelets can discriminate between different isoforms of LPS (18). This suggests that platelets are capable of specifically sensing and responding to various bacterial products. Thus, it is possible that LPS signaling in platelets involves differential NFκB activation, although this has not been investigated in platelets to date.

**SIGNALING MECHANISM OF NFκB IN PLATELETS**

Thrombin activates the NFκB signaling cascade in platelets, although the complete pathway has not yet been elucidated. Several lines of evidence suggest that p38 mitogen-activated protein kinase (MAPK) signaling is upstream of NFκB in platelets, while the extracellular signal-regulated kinase (ERK) pathway is downstream of NFκB activation (19, 20). In human platelets, inhibition of MAPK prevented collagen-induced IKKβ and p65 phosphorylation, while treatment with an ERK inhibitor had no effect. Furthermore, collagen-induced ERK phosphorylation was prevented by pretreatment with an MAPK inhibitor or the NFκB inhibitor BAY, suggesting that NFκB signaling regulates ERK activation. ERK activates phospholipase A2 (PLA2), which releases arachidonic acid, leading to platelet aggregation and mediator release. This explains why arachidonic acid-induced platelet aggregation is unaffected by NFκB inhibitors, while aggregation in response to other agonists is dampened. Interestingly, one study suggests that activation of NFκB by CD40L signaling may be independent of p38 MAPK, but may instead involve TRAF2 activation of IKKβ (15). Additionally, protease-activated receptor 4 (PAR4) stimulation led to ceramide production by sphingomyelin phosphodiesterase (Smase), which in turn activated MAPK, while PAR1 signaling was independent of ceramide (12). This is consistent with the finding that exogenous treatment of platelets with ceramide leads to in vitro activation and enhances thrombosis in vivo. PAR1
and PAR4 are the thrombin receptors on human platelets, with PAR1 having a lower threshold for activation by thrombin than PAR4 (21). PAR1 activation typically induces a rapid, but transient spike in calcium, while PAR4 activation involves a more sustained response, suggesting overlapping, but distinct roles for these receptors (22). These data reveal a novel and distinct signaling pathway for PAR1 and PAR4 receptors, although both converge on NFκB signaling.

Taken together, these data present compelling evidence that NFκB plays an important, albeit complex, role in platelet activation (Figure 1). The data support a model whereby platelet activation through various receptors leads to phosphorylation and activation of IKKβ, release of p65, and subsequent platelet aggregation and granule release. This model, however, is not mutually exclusive of the idea that NFκB may induce a negative inhibitory feedback loop in platelets (8). After dissociation of p65 from its inhibitory complex, PKA is free to induce vasodilator-stimulated phosphoprotein (VASP) phosphorylation, which mediates platelet inhibitory signaling. This may represent a mechanism to fine tune platelet activation after thrombin stimulation. In fact, PIP3 can induce VASP phosphorylation, leading to inhibitory signaling, in addition to activating IKK through protein kinase B, also known as Akt. Thrombin signaling also appears to be unique in that it stimulates GPIb shedding through NFκB and ADAM17, unlike ADP or collagen-induced activation (16). ADAM17 is a sheddase that is critical for platelet surface receptor shedding and can be activated by p38 MAPK (23). Unlike pharmacological inhibition of NFκB in human platelets, IKK-deficient mouse platelets are unable to phosphorylate p38 MAPK after thrombin stimulation (16). This raises the question as to whether defective GPIb shedding in IKKβ-deficient mouse platelets is an artifact of interspecies variability, differences in inhibition versus complete lack of IKKβ, or merely a technical timing issue. Regardless, it will be necessary to investigate the role of ADAM17 and GPIb shedding in human platelet NFκB signaling.

**NON-GENOMIC FUNCTIONS OF NFκB: LESSONS FROM NUCLEATED CELLS**

Nuclear factor kappa B is a versatile family of proteins capable of performing multiple functions in nucleated cells apart from constitutive transcriptional activation. NFκB-mediated non-genomic functions include regulation of Immediate Early Response (IER) genes, activation of the extracellular signal-regulated kinase (ERK) pathway, modulation of cytokine secretion, and control of cell death and survival. These functions are mediated through the rapid translocation of NFκB from the cytoplasm to the nucleus, followed by recognition of specific DNA sequences and regulation of gene expression. Additionally, NFκB has been implicated in the regulation of cell migration, invasion, and angiogenesis. Moreover, NFκB plays a crucial role in the regulation of innate and adaptive immune responses, influencing the development and function of immune cells. The non-genomic functions of NFκB are critical in maintaining cellular homeostasis and are involved in the response to various stimuli, including inflammation, stress, and infection.
from acting as a transcription factor (5). Evaluating these identified non-genomic roles in nucleated cells is likely to translate to important regulatory functions in platelets. Although roles for IKKβ in platelets have already been identified, studies from nucleated cells suggest that there may be more. A strong contender is the role of IKKβ in platelet spreading, as inhibition of IKKβ in human platelets leads to a spreading defect. In epithelial cells and B-lymphocytes, IKKβ-mediated phosphorylation of Dok1 inhibits ERK activation, leading to increased cell motility (24). Platelets are known to transiently activate ERK, which is important for alpha granule release. However, outside-in integrin signaling through the fibrinogen receptor, as is likely to occur during platelet spreading, inhibits ERK signaling (25). Thus, inhibiting IKKβ may impair platelet spreading through a similar Dok1-mediated mechanism. Proteomic data have identified the presence of Dok1 in human platelets, although its function has yet to be studied in this context (26).

The NFκB protein p65 and IκBα, are found in the mitochondria and appear to be differentially regulated compared to their cytoplasmic counterparts (27). Interestingly, canonical NFκB stimulatory signals had no effect on the expression or phosphorylation of mitochondria-localized p65 (28). Conversely, stimulation of liver or Jurkat cells with tumor necrosis factor α (TNFα) or FAS ligand, respectively, led to non-proteasomal degradation of IκBα and induction of apoptosis. IKKβ, on the other hand, was not localized to the mitochondria. To date, most studies of NFκB in platelets have involved the use of IKKβ inhibitors and IKKβ knock-out mice. Investigations in platelets centering on the role of IκBα and p65, in lieu of IKKβ, may reveal novel apoptotic regulatory elements in platelets.

A second intriguing possibility is the role of 14–3–3β in regulating mRNA stability in platelets. 14–3–3β is a highly conserved protein that binds AU-rich elements (ARE) on mRNA, leading to destabilization of mRNA (29). Phosphorylation of 14–3–3β by IKK results in the release of a 14–3–3β-triaretropin complex from the mRNA, thus preventing its destabilizing effects (30). This is interesting in light of the fact that platelets contain mRNA, which can be translated upon stimulation (31). Furthermore, 14–3–3 proteins can interact with p65 and IκBα to facilitate the export of p65 from the nucleus. As proteomic data show that human platelets express 14–3–3β (32), it is possible that it plays an important role in regulating mRNA stability or NFκB signaling in the anucleate platelet.

**PPARγ LIGANDS ARE CARDIOPROTECTIVE**

Thiazolidines (TZDs) are a class of oral antidiabetic drugs that exert their insulin-sensitizing actions through activation of PPARγ. In addition to regulating glucose homeostasis, TZDs, such as rosiglitazone, pioglitazone, and troglitazone, have potent anti-inflammatory properties (35). Type II diabetics taking TZDs have improved glucose metabolism, decreased markers of inflammation, and improved cardiovascular health. Much of this can be attributed to the beneficial actions of TZDs on vascular cells and both direct and indirect actions on circulating platelets. Platelets from type II diabetics exhibit a more activated phenotype and are hyper-responsive to agonist (36, 37). These changes include increased platelet number and mean platelet volume (MPV), which may indicate alterations in megakaryocyte function or increased platelet turnover (38). Additionally, platelets from type II diabetics have enhanced surface expression of the collagen receptor (GPVI) and the fibrinogen receptor (uPARβ3), leading to increased adhesiveness and aggregation (39). Plasma from type II diabetics contains higher levels of the platelet-derived inflammatory mediators, soluble CD40L (sCD40L), soluble P-selectin (s-P-selectin), and C-reactive protein (40, 41). Dysregulated platelet function in type II diabetics is likely due to both inherent changes in the platelet and decreased prostaglandin I (PGI) and nitric oxide (NO) production from endothelial cells, which exert potent anti-platelet effects.

Numerous studies have demonstrated that TZDs beneficially regulate cardiovascular function in type II diabetics. They have consistently been shown to reduce the elevated levels of plasminogen activator inhibitor 1 (PAI-1) (42–44). PAI-1 rapidly binds and inactivates tissue plasminogen activator, thus preventing fibrinolysis and increasing thrombotic risk. Additionally, TZDs reduce markers of cardiovascular disease, which are typically elevated in type II diabetics, including C-reactive protein, serum amyloid A, fibrinogen, and matrix metalloproteinase 9 (45–49). Many of these beneficial changes can be attributed to improved glucose metabolism and restored vascular and endothelial homeostasis, although similar results can be seen in non-diabetic patients (50).

**PPARγ LIGANDS IMPROVE PLATELET FUNCTION IN TYPE II DIABETIC**

Consistent with their ability to reduce thrombotic risk, TZDs reduce markers of platelet activation. Rosiglitazone monotherapy decreased plasma sCD40L and P-selectin levels (51, 52). These data suggest that TZDs can directly or indirectly reduce platelet activation in type II diabetics. Supporting these findings, Khanolkar et al. demonstrated that patients receiving rosiglitazone and metformin had a significantly greater reduction in platelet aggregation compared to patients receiving gliclazide and metformin (53). Similarly, a second study found that pioglitazone in combination with metformin improved platelet function to a greater degree than glimepiride and metformin (49). A major unanswered question from these studies is whether TZDs improve cardiovascular health secondary to improved lipid metabolism or due to the anti-inflammatory actions of PPARγ stimulation. For example, macrophages, which play a key role in atherosclerosis, efflux cholesterol and are less inflammatory in response to PPARγ activation. In an attempt to investigate the insulin-sensitizing independent
effects of TZDs, non-diabetic patients with coronary artery disease were treated with TZDs for 12 weeks. In this setting, TZDs reduced the inflammatory markers, C-reactive protein, tumor necrosis factor α (TNFα), and interleukin-6 (IL-6) (50, 54). Additionally, circulating platelet activity was dampened, as evidenced by fewer P-selectin positive platelets, suggesting an important role for the anti-inflammatory actions of PPARγ.

**PPARγ Ligands Dampen Platelet Function from Healthy Donors**

Due to the global effects of TZDs, it is difficult to tease out indirect actions from potential direct effects of TZDs on platelet function. However, many recent studies have aimed to elucidate the direct effects of TZDs on platelet function. Our lab was the first to show that human platelets and megakaryocytes express functional PPARγ (55). The PPARγ ligands 15d-PGJ2 and rosiglitazone potently inhibit thrombin-induced CD40L surface expression and thromboxane B2 (TXB2) production and dampen ADP-induced aggregation (56). Other studies have confirmed these findings and further demonstrated that rosiglitazone and 15d-PGJ2 inhibited collagen-induced aggregation and prevented P-selectin exposure in vitro and in vivo (54, 57). Utilizing the specific PPARγ antagonist, GW9662, these effects were partially mediated through PPARγ in platelets from healthy donors (58). Moreover, pioglitazone was protective in a mouse model of thrombosis (57, 59). Similarly, using platelets from type II diabetics, which are hyper-responsive to agonist, rosiglitazone reduced aggregation (56). Other studies have confirmed these findings and further demonstrated that rosiglitazone and 15d-PGJ2 inhibited collagen-induced aggregation and prevented P-selectin exposure in vitro and in vivo (54, 57). Utilizing the specific PPARγ antagonist, GW9662, these effects were partially mediated through PPARγ in platelets from healthy donors (58). Moreover, pioglitazone was protective in a mouse model of thrombosis (57, 59).

Interestingly, PPAR-independent pathways are evident upon treatment with some ligands. 15d-PGJ2 is an electrophilic compound that is known to form adducts with other cellular proteins, and could explain some of the PPAR-independent effects (61). Most interestingly, the mechanism of troglitazone differs from that of the structurally similar TZD, pioglitazone, in platelets. Although troglitazone and pioglitazone decreased platelet activation in vitro, only troglitazone directly inhibited platelet aggregation in vitro (62). However, in this study, only 1 µM of each TZD was examined for their effects on platelet function. In some cell systems, troglitazone is more potent than pioglitazone, despite having a higher EC50 for binding PPARγ and this may also be the case in platelets (63). It is possible that higher concentrations of pioglitazone would exhibit similar effects as troglitazone. Another possibility is that there may be PPAR-independent effects or differential signaling of PPARγ in human platelets. Clinical data points to some PPAR-independent actions of TZDs as pioglitazone has been shown to decrease the risk of myocardial infarction and stroke in type II diabetics, while rosiglitazone had no effect and may actually increase the relative risk (64).

**Signaling Mechanism of PPARγ in Platelets**

Differential signaling of PPARγ is not an unprecedented finding, as PPARγ is known to recruit various co-activators after stimulation with different ligands. Although most TZDs bind identical binding pockets in PPARγ, their biological profiles are distinct (65, 66). This is in part due to differential recruitment of co-activators, but also possibly due to variations in availability of cofactors. In cell-based systems, PPARγ ligands can act as partial agonists in some cell types and full agonists in others (67). Additionally, different PPARγ ligands can recruit different co-activators in the same cell type, leading to different outcomes (68–70). These differences likely explain many of the adverse effects observed with some TZDs in clinical trials. Although still poorly understood, differential binding and recruitment of cofactors may explain the complex and sometimes contradictory actions of PPARγ in platelets, although no studies have evaluated this role of PPARγ in platelets to date.

Although a transcription factor, PPARγ has been shown to serve many other important non-genomic roles in platelets and nucleated cells (Figure 2). Specifically in platelets, collagen stimulation results in PPARγ recruitment to the GPVI signalsome and interacts with the adapter molecule, Syk (71). As a result, linker of activated T cells (LAT) is recruited and forms a complex with Syk and PPARγ. In this setting, PPARγ appears to be necessary for enhancement of GPVI-mediated activation as treatment with PPARγ antagonists only partially blocks phosphorylation of LAT and the downstream targets phospholipase Cγ (PLCγ), PI3K, and Akt. Interestingly, several other groups have demonstrated that cytosolic platelet PPARγ is released into the supernatants and in platelet-derived microparticles rapidly upon activation, leaving very low levels of PPARγ in the activated platelets (34, 72). This may suggest that early signaling...
roles of PPARγ include recruitment to the LAT/Syk signaling complex, which results in its packaging and export into microparticles, possibly due to its proximity to the cell membrane. Alternatively, there may be different pools of PPARγ that could have different subcellular localization patterns. It is not known whether platelets contain an unknown endogenous PPARγ ligand that plays a role in this signaling pathway. However, exogenous treatment of activated platelets with PPARγ ligands inhibits its interaction with the LAT/Syk signaling complex and reduces the amplitude of the signal, thus reducing aggregation and mediator release. Similarly, PPARγ ligands also blunt release of PPARγ into the supernatants and platelet-derived microparticles (34).

Separate studies have suggested that PPARγ interacts with ERK and p38 MAPK, which are common downstream mediators of platelet activation, leading to granule secretion (73). Stimulation of human platelets with collagen led to phosphorylation of PPARγ and its subsequent association with p-ERK and p38 MAPK. Treatment with PPARγ ligands prevented these interactions, reducing granule release. Similarly, PPARγ has been shown to interact with PKCα in platelets in response to PPAR agonists, consequently reducing the activation of PKCα (74). Although complex, there is sufficient evidence to suggest that PPARγ plays an important role in platelet signaling. PPARγ knockout mice are embryonic lethal; thus, a megakaryocyte and platelet-specific PPARγ is sufficient evidence to suggest that PPARγ plays an important role in platelet production from megakaryocytes and could potentially play an important role in inhibiting platelet activation.

**PPARα**

PPARα is a major regulator of fatty acid homeostasis and inflammation. It is highly expressed in brown adipose tissue, liver, kidney, heart, and skeletal muscle (75). PPARα agonists are used as a treatment for elevated plasma lipid levels and for their ability to increase the uptake of fatty acids and improve the high-density lipoprotein (HDL) to low-density lipoprotein (LDL) ratio. Little is known about the function of PPARα in regulating hemostasis, but its presence was recently discovered in platelets. Statins and fibrates are PPARα agonists and are widely prescribed for the prevention of coronary artery disease and atherosclerosis, thus reducing the risk of thrombotic events (74). In addition to their effects on lipid metabolism, statins and fibrates have been shown to activate PPARα (79). Specifically, fenofibrate decreased agonist-induced platelet activation and increased bleeding times in mice (74). Fenofibrate’s ability to inhibit platelet aggregation was abolished in the presence of a specific PPARα antagonist. Additionally, bleeding times in PPARα knockout mice were not affected by treatment with fenofibrate, unlike control mice. Interestingly, the baseline bleeding times in PPARα knockout mice were longer than control mice, suggesting an additional role for PPARα in maintaining hemostasis. Due to the usage of PPARα global knockout mice, it is unclear whether this difference was due to platelet-specific or multivariate effects. Although the mechanisms of action of PPARα in platelets have not been thoroughly investigated, some evidence suggests that it may regulate PKCα activity, similar to PPARγ and PPARβ/δ (80).

**NON-GENOMIC FUNCTIONS OF PPARs: LESSONS FROM NUCLEATED CELLS**

The wide use of PPAR ligands in the clinic has led to the discovery of numerous pleotropic effects of these compounds. These include PPAR-dependent, non-genomic actions in addition to PPAR-independent signaling of ligands (81, 82). The PPAR-independent signaling mechanisms of PPAR ligands will not be discussed here, but these effects must be kept in mind when interpreting data investigating the effects of PPAR ligands on platelet function. In fact, these likely play an important role in regulating
platelet function, perhaps through affecting mitochondrial activity, as many effects of PPAR ligands in platelets cannot be reversed using PPAR antagonists. Additionally, many PPAR ligands, such as unsaturated fatty acids, can cross-react with all PPAR isoforms at higher concentrations, further complicating the interpretation of these findings (83). It may be possible to generate useful hypotheses about how PPARs signal in platelets based upon similar studies in nucleated cells.

**PPARγ–PKCα Crosstalk**

In human epithelial colorectal adenocarcinoma cells, PPARγ has been shown to regulate NFκB activity in a non-genomic fashion involving their direct association (84). In this model, PPARγ bound to p65 in the nucleus, resulting in export of both proteins to the cytoplasm. Consequently, cytoplasmic localization of NFκB prevented its proinflammatory transcription-dependent effects. It is possible that PPARγ can also physically interact with p65 in platelets. This interaction could inhibit NFκB activity in platelets, which would exert anti-inflammatory and anti-platelet effects. PPARγ has also been shown to form a direct interaction with PKCα upon stimulation with various PPARγ ligands in macrophages (85). The physical association of PKCα with PPARγ prevented PKCα translocation to the membrane and subsequent degradation. However, in this system PPARγ was only able to negatively regulate PKCα activation in response to low-dose PMA stimulation. This suggests that the inhibitory effect of PPARγ can be overridden with stronger stimulation.

**PPARα–PKCα Crosstalk**

Interestingly, PPARα has also been shown to directly interact with PKCα in macrophages (86). In response to LPS stimulation, PKCα bound and phosphorylated PPARα. However, in the presence of the PPARα ligand, simvastatin, PPARα no longer bound PKCα and consequently was able to transrepress NFκB activation. In this model, PKCα likely acts to deactivate PPARα’s inhibitory actions on the proinflammatory NFκB signaling. This may represent a mechanism by which PPARα ligands, such as statins, exert potent and rapid anti-inflammatory actions. Although the interaction of PPARα with PKCα was postulated to negatively regulate PPARα’s function in this system, it is possible that this complex serves additional and possibly proinflammatory functions. Similarly, these data suggest that in platelets, ligand-induced activation of PPARα may function differently than phosphorylation-induced activation, resulting in different or possibly contradictory actions. This may even explain the discrepancy between the bleeding phenotype observed in PPARα knockout mice, while control mice also had increased bleeding times with PPARα activation. Perhaps, PPARα, independent of ligand, acts as a positive regulator of platelet hemostatic function by enhancing activation of PKCα, while ligand-activated PPARα sequesters and inhibits NFκB thus, negatively regulating platelet activation (Figure 3).

**PPARγ and PPARα Regulate MAP Kinases**

Another possible role of PPARα in platelets involves regulation of MAP kinases. In T cells, PPARα was shown to inhibit p38 MAPK and PKCα, leading to increased activation. Treatment with PPARα ligands prevents PPARα binding to PKCα, but instead enhances its interaction with NFκB, thus functionally sequestering PPARα and NFκB from participating in activation signaling.

---

**FIGURE 3 | Possible mechanism of PPARα and NFκB regulation in platelets.** Platelet activation leads to NFκB phosphorylation and activation, which potentiates activation signals. PKCα is also phosphorylated and activated in response to stimulation. This may lead to an interaction between PPARα and PKCα, leading to increased activation. Treatment with PPARα ligands prevents PPARα binding to PKCα, but instead enhances its interaction with NFκB, thus functionally sequestering PPARα and NFκB from participating in activation signaling.
activation, but only in the absence of ligand (87). Additionally, this regulation of p38 MAPK appeared to be independent of direct interaction with PPARα, but likely required an unknown secondary mediator. Activation of p38 MAPK occurs early in platelet activation, albeit transiently, to induce granule release that stimulates the second wave of aggregation (88, 89). Subsequent ligand binding to αIIbβ3 downregulates active p38 MAPK (90). Perhaps, PPARα mediates the regulation of p38 MAPK in these conditions or possibly under shear stress, whereby p38 MAPK mediates adhesion in flow.

PPARγ has also been shown to regulate MAPK pathways in nucleated cells through non-genomic mechanisms (91). PPARγ reversibly interacts with MEK1 via its AF2 domain, resulting in export from the nucleus to the cytoplasm. This corroborates evidence suggesting that PPARγ ligands regulate downstream ERK signaling (92). Interestingly, PPARγ activation led to rapid ERK phosphorylation in human prostate cancer cells, vascular smooth muscle cells, and human microvascular endothelial cells, but inhibited ERK activation in adrenocortical cancer cells (93–95). Similarly, PPARγ enhanced ERK activation in rabbit renal cortex cells, but had no effect in mouse cells (96). These data suggest cell type-specific and species-specific effects of PPARγ, possibly through availability of different co-activators. The effects of PAPRγ activation on the MEK/ERK pathway in platelets has not been investigated, but represents a promising avenue of further research.

**LIVER X RECEPTORS**

LXR (Liver X Receptors) are transcription factors that play key roles in cholesterol homeostasis by regulating genes, such as apolipoprotein E and cytochrome P450 7α-hydroxylase 1 (Cyp7a1) (97, 98). Recent studies have demonstrated that platelets express LXRβ and its ligands inhibit collagen-induced aggregation (99). Thrombin and fibrinogen-induced activation was affected to a lesser degree, requiring high doses of LXR ligands to exert comparable inhibitory effects. Collagen-induced Syk phosphorylation was strongly inhibited by pretreatment with LXR ligands, while LAT and PLCγ phosphorylation were inhibited to a lesser degree, which could be explained by the high concentration of collagen used for activation (50 ng/mL). Interestingly, and opposite of the results seen with PPARγ (71), LXR ligands induced association of LXR with Syk and PLCγ. Moreover, stimulation of platelets with PPARγ or LXR ligands led to an association between the two transcription factors, which was diminished with higher concentrations of ligand. Taken together, these data suggest that LXR and PPARγ can physically interact in platelets and this may represent a novel regulatory mechanism of collagen-induced activation (Figure 4). In cell free systems, LXRβ was able to bind all three PPAR isoforms with different affinities (100). Moreover, different PPAR ligands altered the affinities of these interactions. For example, troglitazone inhibited PPARγ/LXRβ interactions, while GI262570, a PPARγ ligand with high binding affinity, had no effect on this interaction. This may help to explain why different

---

**FIGURE 4 | Regulation of collagen signaling by LXRβ and PPARγ.** Collagen signaling leads to the phosphorylation of Syk and PPARγ. Ligand-activated LXR binds Syk, preventing its interaction with PPARγ, thus decreasing platelet activation. Similarly, PPARγ ligands or LXR ligands can induce the interaction of PPARγ and LXR, reducing the availability of PAPRγ to participate in collagen signaling.
PPAR ligands exert different effects on platelet signaling and function.

RETINOID X RECEPTORS
Retinoid X receptors comprise a group of nuclear receptors that recognize vitamin A metabolites (101). The three different isoforms of RXRs (α, β, and γ) are expressed in different tissues, with RXRa and RXRβ being fairly widely expressed and RXRγ is mainly expressed in skeletal muscle and heart tissue (102). RXRα is the heterodimeric binding partner of PPARγ, which is essential for mediating the genomic effects of PPARγ. Platelets express the RXRa and β isoforms, but not RXRγ, and RXRs immunoprecipitate with PPARγ in platelets. (103) Similar to PPARγ ligands, the RXR ligand, 9-cis-retinoic acid (9cRA), inhibited agonist-induced platelet activation. Interestingly, however, 9cRA preferentially inhibited ADP and U46619 (a thromboxane mimetic)-induced aggregation, but did not alter collagen-induced activation. This is surprising in light of the fact that PPARγ ligands dampen signaling through the collagen receptor (Figure 2), suggesting that RXR in platelets can signal non-genomically independent of PPARγ. RXRα co-immunoprecipitated with Gq11 in resting platelets and this interaction was enhanced when platelets were stimulated with 9cRA. In this manner, ligand-bound RXRα may sequester Gq from its traditional role in propagating signals from G-protein coupled receptors, such as the ADP and thromboxane receptors.

GLUCOCORTICOID RECEPTORS
The glucocorticoid receptor (GR) is a nuclear hormone receptor that binds glucocorticoids, such as dexamethasone and prednisolone, resulting in an anti-inflammatory response (104). Human platelets express GR and differentially respond to its ligands (105). One study found that human platelets were less responsive to activation than treated with prednisolone, but not dexamethasone (106). Furthermore, the inhibitory effects of prednisolone on platelet function could be prevented by treatment with the GR antagonist, RU486. Interestingly, binding assays in human platelets revealed that both glucocorticoids could bind GR and increase its association with the chaperone protein, HSP90. In nucleated cells, ligand binding to GR causes HSP90 dissociation from the GR complex, which reveals its nuclear localization signal (107). However, the opposite effect was found in platelets, suggesting a different method of regulation. Moreover, a unique dimerization was identified in human platelets between GR and the mineralocorticoid receptor (MR), which can bind some of the same ligands as GR, such as dexamethasone. This may help to explain why dexamethasone affected platelet function differently from the more specific GR agonist, prednisolone. Additionally, mineralocorticoids activate P38K, consistent with the finding that mineralocorticoid actions are associated with increased risk of vascular disease (108).

ARYL HYDROCARBON RECEPTOR
The aryl hydrocarbon receptor (AHR) is a well-known toxicant-sensing receptor that also plays essential immune functions and hematopoiesis (109). Common AHR ligands include polycyclic aromatic hydrocarbons, lipoxin A4, and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Mouse platelets express AHR, which is known to play an important role in hematopoiesis and megakaryocyte development (110). Platelets from AHR knockout mice exhibit defective collagen signaling, with inhibited collagen-induced aggregation and spreading. Of note, platelets from AHR knockout mice had lower levels of the collagen receptor, GPVI, and undetectable levels of Vav proteins. Vav1 and Vav3 are activated downstream of many signaling pathways in platelets, and play a role in responding to collagen (111). Although the defects in platelet collagen signaling in AHR knockout mice are intriguing and may suggest an important role for this transcription factor in platelet biology, a more thorough investigation is needed. It is still unknown whether platelets respond to AHR ligands and whether they affect agonist-induced activation. Moreover, it is possible that the defects in collagen signaling in AHR knockout mice could be solely attributed to defective thrombopoiesis and altered platelet composition. Limited studies have evaluated the effects of AHR ligands in hemostasis. Polychlorinated biphenyls, which are non-specific AHR ligands, enhanced human platelet activation (112). Additionally, the specific AHR ligand, TCDD, was shown to induce thrombocyte aggregation in zebrafish (113). This can likely be attributed to its non-genomic actions in thrombocytes, as these effects were seen as early as 30 min post-treatment. Further research investigating the effects of AHR activation in platelets may reveal novel actions of environmental toxicants on hemostasis and cardiovascular risk.

The AHR has been shown to form a complex with the p65 subunit of NFκB in breast cancer cells (114). Furthermore, this interaction was found to be functionally relevant, resulting in proliferation and tumorigenesis. Additional roles for the AHR in regulation of NFκB signaling have been shown by our group in fibroblasts (115, 116). We found that absence of the AHR coincided with decreased expression of the non-canonical NFκB member, RelB. Signal-specific phosphorylation of RelB targets it for proteosomal degradation. It is possible that physical association between the AHR and RelB prevents its proteosomal degradation and allows it to exert anti-inflammatory effects, similar to its regulation in T cells (117).

SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTIONS
The signal transducer and activator of transcription (STAT) family of proteins were some of the first identified functional transcription factors in platelets (118). Human platelets express STATs 1, 3, and 5, which traditionally play a role in transducing signals from cytokine receptors to elicit an immune response (119). Early on, it was known that thrombopoietin could act on human platelets and this resulted in STAT3 phosphorylation, although the biological significance was unclear. Recently, the role of STAT3 in platelet signaling has been investigated more thoroughly, uncovering an important role for it in GPVI signaling (120). Pharmacologic inhibition of STAT3 signaling or dimerization resulted in a hyporesponsive phenotype to low-dose collagen activation. Additionally, platelets from platelet and megakaryocyte-specific STAT3 knockout mice had lower levels of collagen-induced aggregation, decreased P-selectin expression, and slowed thrombus formation. Interrogation of the signaling cascade revealed that STAT3 was activated by JAK2, in the same manner as in nucleated cells.
Traditionally, STAT3 homodimerizes upon phosphorylation by JAK2, then translocates to the nucleus where it acts as a traditional transcription factor. However, in platelets, phosphorylation of STAT3 by JAK2 resulted in dimerization and activation of PLCγ. Syk was found to be an upstream activator of this pathway and was complexed with STAT3 and PLCγ after activation with collagen (121). These data suggest that STAT3 plays an important regulatory role in platelet activation downstream of collagen and thrombopoietin signaling.

THE DIVERGENT ROLES OF TRANSCRIPTION FACTORS
The investigation of transcription factors in platelets has often led to convoluted and sometimes contradictory findings. This can most clearly been seen in the evaluation of the roles of PPARα in platelets. In this case, PPARα appears to play an important role in platelet activation, but has the opposite effect in the presence of a PPARα ligand (74). Additionally, phosphorylated PPARγ positively regulates collagen signaling, while ligand-bound PPARγ inhibits activation (71). These data support the hypothesis that ligand-induced activation of transcription factors can function differently than phosphorylation-induced activation (Figure 3).

MICROPARTICLES AS TRANSPORTERS OF TRANSCRIPTION FACTORS
Microparticles are plasma membrane-derived vesicles ranging in diameter from 0.1 to 1 µm that are present at levels of approximately 5–50 µg/mL in blood plasma (122). Platelets and megakaryocytes are the primary source of microparticles in the blood circulation (about 80%), whereas other microparticles are derived from erythrocytes, endothelial cells, and granulocytes (122–124). Since their discovery, the role of platelet microparticles in coagulation was evident, and later was supported by identification of tissue factor expression (123) and a phosphatidylserine-rich outer membrane (125) that binds coagulation factors to aid in their assembly and enzymatic processing. Microparticles are produced from resting cells, during apoptosis or during cell activation, likely resulting in different internal and surface composition. Microparticles were shown to differ in composition between human samples and between microparticle size classes (126, 127). Packaging mechanisms for microparticles have not yet been identified and such studies are crucial for the understanding of microparticle influences on their environment. Whether microparticle packaging is a passive or active process, increased proximity of mediators to the cell plasma membrane would likely increase their chances of becoming encapsulated by the released microparticles.

MICROPARTICLE FUNCTIONS AND ROLES IN INFLAMMATION
Microparticles are postulated to have several means to influence their environment. Burger et al. described their ability to (1) promote coagulation, (2) scavenge NO, (3) generate reactive oxygen species, (4) cleave cellular surface proteins via metalloproteinases, (5) signal cells via surface proteins, and (6) deliver cargo via transfer of membrane and internal contents (128). Additionally, microparticles are postulated to contribute to, and sometimes exacerbate inflammation. It is likely that surface interactions and the less studied delivery of internal microparticle contents are both contributing to the influences of microparticles on their environment.

TRANSCELLULAR COMMUNICATION
Circulating microparticles can interact with other blood cells they encounter, such as leukocytes, lymphocytes, platelets, and with endothelial cells. Specific modes of cell–microparticle interactions include surface receptor signaling, plasma membrane fusion, or internalization of microparticles (129). The fusion of platelet microparticle membranes to target cell membranes was demonstrated to transfer the surface protein CXCR4 to cells causing recipient cell susceptibility to human immunodeficiency virus infection (130). Membrane fusion or internalization of microparticles could also cause microparticle internal composition to be transferred into the recipient cell cytoplasm. Lipids (131), RNA, and protein (129) have been seen to be delivered in this fashion, and multiple examples of each are reviewed by Mause and Weber (132). Arachidonic acid is a lipid mediator delivered by platelet microparticles that can be further processed into thromboxane A2 by recipient platelets and contribute to their activation (131). Overall, platelet microparticles contain various mediators that can be delivered to surrounding cells to impact their function.

TRANSCRIPTION FACTORS IN MICROPARTICLES AND ROLES IN TRANSCELLULAR COMMUNICATION
The influence of microparticles on recipient cell function is based on microparticle composition. Not surprisingly, blood microparticle protein composition was found to be highly variable between healthy humans (127). Our work showed that platelet microparticles contain transcription factors, such as PPARγ, derived from parent cells (34). Furthermore, proteomic analysis has led to the discovery of three other transcription factors in platelet microparticles, RuvB-like 2, STAT3, and STAT5A (133).

Transcription factors are transported from cells through microparticles and retain function within the recipient cells (34, 72). Our lab was the first to show this ability with platelet-derived microparticles delivering functional PPARγ to THP-1 monocytes, detected through an electrophoresis mobility gel shift assay (134). We have since developed a novel platform technology to engineer microparticles through overexpression of PPARγ in platelet and megakaryocyte-derived microparticles obtained from the cultured megakaryoblastic cell line, Meg-01 cells. We showed that these engineered microparticles could be taken up by THP-1 monocytes, and that the transferred PPARγ was functional within recipients shown by induction fatty acid binding protein-4 (FABP4) expression, a unique PPARγ-specific target gene (72) (Figure 5).

To identify the significance of the transferred PPARγ to recipient cells, we compared recipient cell responses incubated with microparticles that did or did not contain PPARγ, to inflammatory stimuli (135). Monocytes that received PPARγ-containing microparticles had decreased inflammatory mediator production compared to the control microparticles. PPARγ activation has been shown to induce cell differentiation (136, 137). Our work also supported the influence of PPARγ on monocyte differentiation as the cells receiving PPARγ-containing microparticles became more adherent through increased integrin expression and fibronectin.
FIGURE 5 | Platelet microparticles as a method of transcellular delivery of transcription factors. Platelet-derived PPARγ is packaged into platelet microparticles, which can deliver intact PPARγ protein to target cells, such as monocytes. PPARγ can then bind to peroxisome proliferator response elements (PPRE) in the nucleus to affect transcription of target genes. Transfer of PPARγ to monocytes via microparticles has been shown to decrease LPS-induced inflammatory mediator release.

Overall, platelet microparticles are abundant and influential transcellular vesicles. They contain proteins, lipids, and RNA derived from their parent cells. Therefore, these circulating biomarkers provide insight into the cumulative activation and inflammatory state of all blood cell populations. Importantly, microparticles have repeatedly been shown to not just be cell byproducts but rather influential delivery mechanisms. Transportation of transcription factors to cells could influence several ongoing or initiate new pathways causing profound impacts within recipient cells. Transcellular communication involving transcription factors via platelet microparticles substantiates another possible key role of transcription factors presence in platelets.

CONCLUSION
Transcription factors play numerous important and previously unrecognized roles in regulating platelet function in a non-genomic manner. Additionally, transfer of intact and functional transcription factors to other cells via microparticles may serve as a novel regulatory mechanism for inflammation. The initial discovery of transcriptional regulatory elements in a particular protein does not exclude the possibility of non-genomic roles for that protein as well. The anucleate platelet can serve as an important model system to study non-genomic roles of transcription factors. Further elucidation of the non-genomic functions of transcription factors will yield important discoveries that have the potential to illuminate platelet biology and also help to explain the pleotropic effects of pharmaceuticals that target these pathways.

ACKNOWLEDGMENTS
This work was supported in part by ES001247, HL095467, T32-AI007285, NS066891, T90 DE0 21985 and HL128129 a University of Rochester grant from Howard Hughes Medical Institute through the Med into Grad Initiative, and UL1RR024160 and UL1TR000042 from the National Center for Research Resources. The content is solely the responsibility of the authors and does not necessarily represent the views of NCRR or NIH.
REFERENCES

1. Rondina MT, Weyrich AS, Zimmerman GA. Platelets as cellular effectors of inflammation in vascular diseases. Circ Res (2013) 112(1):1506–19. doi:10.1161/CIRCRESAHA.113.300512

2. Morrell CN, Aggery AA, Chapman LM, Modjeski KL. Emerging roles for platelets as immune and inflammatory cells. Blood (2014) 123(18):2759–67. doi:10.1182/blood-2013-11-462432

3. Cognassse F, Hamzeh-Cognassse H, Pozzetto B, Cavaillon JM, Cognasse F. Bench-to-bedside review: platelets and active immune functions—new clues for immunopathology? Crit Care (2013) 17(4):236. doi:10.1186/cc12716

4. Spinnenli SL, Maggirwar SB, Bumberg N, Phipps RP. Nuclear encapsulation: a platelet tour de force. Sci Signal (2010) 3(144):e37. doi:10.1126/scisignal.314pe37

5. Garraud O, Hamzeh-Cognassse H, Pozzetto B, Cavaillon JM, Cognasse F. Bench-to-bedside review: platelets and active immune functions—new clues for immunopathology? Crit Care (2013) 17(4):236. doi:10.1186/cc12716

6. Spinelli SL, Casey AE, Pollock SJ, Gertz JM, McMillan DH, Narasipura SD, et al. Platelets and megakaryocytes contain functional nuclear factor-kappaB. Arterioscler Thromb Vasc Biol (2010) 30(5):591–8. doi:10.1161/ATVBAHA.109.197343

7. Malave E, Romaniuk MA, D’Atri LP, Pozner RG, Negrotto S, Benzadón R, et al. Platelets B kinase phosphorylates Dok1 serines in response to TNF, IL-1, or gamma radiation. Proc Natl Acad Sci U S A (2004) 101(5):17416–21. doi:10.1073/pnas.0408061101

8. Garraud O, Hamzeh-Cognassse H, Pozzetto B, Cavaillon JM, Cognasse F. Bench-to-bedside review: platelets and active immune functions—new clues for immunopathology? Crit Care (2013) 17(4):236. doi:10.1186/cc12716

9. Lee HS, Kim SD, Lee WM, Endale M, Kamruzzaman SM, Oh WJ, et al. A noble role of B kinase beta phosphorylates Dok1 serines in response to TNF, IL-1, or gamma radiation. Proc Natl Acad Sci U S A (2004) 101(5):17416–21. doi:10.1073/pnas.0408061101

10. Chang CC, Lu WJ, Ong ET, Chiang CW, Lin SC, Huang SY, et al. A novel role of nuclear factor-kappaB signaling in platelets in a p38-dependent fashion. Cardiovasc Res (2009) 84(1):137–44. doi:10.1093/cvr/cvp176

11. Lee HS, Kim SD, Lee WM, Endale M, Kamruzzaman SM, Oh WJ, et al. A noble role of B kinase beta phosphorylates Dok1 serines in response to TNF, IL-1, or gamma radiation. Proc Natl Acad Sci U S A (2004) 101(5):17416–21. doi:10.1073/pnas.0408061101

12. Chen WF, Lee JJ, Chang CC, Lin KH, Wang SH, Sheu JR. Platelet protease-activated receptor (PAR)4, but not PAR1, associated with neutral sphingomyelinase in platelets in a p38-dependent fashion. J Biol Chem (2010) 285(24):18352–63. doi:10.1074/jbc.M109.077602

13. Pierce JW, Schoenleber R, Jesmok G, Best J, Moore SA, Collins T, et al. Novel inhibitors of cytokine-induced IkappaBalpha phosphorylation and endothelial cell adhesion molecule expression show anti-inflammatory effects in vivo. J Biol Chem (1997) 272(34):21096–103. doi:10.1074/jbc.272.34.21096

14. Karim ZA, Zhang J, Banerjee M, Chicka MC, Al Hawas R, Hamilton TR, et al. I kappaB kinase phosphorylation of SNAP-23 controls platelet secretion. Blood (2013) 121(22):4567–74. doi:10.1182/blood-2012-11-470468

15. Bachem A, Yokoub D, Zaid Y, Mourad W, Merhi Y. Involvement of nuclear factor-kappaB in platelet CD40 signaling. Biochem Bioph Res Com (2013) 425(1):58–63. doi:10.1016/j.bbrc.2012.07.049

16. Wei S, Wang H, Zhang G, Lu Y, An X, Ren S, et al. Platelet I kappaB alpha, the NF-kappaB inhibitory subunit, interacts with ANT, the mitochondrial ATP/ADP translocator. J Biol Chem (2001) 276(24):21317–24. doi:10.1074/jbc.M005850200

17. Cognassse F, Garcia-Larco JJ, Van Het Hof B, Van Dijk W. Low-dose rosiglitazone exerts an antiinflammatory effect with an increase in adiponectin independently of free fatty acid fall and insulin sensitization in obese type 2 diabetics. J Clin Endocrinol Metab (2008) 93(1):3555–8. doi:10.1210/jc.2005-0269

18. Sambrano GR, Weiss EJ, Zheng YW, Huang W, Coughlin SR. Role of thrombin in platelet activation. J Biol Chem (2010) 285(24):18352–63. doi:10.1074/jbc.M109.077602

19. Lu WJ, Lin KH, Hsu MJ, Chou DS, Hsiao G, Sheu JR. Suppression of NF-kappaB signaling by andrographolide with a novel mechanism in human platelets: regulatory roles of the p38 MAPK-hydroxyl radical-ERK2 cascade. Biochem Pharmacol (2012) 84(7):914–24. doi:10.1016/j.bcp.2012.06.030

20. Lu WJ, Lin KH, Hsu MJ, Chou DS, Hsiao G, Sheu JR. Suppression of NF-kappaB signaling by andrographolide with a novel mechanism in human platelets: regulatory roles of the p38 MAPK-hydroxyl radical-ERK2 cascade. Biochem Pharmacol (2012) 84(7):914–24. doi:10.1016/j.bcp.2012.06.030

21. Lannan et al. Transcription factors in platelets and platelet-derived microparticles
37. Watala C, Boncler M, Gresner P. Blood platelet abnormalities and pharmaco-
    cological modulation of platelet reactivity in patients with diabetes mellitus. P
    harmacol Rep (2005) 57(Suppl):42–58. doi:10.2174/1871127195049804
38. Gasparyan AV, Ayvazyan L, Mikhailidis DP, Kitas GD. Mean platelet vol-
    ume: a link between thrombosis and inflammation? Curr Pharm Des (2011)
    17(1):47–58. doi:10.2174/138161211795049804
39. Trovati M, Anfossi G. Mechanisms involved in platelet hyperactivation and
    platelet-endothelium interrelationships in diabetes mellitus. Curr Diab Rep
    (2003) 3(1):316–22. doi:10.1007/bf03630202-0020-27
40. Hetzel J, Balletshofer B, Rittig K, Walcher D, Kratzer W, Hombach V, et al. Rapid
    effects of rosiglitazone treatment on endothelial function and inflammatory
    biomarkers. Arterioscler Thromb Vasc Biol (2005) 25(9):1804–9. doi:10.1161/1
    0341.0104.017612.1965.9a
41. Pfützner A, Schöndorf T, Seidel D, Winkler K, Matthaei S, Hamann A,
    et al. Improvement by the peroxisome proliferator-activated receptor-gamma
    agonist rosiglitazone reduces circulating platelet activity in patients without
    diabetes mellitus who have coronary artery disease. Am Heart J (2004) 147(6):e25.
    doi:10.1016/j.amjheart.2003.12.035
42. Kato K, Y amada D, Midorikawa S, Sato W, Watanabe T. Contribution of
    platelet-endothelium interrelationships in diabetes mellitus. Curr Pharm Des
    (2005) 11(5):646–50. doi:10.2174/1381612054367337
43. Trovati M, Anfossi G. Mechanisms involved in platelet hyperactivation and
    platelet-endothelium interrelationships in diabetes mellitus. Curr Diab Rep
    (2003) 3(1):316–22. doi:10.1007/bf03630202-0020-27
44. Kato K, Y amada D, Midorikawa S, Sato W, Watanabe T. Improvement by the
    peroxisome proliferator-activated receptor-gamma agonist rosiglitazone
    reduces circulating platelet activity in patients without diabetes mellitus who
    have coronary artery disease. Am Heart J (2004) 147(6):e25. doi:10.1016/j.amjheart.2003.12.035
45. Akbijik F, Ray DM, Gettings KJ, Blumberg N, Francis CW, Phipps RP. Human
    bone marrow megakaryocytes and platelets express PPARgamma, and
    PPARgamma agonists blunt platelet release of CD40 ligand and thrombosanols.
    Blood (2004) 104(1):1361–8. doi:10.1182/blood-2004-03-0926
46. Hishinuma T, Y amazaki T, Mizugaki M. Rosiglitazone has a reducing effect
    on thromboxane production. Prostaglandins Other Lipid Mediat (2000)
    62(1):135–43. doi:10.1016/s0090-6980(00)00059-9
47. Bodary PF, Vargas FG, King SA, Jongeward KI, Wickenheiser KI, Fitzman DT.
    Rosiglitazone protects against thrombosis in a mouse model of obesity and
    insulin resistance. J Thromb Haemost (2005) 3(10):2149–53. doi:10.1111/j.
    1538-7836.2005.01551.x
48. Rao F, Yang RQ, Chen XS, Xu JS, Fu HM, Su H, et al. PPARγ lig-
    ands decrease hydrostatic pressure-induced platelet aggregation and pro-
    inflammmatory activity. PLoS One (2014) 9(2):e89654. doi:10.1371/journal.pone.
    0898654
49. Liu D, Chen K, Sinha N, Zhang X, Wang Y, Sinha AK, et al. The effects of
    PPAR-gamma ligand pioglitazone on platelet aggregation and arterial throm-
    bus formation. Cardiovasc Res (2005) 65(4):907–12. doi:10.1016/j.cardiores.
    2004.11.027
50. Vareo N, Vincent D, Libby P, Nuovo R, Calle-Pascual AL, Bernal MR, et al.
    Elevated plasma levels of the atherogenic mediator soluble CD40 ligand in
    diabetic patients: a novel target of thiazolidinediones. Circulation (2003)
    107(21):2664–9. doi:10.1161/01.cir.0000074043.46437.44
51. Surh YJ, Na HK, Park JM, Lee HN, Kim WY, Yoon IS, et al. 15-Deoxy-12,14-
    prostaglandin J2, an electrophilic lipid mediator of anti-inflammatory and pro-
    resolving signaling. Biochem Pharmacol (2011) 82(10):1335–51. doi:10.1016/j.
    bcp.2011.07.100
52. Ishii T, Itoya S, Wada H, Ishizawa M, Kimura M, Kajita K, et al. Differen-
    tial effect of the antidiabetic thiazolidinediones troglitazone and pioglitazone
    on human platelet aggregation mechanism. Diabetes (1998) 47(9):1494–500.
    doi:10.2337/diabetes.47.9.1494
53. Smith NJ, Stoddart LA, Devine NM, Jenkins L, Milligan G. The action and
    mode of binding of thiazolidinedione ligands at free fatty acid receptor 1.
    J Biol Chem (2009) 284(26):17527–39. doi:10.1074/jbc.m109.012849
54. Simó R, Rodríguez A, Caveda E. Different effects of thiazolidinediones on
    cardiovascular risk in patients with type 2 diabetes mellitus: pioglitazon-
    e versus rosiglitazone. Curr Drug Saf (2010) 5(3):234–44. doi:10.1080/1
    7584687091683532
55. Pickavance L, Widdowson PS, King P, Ishii S, Tanaka H, Williams G. The de-
    velopment of overt diabetes in young Zucker diabetic fatty (ZDF) rats and the
    effects of chronic MCC-555 treatment. Br J Pharmacol (1998) 125(4):767–70.
    doi:10.1038/bj.1998.2158
56. Choi J, Park Y, Lee HS, Yang Y, Yoon S. 1,3-diphenyl-1H-pyrazole derivatives as
    new series of potent PPARα partial agonists. Bioorg Med Chem (2010)
    18(23):8315–23. doi:10.1016/j.bmc.2010.09.068
57. Camp HS, Li O, Wise SC, Hong YH, Frankowski CL, Shen X, et al. Differential
    activation of peroxisome proliferator-activated receptor-gamma by troglita-
   zone and pioglitazone. Diabetes (2000) 49(4):539–47. doi:10.2337/diabetes.
    49.4.539
58. Guan HP, Ishizuka T, Chui PC, Lehrke M, Lazar MA. Corepressors selectively
    control the transcriptional activity of PPARgamma in adipocytes. Genes Dev
    (2005) 19(9):453–61. doi:10.1101/gad.126305
59. Kodera Y, Takeyama K, Murayama A, Suzawa M, Masuhiro Y, Kato S. Lig-
    and-specific interactions of peroxisome proliferator-activated receptor gamma
    with transcriptional coactivators. J Biol Chem (2000) 275(43):33201–4.
    doi:10.1074/jbc.c000517200
60. Leonard DM, O’Malley BW. Nuclear receptor coregulators: modulators of
    pathology and therapeutic targets. Nat Rev Endocrinol (2012) 8(10):598–604.
    doi:10.1038/nrendo.2012.100
61. Moraes LA, Spyridon M, Kaiser WJ, Jones CI, Sage T, Atherton RE, et al. Non-
    genomic effects of PPARgamma ligands: inhibition of GPVT-stimulated platelet
    activation. J Thromb Haemost (2010) 8(3):577–87. doi:10.1111/j.1538-7836.
    2009.03732.x
72. Sahler J, Wöhrle C, Spinnelli S, Blumberg N, Phipps R. A novel method for over-
expression of peroxisome proliferator-activated receptor-γ in megakaryocyte
and platelet microorganisms achieves transcellular signaling. J Thromb Haemost
(2012) 10(12):2563–72. doi:10.1111/jth.12017

73. Du H, Hu H, Zheng H, Hao J, Yang J, Cui W. Effects of peroxisome proliferator-
activated receptor γ in sсанvastatin antiplatelet action: influences on cAMP and
mitogen-activated protein kinase. Thromb Res (2014) 134(1):111–20.
doi:10.1016/j.thromres.2014.05.005

74. Ali FY, Armstrong PC, Dhanji AR, Tucker AT, Paul-Clark MJ, Mitchell JA, et al.
Antiplatelet actions of statins and fibrates are mediated by PPARs. Ar
tieroscler Thromb Vasc Biol (2009) 29(5):706–11. doi:10.1161/ATVBAHA.108.
183560

75. Kliever SA, Forman BM, Blumberg B, Ong ES, Borgmeyer U, Mangelsdorf DJ, et al.
Different expression and activation of a family of murine peroxisome proliferator-
activated receptors. Proc Natl Acad Sci U S A (1994) 91(15):7355–9.
doi:10.1073/pnas.91.15.7355

76. Ali FY, Davidson SJ, Moraes LA, Traves SL, Paul-Clark M, Bishop-Bailey D, et al.
Differential expression and activation of a family of murine peroxisome proliferator-
activated receptors. Proc Natl Acad Sci U S A (1994) 91(15):7355–9.
doi:10.1073/pnas.91.15.7355

77. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586

78. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586

79. Li Z, Xi X, Du X. Antinfection-activated protein kinase-dependent signaling path-
way in the activation of platelet integrin alphaIIbbeta3. J Biol Chem (2001)
276(45):42226–32. doi:10.1074/jbc.M106129200

80. Li Z, Zhang G, Feil R, Han J, Du X. Sequential activation of p38 and ERK path-
ways by cGMP-dependent protein kinase leading to activation of the platelet
integrin alphaIIbbeta3. Blood (2006) 107(3):965–72. doi:10.1182/blood-2005-
03-1308

81. Feinstein DL, Spagnolo A, Akar C, Weinberg G, Murphy P, Gavrilyuk V, et al.
Peroxisome proliferator-activated receptor gamma (PPARgamma) is required for
modulating endothelial inflammatory response through a nongen-
omic mechanism. Eur J Cell Biol (2010) 89(9):545–53. doi:10.1016/j.ejcb.
2010.04.002

82. Luconi M, Cantini G, Serio M. Peroxisome proliferator-activated receptor
γ signaling pathway in the activation of platelet integrin αIIbb3. J Biol Chem
(2010) 285(15):196–203. doi:10.4049/jimmunol.171.1.196

83. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586

84. Kelly D, Campbell JI, King TP, Grant G, Jansson EA, Coutts AG, et al.
Acute antiinflammatory properties of statins involve peroxisome
phos to desensitize monocytes/macrophages. J Cell Biol (2006)
174(5):681–94. doi:10.1083/jcb.200508117

85. Ali FY, Campbell JI, King TP, Grant G, Jansson E A, Coutts AG, et al.
Acute antiinflammatory properties of statins involve peroxisome
phos to desensitize monocytes/macrophages. J Cell Biol (2006)
174(5):681–94. doi:10.1083/jcb.200508117

86. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586

87. Li S, Li X, Wu C, Zhu J, Cai J, Shen J, et al. Ligand-binding regulation of
nuclear receptor that identifies a novel retinoic acid response pathway.
Nature (1992) 357(6383):693–7. doi:10.1038/357693a0

88. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586

89. Li Z, Xi X, Du X. Antinfection-activated protein kinase-dependent signaling path-
way in the activation of platelet integrin alphaIIbbeta3. J Biol Chem (2001)
276(45):42226–32. doi:10.1074/jbc.M106129200

90. Li Z, Zhang G, Feil R, Han J, Du X. Sequential activation of p38 and ERK path-
ways by cGMP-dependent protein kinase leading to activation of the platelet
integrin alphaIIbbeta3. Blood (2006) 107(3):965–72. doi:10.1182/blood-2005-
03-1308

91. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586

92. Ali FY, Davidson SJ, Moraes LA, Traves SL, Paul-Clark M, Bishop-Bailey D, et al.
Differential expression and activation of a family of murine peroxisome proliferator-
activated receptors. Proc Natl Acad Sci U S A (1994) 91(15):7355–9.
doi:10.1073/pnas.91.15.7355

93. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586

94. Saklatvala J, Rawlinson L, Waller RJ, Sarsfield S, Lee JC, Morton LF, et al. Role
for p38 mitogen-activated protein kinase in platedlet aggregation caused by
coliogen or a thromboxane analogue. J Biol Chem (1996) 271(12):6586–9.
doi:10.1074/jbc.271.12.6586
109. Lindsey S, Papoutskas ET. The evolving role of the aryl hydrocarbon receptor (AhR) in the normophysiology of hematopoiesis. *Stem Cell Rev* (2012) 8(4):1223–35. doi:10.1007/s12015-012-9384-5

110. Lindsey S, Jiang L, Woudie D, Papoutskas ET. Platelets from mice lacking the aryl hydrocarbon receptor exhibit defective collagen-dependent signaling. *J Thromb Haemost* (2014) 12(3):383–94. doi:10.1111/jth.12490

111. Pearce AC, Senis YA, Billadeau DD, Turner M, Watson SP, Vigneri E, Vav and var3 have critical but redundant roles in mediating platelet activation by collagen. *J Biol Chem* (2004) 279(52):53955–62. doi:10.1074/jbc.M410355200

112. Raulif M, König W. In vitro effects of polychlorinated biphenyls on human platelets. *Immunology* (1991) 72(2):287–91.

113. Kim S, Sundaramoorthi H, Jagadeeswaran P. Dioxin-induced thrombocyte aggregation in zebrafish. *Blood Cells Mol Dis* (2014) 54(1):116–22. doi:10.1016/j.bcmd.2014.07.010

114. Kim DW, Gazarian L, Quadri SA, Romieu-Moure-R, Sherr DH, Sonenshein GE. The RelA NF-kappa B subunit and the aryl hydrocarbon receptor (AhR) cooperate to transactivate the c-myc promoter in mammary cells. *Oncogene* (2000) 19(48):5498–506. doi:10.1038/sj.onc.1203345

115. Thatcher TH, Maggirwar SB, Baglole CJ, Lakoitis HF, Gasiewicz TA, Phipps RP, et al. Aryl hydrocarbon receptor-deficient mice develop heightened inflammatory responses to cigarette smoke and endotoxin associated with rapid loss of the nuclear factor-kappaB component RelB. *Am J Pathol* (2007) 170(3):855–64. doi:10.2353/ajpath.2007.060391

116. Baglole CJ, Maggirwar SB, Gasiewicz TA, Thatcher TH, Phipps RP, Sime PJ. The aryl hydrocarbon receptor attenuates tobacco smoke-induced cyclooxygenase-2 and prostaglandin production in lung fibroblasts through regulation of the nuclear factor-kappaB component RelB. *J Biol Chem* (2008) 283(43):28944–57. doi:10.1074/jbc.M800685200

117. Marienfeld R, Berberich-Siebelt F, Berberich I, Denk A, Serfling E, Neu mann M. Signal-specific and phosphorylation-dependent RelB degradation: a potential mechanism of NF-kappaB control. *Oncogene* (2001) 20(56):8142–7. doi:10.1038/sj.onc.1204884

118. Ezumi Y, Takayama H, Okuma M. Thrombopoietin, c-Mpl ligand, induces megakaryocyte and platelet formation. *Am J Pathol* (1993) 89(3):383–94. doi:10.1111/jth.12490

119. Miyakawa Y, Oda A, Druker BJ, Ozaki K, Handa M, Ohashi H, et al. Thrombopoietin receptor interacts with c-Mpl, a member of the cytokine receptor superfamily. *J Biol Chem* (2002) 277(17):15120–7. doi:10.1074/jbc.M111025200

120. Zhou Z, Gushiken FC, Bolgiano D, Salsbery BJ, Aghakasiri N, Jing N, et al. Bicarbonate-induced thrombopoiesis and megakaryopoiesis. *Circ Res* (2010) 107(9):1047–57. doi:10.1161/CIRCRESAHA.110.226456

121. Garcia BA, Smalley DM, Cho H, Shabanozvit L, Ley K, Hunt DF. The platelet microsomal proteome. *J Proteome Res* (2005) 4(2):287–98. doi:10.1021/pr0400760

122. Feldon SE, O’loughlin CW, Ray DM, Landskroner-Eiger S, Seweryniak KE, Phipps RP. Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes. *Am J Pathol* (2006) 169(4):1183–93. doi:10.1016/j.ajpath.2006.060434

123. Sahler J, Woeller CE, Phipps RP. Microvesicles engineered to highly express peroxisome proliferator-activated receptor-y decreased inflammatory mediator production and increased adhesion of recipient monocytes. *PLoS One* (2014) 9(11):e113189. doi:10.1371/journal.pone.0113189

124. Liu H, Shi B, Huang CC, Ekasrko P, Pope RM. Transcriptional diversity during monocytic differentiation to adipocytes. *J Biol Chem* (2007) 282(18):1183–93. doi:10.1074/jbc.M600501200

125. Sahler J, Woeller CE, Phipps RP. Microparticles engineered to highly express peroxisome proliferator-activated receptor-y decreased inflammatory mediator production and increased adhesion of recipient monocytes. *PLoS One* (2014) 9(11):e113189. doi:10.1371/journal.pone.0113189

126. Dean WL, Lee MJ, Cummings TD, Schultz DJ, Powell DW. Proteomic and functional characterisation of platelet microparticle size classes. *Thromb Haemost* (2009) 102(4):711–8. doi:10.1160/TH09-04-243

127. Bastos-Andrade P, Royo F, Gonzalez E, Conde-Vancells J, Palomo-Diez L, Borras FE, et al. Proteomic analysis of microvesicles from plasma of healthy donors reveals high individual variability. *J Proteomics* (2012) 75(12):3574–84. doi:10.1016/j.jprot.2012.03.054

128. Burger D, Schok S, Thompson CS, Montezano AC, Hakim AM, Toszyk RM. Microparticles: biomarkers and beyond. *Clin Sci (Lond)* (2013) 124(7):423–41. doi:10.1042/CS20120309

129. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/micromeres as a mechanism of cell-to-cell communication. *Kidney Int* (2010) 78(9):838–48. doi:10.1038/ki.2010.278

130. Rozmyslowicz T, Majka M, Kijowski J, Murphy SL, Conover DO, Poncz M, et al. Platelet- and megakaryocyte-derived microparticles transfer CXCR4 receptor to CXCR4-null cells and make them susceptible to infection by X4-HIV. *AIDS* (2007) 17(1):33–42. doi:10.1097/00002030-2003010300-00006

131. Barry OP, Pratico D, Lawson JA, FitzGerald GA. Transcellular activation of platelets and endothelial cells by bioactive lipids in platelet microparticles. *J Clin Invest* (1997) 99(9):2118–27. doi:10.1172/JCI119085

132. Masse SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. *Circ Res* (2010) 107(9):1047–57. doi:10.1161/CIRCRESAHA.110.226456

133. Garcia BA, Smalley DM, Cho H, Shabanozvit L, Ley K, Hunt DF. The platelet microsomal proteome. *J Proteome Res* (2005) 4(5):1516–21. doi:10.1021/pr0500760

134. Feldon SE, O’loughlin CW, Ray DM, Landskroner-Eiger S, Seweryniak KE, Phipps RP. Activated human T lymphocytes express cyclooxygenase-2 and produce proadipogenic prostaglandins that drive human orbital fibroblast differentiation to adipocytes. *Am J Pathol* (2006) 169(4):1183–93. doi:10.1016/j.ajpath.2006.060434

135. Sahler J, Woeller CE, Phipps RP. Microparticles engineered to highly express peroxisome proliferator-activated receptor-y decreased inflammatory mediator production and increased adhesion of recipient monocytes. *PLoS One* (2014) 9(11):e113189. doi:10.1371/journal.pone.0113189

136. Liu H, Shi B, Huang CC, Ekasrko P, Pope RM. Transcriptional diversity during monocytic differentiation to adipocytes. *J Biol Chem* (2007) 282(18):1183–93. doi:10.1074/jbc.M600501200

Conflict of Interest Statement: Neil Blumberg has received lecture honoraria and consulting fees from Antek, Inc., Fenwal, Pall BioMedical, and Caridian (Terumo), manufacturers of leukoreduction filters, blood component equipment, and cell wash devices. The other authors have nothing to disclose.