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Review

Protein kinase C-theta in platelet activation

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ABSTRACT

Members of the protein kinase C (PKC) family of serine/threonine kinases have been implicated in several physiological processes regulating the activation response of platelets. They are involved in processes leading to granule secretion, integrin activation, platelet aggregation and spreading, and procoagulation. The protein kinase C θ (PKCθ) isoform, which was originally identified in T lymphocytes, is also expressed at relatively high levels in platelets, wherein it is involved in the regulation of hemostasis and thrombosis. Recent studies suggest a role for PKCθ in protease-activated receptor (PAR)-, glycoprotein VI (GPVI) receptor- and glycoprotein $\alpha_{IIb}\beta_3$ integrin receptor-linked signal transduction pathways. The present review focuses on the latest observations relevant to the role of PKCθ in platelet activation.

1. Introduction

Platelets, also termed thrombocytes, are small, anucleated cells, which are formed in the bone marrow by pinching off cytoplasmic fragments of megakaryocytes. Their average lifespan is 5–9 days, they circulate in the blood, and are critical for hemostasis and clot formation in response to vascular injury. They are also involved in restenosis and inflammatory reactions [1–3]. Upon activation, platelets secrete a multitude of soluble effector molecules that stimulate the deposition of extracellular matrix and promote healing of damaged tissue [1–3]. A decreased number or reduced activity of platelets may result in prolonged bleeding times and increased blood loss following injury, while an increased number or excessive activity of platelets can lead to intravascular clot formation, circulating emboli and occlusion of blood vessels [1–3].

Platelet interaction with soluble blood constituents or vascular wall components is mediated by specific surface receptors, which promote adhesion to adjacent platelets, leukocytes, endothelial cells and extracellular matrices. This can result in platelet activation and amplification of thrombo-inflammatory reactions [4]. Adherence of platelets to injured vasculature induces granule secretion, synthesis of thromboxane A2 (TxA_2), extension of filopodia and lamellipodia and activation of surface adhesion molecules [4]. Many of these functions are regulated, at least in part, by members of the protein kinase C (PKC) family [5]. In this review, we will discuss some of the recent findings concerning the isoform-specific roles of PKCθ in platelets, focusing on results obtained using platelets of PKC-deficient mice.

2. Platelet activation

Platelet activation plays an important role in hemostasis and thrombus formation. Under normal physiological conditions, platelets circulate in the blood as resting cells, where they continuously encounter inhibitory signals provided by endothelial cells that line the inner surface of the blood vessels [6]. Vascular endothelial cells produce nitric oxide that inhibits platelet activation, and soluble ADPase, which degrades the platelet activator, ADP. Endothelial cells also produce the eicosanoid prostacyclin (PGI_2) that can associate with the Gs protein-coupled prostacyclin receptor on the surface of platelets. PGI_2 binding to its cognate receptor, signals

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adenylyl cyclase to produce cAMP that counteract the effects of the platelet activator, TXA_2, thereby inhibiting platelet activation [1,2].

Vascular endothelial cells adhere to the blood vessel basement membrane by physical binding to the collagen. They also associate with the von Willebrand factor (vWF) that functions as a cell adhesion ligand and increases the binding affinity of the endothelial cells to the extracellular matrix [7,8]. Under physiological conditions, the collagen is not exposed to the bloodstream. However, a trauma to the vascular endothelial layer exposes collagen and vWF from the subendothelium to the bloodstream, making them accessible to platelets that respond by a rapid turn-on of several biochemical cascades [7,8]. For example, binding of the platelet receptor glycoprotein VI (GPVI) to collagen initiates a signaling cascade leading to platelet activation, which is characterized by the release of granule-stored mediators and synthesis of thrombin and TXA_2 [9]. Secreted mediators, such as adenosine diphosphate (ADP), serotonin, platelet-activating factor (PAF), vWF, and TXA_2 activate additional platelets and further amplify the intensity of the entire response. In addition, stimulation of platelets results in the expression of proteins that enable them to adhere to specific receptors on the surface of leukocytes and endothelial cells [9]. This mechanism is mediated by proteins such as P selectins (P stands for platelet; also termed CD62P), which are normally stored in α-granules within the cytoplasm of resting platelets, but move to the platelet's outer surface upon their activation. They can then interact with P-selectin glycoprotein ligand-1 (PSGL1; also termed Selectin P ligand (SELPLG) or CD162) and other ligands on the surface of the leukocytes or endothelial cells [10].

Molecules that belong to a different type of a receptor family, termed PARs (protease activated receptors) are G-protein coupled receptors that undergo activation by the action of serine proteases that cleave an extracellular portion of the receptor. The cleaved N-terminal peptide acts as a tethered ligand or agonist, which activates the PAR receptor and initializes a physiological response.

Both GPVI and PARs receptors can stimulate phospholipase C (PLC), leading to the generation of second messengers, which activate PKC and stimulate the release of calcium ions from intracellular stores, respectively [11]. Activation of this signaling pathway is an early step in biochemical processes regulating many of the functional responses of platelets.

Platelet activation also results in scramblase-mediated transport of negatively charged phospholipids to the platelet surface. These phospholipids provide a catalytic surface (with the charge provided by phosphatidylserine and phosphatidylethanolamine) for the tenase complex (formed by the activated forms of the blood coagulation factor VIII and factor I).

Platelet aggregation involves an additional type of receptor that specifically associates with fibrinogen, the glycoprotein $\alpha_{IIb}\beta_3$ integrin receptor. Fibrinogen binding to platelet $\alpha_{IIb}\beta_3$ triggers 'outside-in' signals that promote actin polymerization and cell spreading [12]. In contrast, $\alpha_{IIb}\beta_3$ inhibitors prevent platelet aggregation and thrombus formation and are therefore useful for treating patients with acute coronary syndromes.

Platelets contain two types of morphological distinct storage granules, the α-granules and the dense granules. Release of soluble mediators from the α-granules amplify the coagulation cascade at the site of the vascular injury and increase the platelets' procoagulation activity. Release of soluble mediators from the dense granules, predominantly the ADP, promotes the recruitment of additional platelets to the site of injury.

3. Protein kinase C (PKC)

PKC is a ubiquitous family of structurally related serine/threonine kinases that are part of the AGC-type kinase (PKC/PKG/PKC) superfamily [13]. The PKC family consists of at least 10 distinct isoforms that are grouped into three classes: the classical (cPKC), novel (nPKC) and atypical (aPKC) enzymes. Members of the cPKC (α, βI, βII, and γ) utilize both Ca^{2+} ions and diacylglycerol (DAG) as cofactors for activation, the nPKC (δ, ε, η, and θ) utilize DAG, but are Ca^{2+}-independent, and the aPKC (ζ and ι/λ) that are active independent of Ca^{2+} or DAG. Different PKC isoforms exhibit some overlapping functions as well as distinct, non-redundant functions, which depending on the experimental system used, can exhibit specific biochemical properties, expression profiles, and physiological functions. They can differ also in subcellular localization, and although the majority of PKC enzymes reside in the cytoplasm of resting cells, they translocate to the plasma membrane [14,15], nuclear membrane [16], or other subcellular compartments [17–19], following stimulation of cells with a large variety of physiological agonists. The redistribution of distinct PKC isoforms to specific subcellular compartments is critical for the induction of PKC catalytic activity, since the specific location determines the enzyme's ability to interact with selected cofactors and has access to specific substrates. The differential subcellular distribution of PKC is, at least partially, regulated by PKC-binding proteins, such as RACKs (receptor for activated C kinase) [20], scaffold proteins, including AKAP (a kinase-anchoring protein) [21], and cytoskeletal elements, including actin [18,22]. PKC enzymes play a major role in the signaling networks that translate environmental signals into cellular behavior. By phosphorylating specific substrates and altering their conformation and/or biological activity PKC can regulate multiple cellular functions.

4. PKCθ

The human PKCθ gene (termed *PRKCQ*) was first discovered in 1993, in a search for new PKC isoforms that are potentially involved in the regulation of T cell responses [23]. Relatively high expression levels of PKCθ were found in T lymphocytes and lymphoid organs, skeletal muscle, lung and kidney [23–26]. Among the hematopoietic cell lineages tested, high levels of PKCθ were observed in T but not B lymphocytes and in representatives of the megakaryoid cell lineage, including megakaryocytes and platelets [24,27,28].

PKCθ is unique among the PKC isoforms in its ability to translocate from the cytosol to the center of the immunological synapse of activated T cells where it colocalizes with the TCR [29,30]. It is also involved in cytoskeletal remodeling and contributes to the formation of the rearranged synapse [31]. Translocation of PKCθ was found to be regulated by the Lck protein tyrosine kinase and correlated with the catalytic activation of PKCθ by inducible cofactors that are produced along the PI3K- and Vav-dependent pathway [32].

Inherited deficiency of PKCθ in mice has no effect on thymocyte development but it impairs TCR-induced activation response in mature T cells [33]. As a result, proliferative responses of antigen-triggered PKCθ-deficient mature T cells are inhibited, predominantly because of reduced production of IL-2 and decreased expression of CD25, the high affinity subunit of the IL-2 receptor [33]. It is suggested therefore that PKCθ plays a critical role in transduction of essential signals downstream of activated T cell antigen receptors [34]. This suggestion is in agreement with the fact that TCR-stimulated PKCθ-deficient mature T cells fail to respond by upregulation of the AP-1, NF-AT and NF-kB transcription factors [33,35]. These effects may possibly account for the defect in IL-2 production, since the *IL-2* gene promoter possesses critical binding sites for AP-1, NF-kB and NF-AT transcription factors, in addition to the Oct-1 [36].

More recent studies with PKCθ-deficient mice demonstrated the need for PKCθ during the differentiation of T cells into specific cell lineages. For example, PKCθ was found to be essential for the