Arch. Immunol. Ther. Exp. (2011) 59:79–87
DOI 10.1007/s00005-011-0112-7

REVIEW

Protein Kinase C Isoforms in Neutrophil Adhesion and Activation

Anna Bertram · Klaus Ley

Received: 14 June 2010 / Accepted: 8 October 2010 / Published online: 6 February 2011

Abstract Neutrophils are the first line of defense against bacterial and mycotic pathogens. In order to reach the pathogens, neutrophils need to transmigrate through the vascular endothelium and migrate to the site of infection. Defense strategies against pathogens include phagocytosis, production and release of oxygen radicals through the oxidative burst, and degranulation of antimicrobial and inflammatory molecules. Protein kinase C (PKC)-δ is required for full assembly of NADPH oxidase and activation of the respiratory burst. Neutrophils also express PKC-α and -β, which may be involved in adhesion, degranulation and phagocytosis, but the evidence is not conclusive yet. This review focuses on the potential impact of protein kinase C isoforms on neutrophil adhesion and activation.

Keywords Neutrophil · Protein kinase C · Integrin activation · Adhesion · Rolling · NADPH oxidase

Abbreviations

BPI	Bactericidal permeability increasing protein
CXCL	CXC ligand
DAG	Diacylglycerol
ERM	Ezrin/radixin/moesin
fMLP	Formyl-methionyl-leucyl-phenylalanine
GEF	Guanine nucleotide exchange factor
GPCR	G-protein coupled receptor
ICAM	Intercellular adhesion molecule
IL	Interleukin
LFA-1	Lymphocyte function-related antigen-1
Mac-1	Macrophage receptor-1
MAPK	Mitogen-activated protein kinase
PKC	Protein kinase C
PLC	Phospholipase C
PMA	Phorbol 12-myristate 13-acetate
PI3K	Phosphoinositide 3-kinase
PS	Phosphatidylserine
PSGL-1	P-selectin glycoprotein ligand-1
TNF-α	Tumor necrosis factor-α

A. Bertram
Department of Nephrology, Hannover Medical School, Carl-Neuberg-Str. 1, 30625 Hannover, Germany

K. Ley (✉)
Division of Inflammation Biology, La Jolla Institute for Allergy and Inflammation, 9420 Athena Circle, La Jolla, CA 92037, USA
e-mail: klaus@liai.org

Introduction

Protein kinase C (PKC) isoforms are important intracellular signaling molecules in cell differentiation, migration, proliferation and activation. These threonine/serine kinases are known to be activated by G-protein coupled receptors (GPCRs), tyrosine kinase receptors and growth factor receptors (Steinberg 2008). Using knockout strategies, PKC isoforms have been implicated in a multitude of diseases and organ dysfunctions, such as oxidative stress, fibrosis, diabetes, hypertension, cancer, thrombosis, cardiomyopathy, autoimmune diseases, and other inflammatory processes (Chaudhary and Kasaian 2006; Konopatskaya and Poole 2010; Meier et al. 2009; Salamanca and Khalil 2005). Although inflammation is critical for survival, massive inflammation can also be deleterious, leading to systemic inflammatory response syndrome or other disorders.

A critical step in the inflammatory response is the transmigration of leukocytes through the vascular endothelium

and their migration to the site of inflammation. Neutrophil granulocytes (neutrophils) migrate to the site of injury in response to bacterial or fungal infection or other inflammatory stimuli. Once migrated out of the vascular system, neutrophils chemotax, find the site of injury, phagocytose foreign material, attack bacteria with superoxide, hydrogen peroxide and other oxygen radicals produced by their NADPH oxidase, degranulate to secrete bactericidal molecules, and release inflammatory cytokines and chemokines into the tissue. PKC isoforms contribute to migration and stimulation of various cell types and are implicated in oxidative stress (Geraldes and King 2010). Neutrophils are strongly activated by PKC activating phorbol esters (DeChatelet et al. 1976; Oseas et al. 1981; Wright and Meyer 1986), which together with the expression of several PKC isoforms in neutrophils suggests a role for PKC in neutrophil functions. However, the specific role of the many PKC isoforms in neutrophils is still not clear. This work aims to systemically review what is known about PKC isoforms in neutrophil function, focusing on neutrophil adhesion and activation.

PKC Families and PKC Expression in Neutrophils

The mammalian PKCs have been grouped into three subfamilies according to their domain structure and their regulation (Fig. 1). The conventional PKCs (cPKCs) include PKC-α, the two splice variants PKC-βI and -βII, and PKC-γ. The cPKC isoforms require binding of phosphatidylserine (PS), diacylglycerol (DAG) and calcium for their activation and contain two regulatory domains: C1, which binds to PS and DAG, and the calcium-binding C2 domain. The C1 domain is also a target for the tumor-promoting phorbol ester phorbol 12-myristate 13-acetate (PMA) that activates cPKC by eliminating the requirement for DAG and by decreasing the concentration of calcium necessary for activation (Castagna et al. 1982). The novel PKC (nPKC) isoforms are PKC-δ, PKC-ε, PKC-θ, and PKC-η. These kinases lack the C2 domain and are therefore calcium-insensitive, but they still have a C1 domain and are activated by DAG or phorbol esters (Ono et al. 1988). The atypical PKCs (aPKCs) include the isoforms PKC-ζ and PKC-ι/λ. Since aPKCs lack both the C1 and C2 domain, they are calcium-insensitive and do not respond to PMA or DAG (Ono et al. 1988). Instead, they are activated by the phosphoinositide-dependent protein kinase-1 (PDK-1) downstream of phosphoinositide 3-kinase (PI3K) and its product phosphatidylinositol-3,4,5-trisphosphate (Chou et al. 1998). In order to achieve catalytic competence, the PKC molecule first needs to become phosphorylated on its priming sites (Liu and Heckman 1998). Phosphorylation of PKC and subcellular translocation to the membrane compartment have become the standard parameters to assess PKC activity (Sando et al. 2003).

Neutrophils express PKC-α, PKC-β, and PKC-δ (Balasubramanian et al. 1998; Devalia et al. 1992; Smallwood and Malawista 1992). The expression of other isoforms, namely PKC-ζ, PKC-ι/λ, and PKC-θ, in neutrophils is still a matter of discussion (Balasubramanian et al. 2002; Dang et al. 1994). Specific inhibition of PKC-ζ with a synthetic pseudosubstrate peptide was shown to affect neutrophil function (Laudanna et al. 1998), suggesting that this isoform is also expressed. PKC isoforms are thought to be involved in various neutrophil functions, such as oxidative burst (Kilpatrick et al. 2010), cell apoptosis (Geraldes and King 2010), intracellular pH change during adhesion (Galkina et al. 1996), and early signaling events such as tyrosine phosphorylation following neutrophil stimulation (Brumell et al. 1997).

Neutrophil Rolling and the Impact of PKC on Selectin Signaling

During inflammation, neutrophils roll along the vascular endothelium through a mechanism driven by blood flow and controlled by a continuous succession of forming and breaking molecular bonds. Neutrophil rolling along the endothelium is mediated by selectins, which encompass P-selectin, E-selectin and L-selectin. Of these three members, only L-selectin is expressed on neutrophils. E-selectin

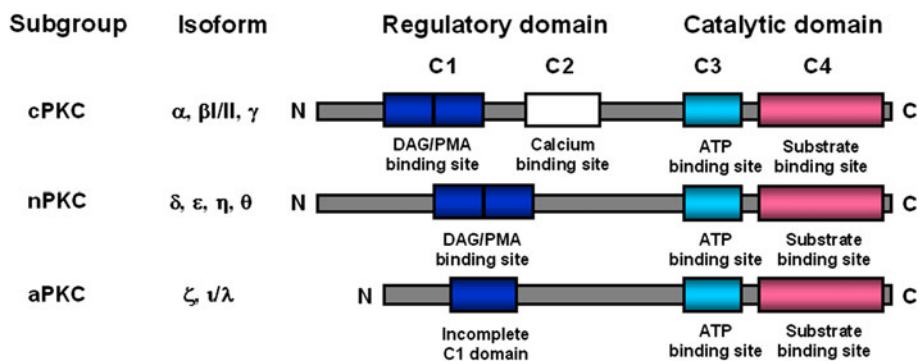

Fig. 1 Domain structure of the conventional, novel and atypical PKC subfamilies cPKC, nPKC, and aPKC. The cPKC isoforms are activated by calcium and DAG or phorbol esters. The nPKCs respond to DAG, but are calcium insensitive, and aPKC isoforms do not require either DAG or calcium. Modified from Meier et al. (2009)

and P-selectin are expressed on endothelial cells. The main binding partner for selectins on leukocytes is P-selectin glycoprotein ligand-1 (PSGL-1), which is also expressed on neutrophils and mediates neutrophil–endothelium interaction by binding P-selectin or E-selectin and neutrophil-neutrophil interaction by binding L-selectin. The initial capture of neutrophils on the endothelium is mediated by binding of PSGL-1 to endothelial selectins (Xia et al. 2002). Reversible binding of P-selectin to PSGL-1 results in rolling velocities of about 40 μm/s (Jung et al. 1996). Under inflammatory conditions, e.g. stimulation with tumor necrosis factor (TNF)-α, E-selectin expression on endothelial cells is upregulated, which leads to a slower rolling of less than 10 μm/s (Kunkel and Ley 1996). This neutrophil deceleration requires expression of intercellular adhesion molecule-1 (ICAM-1) on endothelial cells. ICAM-1 binds to the neutrophil β_2-integrins lymphocyte function-related antigen-1 (LFA-1) and macrophage receptor-1 (Mac-1) in vivo (Dunne et al. 2003). In flow chamber models in vitro, only LFA-1 has been shown to contribute to slow rolling (Zarbock et al. 2007). Engagement of L-selectin in neutrophil-neutrophil interaction is followed by intracellular signaling events leading to increased adhesion via activation of β_2-integrins (Simon et al. 1999). Engagement of PSGL-1 promotes tyrosine phosphorylation and mitogen-activated protein kinase (MAPK) activation via interaction with the ezrin/radixin/moesin (ERM) group (Urzainqui et al. 2002). Recent studies using various gene-deficient mice elucidated the signaling events following binding of endothelial E-selectin to PSGL-1. This signaling cascade starts with the Src family tyrosine kinase that phosphorylates the adaptor molecules DAP-12 and FcR-γ (Zarbock et al. 2008), which in turn recruit spleen tyrosine kinase SYK (Zarbock et al. 2007). SYK phosphorylates Bruton's tyrosine kinase (BTK), which activates phospholipase C (PLC)-γ and PI3K-γ (Mueller et al. 2010). This pathway eventually leads to integrin extension, but does not induce the high affinity conformation of LFA-1 (Kuwano et al. 2010).

It is not known whether PKC isoforms are involved in neutrophil rolling. However, there is evidence for a role of PKC in selectin signaling in T cells. Kilian et al. (2004) showed that PKC-θ and PKC-ι phosphorylate the cytoplasmic domain of L-selectin in Jurkat T cells in vitro, which induced association of PKC-α with L-selectin. Binding of PKC-α and PKC-θ to L-selectin was induced by PMA or T cell receptor stimulation (Kilian et al. 2004). The cytoplasmic domain of L-selectin is crucial for leukocyte tethering, probably by linking L-selectin to the cytoskeleton via interaction with ERM (Ivetic et al. 2004; Kansas et al. 1993). In addition to L-selectin, PKC isoforms were implicated in PSGL-1 stimulated adhesion of T cells on ICAM-1, which was blocked by pharmacological PKC inhibition. The data relying on PKC inhibitors were substantiated by evidence for activation of cPKC isoforms using phospho-PKC antibodies following PSGL-1 cross-linking in T cells (Atarashi et al. 2005). In contrast to the findings implicating PKC isoforms in selectin signaling in T cells, there are no convincing studies investigating the role of PKC in neutrophil tethering, rolling, or selectin signaling.

Chemokine Receptor Signaling can Induce Neutrophil Arrest and Adhesion

During inflammation, many different chemokines are presented on the endothelium. The most relevant ones for neutrophils are the CXC chemokines CXC ligand 1 (CXCL1), also known as growth-regulated oncogene α, and CXCL8, also known as interleukin (IL)-8. These immobilized chemokines are thought to bind to their cognate GPCRs CXCR1 and CXCR2, which are expressed on the neutrophil surface and may come in transient contact with the chemokines during rolling. This initiates an intracellular signaling cascade that results in neutrophil arrest within less than a second (Laudanna and Alon 2006). The final step in this signaling cascade is a conformational change of β_2-integrin conformation through inside-out signaling, which enables the integrin to form a firm bond with endothelial ligands such as ICAM-1. Although LFA-1 in its low affinity conformation cannot bind ICAM-1, it can change into an intermediate conformation after engagement of E-selectin and into a high affinity state after binding of chemokines to GPCRs, thereby mediating slow rolling and arrest, respectively (Zarbock et al. 2007). The signaling cascade leading to GPCR-mediated integrin conformational change involves PLC-γ, guanine nucleotide exchange factors (GEFs) that activate small GTPases, such as Rap1 and RhoA, and actin-binding molecules including talin-1, kindlin-3 and α-actinin (Ley et al. 2007). These actin-binding molecules are thought to be a critical component in stabilizing the integrin high affinity conformation and, therefore, its bond to ICAM-1. However, the complex signaling events involved in GPCR-mediated rapid neutrophil arrest are still far from being completely understood.

Some findings point to a role of PKC isoforms in GPCR-mediated integrin activation. Upon stimulation with the GPCR ligand formyl-methionyl-leucyl-phenylalanine (fMLP), PKC-δ in human neutrophils becomes activated as measured by translocation of the enzyme to the membrane (Kent et al. 1996), thus implicating the involvement of PKC-δ in signaling downstream of GPCR binding. In another study, adhesion of neutrophils from PKC-δ gene deficient mice was investigated in vitro. Upon stimulation

with fMLP or IL-8, PKC-δ gene deficient neutrophils showed markedly reduced adhesion to plates coated with fetal calf serum in comparison to wild type neutrophils (Chou et al. 2004). This type of "static" adhesion assay cannot distinguish whether PKC-δ is involved in inside-out signaling triggering integrin conformational change or in post-adhesion events such as cell spreading and integrin outside-in signaling. Post-adhesion outside-in signaling strongly activates neutrophils through Src family kinases and SYK (Mócsai et al. 1999, 2006). Based on these data, it is not clear whether PKC-δ is located upstream or downstream of neutrophil β_2-integrin activation.

The same is true for PKC-ζ, which was implicated in GPCR-induced adhesion of human neutrophils to fibrinogen (Laudanna et al. 1998). In that work, inhibitory peptides for the PKC-α and PKC-ζ pseudosubstrate regions were used to specifically inhibit these PKC isoforms. While the PKC-α inhibitory peptide had no effect on neutrophil adhesion, the PKC-ζ inhibitory peptide led to a marked reduction in fMLP or IL-8-induced adhesion on fibrinogen. Using pharmacological inhibition with C3 transferase, small GTPases were implicated in PKC-ζ activation in this context (Laudanna et al. 1998). However, whether PKC-ζ is upstream or downstream of β_2-integrin activation remains unclear. Support for the involvement of PKC isoforms in inside-out signaling between GPCR and β_2-integrin comes from a study by Fagerholm et al. (2002). In leukocyte extracts and T cell lysates, PKC-α, PKC-β, PKC-δ, and PKC-η phosphorylated the cytoplasmic domain of the β_2-integrin chain at different, partially overlapping serine and threonine residues. However, PMA-induced β_2-integrin tail phosphorylation does not regulate binding of ICAM-1 (Hibbs et al. 1991), so the physiologic significance of PKC-mediated integrin phosphorylation remains unknown.

Adhesion Strengthening and Crawling

After arrest, adhesion strengthening is the next step to prepare neutrophil migration to the site of inflammation. The activated and bound β_2-integrins play a major role in this step by initiating intracellular signaling events that result in morphological changes of the neutrophil, resulting in spreading and adhesion strengthening. Much of this depends on integrin outside-in signaling (Mócsai et al. 1999, 2006). Other integrin-dependent cellular functions include apoptosis, phagocytosis, and cell migration (Luo et al. 2007; Mayadas and Cullere 2005). The outside-in signaling events downstream of β_2-integrins involve SYK and Src kinases, PI3K, the GEFs VAV1 and VAV3, and small GTPases, such as Cdc42, RhoA, or Rac1 (Gakidis et al. 2004; Giagulli et al. 2006; Mócsai et al. 1999, 2006; Smith et al. 2006). Blocking or knocking out any of these molecules leads to defective neutrophil resistance to shear stress from the blood flow, resulting in premature detachment from the endothelial surface and impaired transmigration. Outside-in signaling events following integrin activation involve rearrangement of integrins on the cell surface (clustering). Restructuring of the basal cytoskeleton probably leads to flattening of surface protrusions (microvilli), which increases available surface area and brings the neutrophil into closer contact with the endothelial cell. Most likely, outside-in signaling also initiates neutrophil polarization, generating a lamellipod in the front and a uropod at the rear (Green et al. 2004). Neutrophil polarization requires actin polarization, which involves the small GTPases RhoA, Cdc42 and Rac2 (Zhelev and Alteraifi 2002). In order to find the right spot to transmigrate through the endothelium, the neutrophils crawl on the endothelial cells. Crawling is dependent on interaction between neutrophil Mac-1 and endothelial ICAM-1. If this step in the adhesion cascade is disturbed, neutrophils start to transmigrate where they adhere, which results in less efficient transmigration (Phillipson et al. 2006).

The impact of PKC isoforms on neutrophil adhesion strengthening is not clear. As described in the previous section, reduced adhesion of PKC-δ gene deficient neutrophils after GPCR ligand activation in vitro could be caused by defective signaling downstream of integrin engagement (Chou et al. 2004). Evidence for the involvement of PKC isoforms in integrin-dependent outside-in signaling comes from platelets, in which fibrinogen binding causes spreading via outside-in signaling downstream of adhesion via the platelet integrin $\alpha_{IIb}\beta_3$. Buensuceso et al. (2005) demonstrated that spreading of platelets on fibrinogen was diminished by broad spectrum PKC inhibitors. They also showed defective spreading of PKC-β deficient platelets on fibrinogen (Buensuceso et al. 2005). Another study implicated PKC-θ in platelet $\alpha_{IIb}\beta_3$-integrin outside-in signaling, and PKC-θ was found to be constitutively associated with the $\alpha_{IIb}\beta_3$-integrin (Soriani et al. 2006). Stimulated by fibrinogen binding to the $\alpha_{IIb}\beta_3$-integrin, PKC-θ then associated with the kinases BTK and SYK, which were required for actin remodeling. Mouse platelets deficient in PKC-θ or BTK failed to spread on fibrinogen (Soriani et al. 2006). In addition to platelets, PKC isoforms were also implicated in integrin outside-in signaling in lymphocytes. LFA-1 clustering induced by the chemokine CCL-21 was markedly reduced by a PKC-ζ pseudosubstrate inhibitory peptide (Giagulli et al. 2004). For neutrophils, a similar approach was used by Chakrabarti and Patel (2008), who examined IL-8-induced Mac-1 clustering with or without the same PKC-ζ inhibitory peptide. By blocking PKC-ζ with that method, Mac-1

clustering was prevented (Chakrabarti and Patel 2008). These findings suggest the involvement of PKC isoforms in outside-in signaling, because clustering of β_2-integrins in neutrophils is mediated via outside-in signaling involving Src family kinases (Mócsai et al. 1999, 2006; Piccardoni et al. 2004). In monocytes, Mac-1, but not LFA-1 clustering was shown to result in phosphorylation and activation of PKC-δ in a Src kinase dependent manner (Xue et al. 2010). Possibly, the reduced static adhesion of PKC-δ gene deficient neutrophils (Chou et al. 2004) is a consequence of defective signaling downstream of Mac-1 clustering, as well. In addition to spreading and adhesion strengthening, PKC isoforms were implicated in integrin-dependent events that are mediated by outside-in signaling, such as superoxide generation and neutrophil degranulation (see next sections).

Phagocytosis

One major task of neutrophils in innate immunity is the phagocytosis of attacking bacteria. There are many different types of phagocytosis, many of which are specific to certain pathogens. Among the best-described mechanisms of phagocytosis are Fcγ and complement receptor dependent ones. Fcγ receptors are responsible for phagocytosis of IgG-coated particles and initiate a signaling cascade involving Src family kinases, PI3K and small GTPases (Huang et al. 2006; Paul et al. 2008). The GTPases RhoA, Cdc42 and Rac1 play distinct roles in the cytoskeletal rearrangement that lead to incorporation of the foreign object (Caron and Hall 1998; Massol et al. 1998; Park and Cox 2009). Fcγ receptor mediated phagocytosis initiates the respiratory burst within the phagosome (Witko-Sarsat et al. 2000). Complement receptors, especially CR3, which is identical to the β_2-integrin Mac-1, are responsible for phagocytosis of C3bi-coated particles. However, C3bi-coated particles can only be phagocytosed if the neutrophils are previously activated by GPCR stimulation, for example by fMLP, or by PKC activation through PMA. Usually, foreign particles are not coated by C3bi alone, but by IgG as well, so the phagocytosis may involve a combination of Mac-1 dependent and Fcγ receptor dependent mechanisms (Witko-Sarsat et al. 2000).

PKC isoforms have been implicated in phagocytosis by macrophages. Although there may be differences between phagocytosis of macrophages and neutrophils, these two "professional" phagocytic cell types also share similarities (Silva 2010). Thus, involvement of PKC isoforms in macrophage phagocytosis potentially suggests a role of PKC in neutrophil phagocytosis. Campos et al. (2009) induced Fcγ receptor mediated phagocytosis in alveolar macrophages by activation with leukotrienes. Studies with pharmacological PKC inhibitors implicated PKC isoforms in the signaling pathway leading to leukotriene-stimulated phagocytosis. Consistently, phosphorylation of PKC-α and PKC-δ was reduced after leukotriene binding (Campos et al. 2009).

Among the PKC isoforms expressed in macrophages, i.e. at least PKC-α, PKC-β, PKC-δ, and PKC-ι/λ, PKC-δ is upregulated by acute and downregulated by chronic PMA treatment. This is accompanied by inverse activity of PI3K and Akt phosphorylation. Knockdown of PKC-δ with shRNA increased Fcγ receptor mediated phagocytosis, which was associated with enhanced PI3K activity. These findings suggest an inhibitory role of PKC-δ in Fcγ receptor mediated phagocytosis (Hazeki et al. 2009). However, this remains to be tested in neutrophils.

Oxidative Burst

After phagocytosis, bacteria are killed by secretion of bactericidal molecules into the phagosome and by the respiratory burst. The latter is elicited by the activation of NADPH oxidase. The neutrophil NADPH oxidase consists of the catalytic subunit $gp91^{phox}$, which is located at the phagosome membrane together with $p22^{phox}$. The assembly of $gp91^{phox}$ and $p22^{phox}$ with the cytosolic subunits $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ and the small GTPase Rac2 results in rapid production of superoxide that is released into the phagosome. Starting with superoxide, H_2O_2 is generated by superoxide dismutase, which is then metabolized to hypochloric acid and other reactive oxygen species by myeloperoxidase. Thereby, the neutrophil possesses a whole array of reactants to damage the ingested attackers (Robinson 2009). The respiratory burst can be initiated by activation of β_2-integrins, which involves signaling via Src family kinases, VAV and PLC-γ (Graham et al. 2007; Paul et al. 2008).

PKC isoforms were implicated in the oxidative burst as the potential key activators of NADPH oxidase, because stimulation of neutrophils with phorbol esters activates PKC and leads to a strong oxidative response (DeChatelet et al. 1976). PKC isoforms are required for phosphorylation of $p47^{phox}$, resulting in the assembly and activation of phagocytic NADPH oxidase (El-Benna et al. 2009). In a cell-free system with recombinant cytosolic proteins, PKC-α, -β, -δ, and -ζ phosphorylated $p47^{phox}$ and initiated superoxide generation. Phosphopeptide mapping showed that PKC-α, -β, and -δ phosphorylated all phosphorylation sites on $p47^{phox}$, and PKC-ζ could phosphorylate a few (Fontayne et al. 2002). PKC-α was further implicated in fMLP- and PMA-induced superoxide generation based on siRNA evidence in HL-60 cells differentiated to resemble granulocytes (Korchak et al. 2007). Brown et al. (2003)

substantiated the role of PKC-δ in NADPH oxidase activation and superoxide generation in neutrophils after stimulation with PMA, and Chou et al. (2004) showed a marked decrease of TNF-α-induced superoxide production in PKC-δ gene deficient neutrophils.

The molecular mechanism by which PKC-δ activates phagocytic NADPH oxidase was elucidated by Cheng et al. (2007). Using COS cells transfected with the essential subunits of phagocytic NADPH oxidase, they showed that PKC-δ, but not PKC-β, is required for fMLP-induced NADPH oxidase activation via phosphorylation of $p47^{phox}$. PKC-δ undergoes autophosphorylation of its activation loop following stimulation with fMLP. Without autophosphorylation, PKC-δ loses its ability to phosphorylate $p47^{phox}$ and to activate phagocytic NADPH oxidase. These results were confirmed for granulocyte-like PLB-985 cells (Cheng et al. 2007). However, Kilpatrick et al. (2010) could not find any reduction in fMLP-induced superoxide production using a PKC-δ selective inhibitory peptide in isolated human neutrophils, while TNF-α-induced superoxide production was delayed. Furthermore, stimulation with TNF-α led to association of PKC-δ with $p47^{phox}$ (Kilpatrick et al. 2010). The discrepant findings regarding fMLP-induced superoxide production could be explained by the use of different cell types, namely the cell line PLB-985 (Cheng et al. 2007) and isolated human neutrophils (Kilpatrick et al. 2010). Although the existing data provide strong evidence for a role of PKC-δ in the oxidative burst, the impact of PKC isoforms in the distinct pathways leading to superoxide generation remains to be clarified.

Degranulation

Neutrophils contain a multitude of bactericidal molecules that are stored in granules, giving the cell the name (neutrophil) granulocyte. Upon activation, the contents of these granules can be released into phagosomes or into the environment. The molecules stored in the granules include lactoferrin, elastase, bactericidal permeability increasing protein (BPI), defensins, myeloperoxidase, cathepsins, and others (Soehnlein 2009). For instance, BPI binds bacterial lipopolysaccharide and is, therefore, a highly effective weapon against Gram-negative bacteria (Schultz et al. 2007). The proteases that are stored in neutrophil granules, such as collagenase or elastase, play a role in the physiological cleanup machinery. Furthermore, granules contain neutrophil surface molecules, among them additional Mac-1, which can be brought to the cell membrane upon activation (Nauseef 2007). Neutrophil degranulation can be initiated by rapid GPCR activation, e.g. via fMLP, or by activation with PMA (Sengeløv et al. 1993). Binding of the neutrophil adhesion molecules L-selectin and Mac-1 seems to enable neutrophil degranulation via outside-in signaling. Mac-1-induced degranulation is mediated by Src family kinase signaling and L-selectin-induced degranulation by MAPK signaling (Mócsai et al. 1999; Smolen et al. 2000).

Considering the role of PKC isoforms in the oxidative burst, the involvement of PKC isoforms in neutrophil degranulation appears possible. In addition to defective adhesion and superoxide generation, PKC-δ gene deficient neutrophil showed markedly reduced lactoferrin degranulation following stimulation with fMLP or PMA (Chou et al. 2004). Popa-Nita et al. (2009) implicated PKC isoforms in urate crystal-induced activation and degranulation of neutrophils, which play a crucial role in gouty inflammation. The cPKC isoforms PKC-α and -β were shown to translocate to the membrane after exposure of the neutrophils to urate crystals in vitro. Knockdown of PKC-α in the myeloid cell line PLB-985 reduced the release of chemoattractants following urate crystal-induced activation. While Src kinases were implicated in PKC activation in urate crystal stimulated neutrophils, SYK was shown to be involved in the signaling pathway downstream of PKC. So far, the contribution of PKC isoforms to neutrophil degranulation has only been investigated using various PKC inhibitors, showing a strong reduction of lysozyme degranulation by pharmacological inhibition of PKC (Popa-Nita et al. 2009). In neutrophil degranulation triggered by adhesion to immunoglobulins, PKC-α and PKC-β together with SYK accumulated in the adherent membrane at the time of degranulation (Nauclér et al. 2002). These findings suggest a signaling pathway involving PKC isoforms and SYK in the initiation of neutrophil degranulation. However, Korchak et al. (2007) did not find an impact of PKC-α knockdown on degranulation in granulocyte-differentiated HL60 cells.

Further Perspectives

PKC isoforms have been implicated in a multitude of intracellular signaling pathways and, amongst others, cytoskeletal rearrangements in diverse cells. Although various PKC isoforms are expressed in neutrophils and cytoskeletal molecules are crucial for a variety of neutrophil functions, the literature on PKC isoforms in neutrophil activation is sparse. Only the contribution of PKC isoforms, mainly PKC-δ, in triggering the intensely investigated oxidative burst can be considered certain. Thus, further research is needed to establish the role of PKC isoforms in the molecular mechanisms leading to neutrophil adhesion and activation in the context of inflammation.

References

Atarashi K, Hirata T, Matsumoto M et al (2005) Rolling of Th1 cells via P-selectin glycoprotein ligand-1 stimulates LFA-1-mediated cell binding to ICAM-1. J Immunol 174:1424–1432

Balasubramanian N, Advani SH, Zingde SM (1998) Protein kinase C isoforms in normal and chronic myeloid leukemic neutrophils. Distinct signal for PKC alpha by immunodetection on PVDF membrane, decreased expression of PKC alpha and increased expression of PKC delta in leukemic neutrophils. Leuk Res 22:597–604

Balasubramanian N, Advani SH, Zingde SM (2002) Protein kinase C isoforms in normal and leukemic neutrophils: altered levels in leukemic neutrophils and changes during myeloid maturation in chronic myeloid leukemia. Leuk Res 26:67–81

Brown GE, Stewart MQ, Liu H et al (2003) A novel assay system implicates PtdIns(3, 4)P(2), PtdIns(3)P, and PKC delta in intracellular production of reactive oxygen species by the NADPH oxidase. Mol Cell 11:35–47

Brumell JH, Chan CK, Butler J et al (1997) Regulation of Src homology 2-containing tyrosine phosphatase 1 during activation of human neutrophils. Role of protein kinase C. J Biol Chem 272:875–882

Buensuceso CS, Obergfell A, Soriani A et al (2005) Regulation of outside-in signaling in platelets by integrin-associated protein kinase C beta. J Biol Chem 280:644–653

Campos MR, Serezani CH, Peters-Golden et al (2009) Differential kinase requirement for enhancement of Fc gammaR-mediated phagocytosis in alveolar macrophages by leukotriene B4 vs. D4. Mol Immunol 46:1204–1211

Caron E, Hall A (1998) Identification of two distinct mechanisms of phagocytosis controlled by different Rho GTPases. Science 282:1717–1721

Castagna M, Takai Y, Kaibuchi K et al (1982) Direct activation of calcium-activated, phospholipid-dependent protein kinase by tumor-promoting phorbol esters. J Biol Chem 257:7847–7851

Chakrabarti S, Patel KD (2008) The atypical zeta (zeta) isoform of protein kinase C regulates CD11b/CD18 activation in human neutrophils. Microcirculation 15:555–567

Chaudhary D, Kasaian M (2006) PKCtheta: a potential therapeutic target for T-cell-mediated diseases. Curr Opin Investig Drugs 7:432–437

Cheng N, He R, Tian J et al (2007) A critical role of protein kinase C delta activation loop phosphorylation in formyl-methionyl-leucyl-phenylalanine-induced phosphorylation of p47(phox) and rapid activation of nicotinamide adenine dinucleotide phosphate oxidase. J Immunol 179:7720–7728

Chou MM, Hou W, Johnson J et al (1998) Regulation of protein kinase C zeta by PI 3-kinase and PDK-1. Curr Biol 8:1069–1077

Chou WH, Choi DS, Zhang H et al (2004) Neutrophil protein kinase Cdelta as a mediator of stroke-reperfusion injury. J Clin Invest 114:49–56

Dang PM, Hakim J, Périanin A (1994) Immunochemical identification and translocation of protein kinase C zeta in human neutrophils. FEBS Lett 349:338–342

DeChatelet LR, Shirley PS, Johnston RB Jr (1976) Effect of phorbol myristate acetate on the oxidative metabolism of human polymorphonuclear leukocytes. Blood 47:545–554

Devalia V, Thomas NS, Roberts PJ et al (1992) Down-regulation of human protein kinase C alpha is associated with terminal neutrophil differentiation. Blood 80:68–76

Dunne JL, Collins RG, Beaudet AL et al (2003) Mac-1, but not LFA-1, uses intercellular adhesion molecule-1 to mediate slow leukocyte rolling in TNF-alpha-induced inflammation. J Immunol 171:6105–6111

El-Benna J, Dang PM, Gougerot-Pocidalo MA et al (2009) p47phox, the phagocyte NADPH oxidase/NOX2 organizer: structure, phosphorylation and implication in diseases. Exp Mol Med 41:217–225

Fagerholm S, Morrice N, Gahmberg CG et al (2002) Phosphorylation of the cytoplasmic domain of the integrin CD18 chain by protein kinase C isoforms in leukocytes. J Biol Chem 277:1728–1738

Fontayne A, Dang PM, Gougerot-Pocidalo MA et al (2002) Phosphorylation of p47phox sites by PKC alpha, beta II, delta, and zeta: effect on binding to p22phox and on NADPH oxidase activation. Biochemistry 41:7743–7750

Gakidis MA, Cullere X, Olson T et al (2004) Vav GEFs are required for beta2 integrin-dependent functions of neutrophils. J Cell Biol 166:273–282

Galkina SI, Sud'ina GF, Margolis LB (1996) Regulation of intracellular pH by phospholipase A2 and protein kinase C upon neutrophil adhesion to solid substrata. FEBS Lett 393:117–120

Geraldes P, King GL (2010) Activation of protein kinase C isoforms and its impact on diabetic complications. Circ Res 106:1319–1331

Giagulli C, Scarpini E, Ottoboni L (2004) RhoA and zeta PKC control distinct modalities of LFA-1 activation by chemokines: critical role of LFA-1 affinity triggering in lymphocyte in vivo homing. Immunity 20:25–35

Giagulli C, Ottoboni L, Caveggion E et al (2006) The Src family kinases Hck and Fgr are dispensable for inside-out, chemoattractant-induced signaling regulating beta 2 integrin affinity and valency in neutrophils, but are required for beta 2 integrin-mediated outside-in signaling involved in sustained adhesion. J Immunol 177:604–611

Graham DB, Robertson CM, Bautista J et al (2007) Neutrophil-mediated oxidative burst and host defense are controlled by a Vav-PLCgamma2 signaling axis in mice. J Clin Invest 117:3445–3452

Green CE, Pearson DN, Camphausen RT et al (2004) Shear-dependent capping of L-selectin and P-selectin glycoprotein ligand 1 by E-selectin signals activation of high-avidity beta2-integrin on neutrophils. J Immunol 172:7780–7790

Hazeki K, Inoue K, Nigorikawa K et al (2009) Negative regulation of class IA phosphoinositide 3-kinase by protein kinase Cdelta Limits Fcgamma receptor-mediated phagocytosis in macrophages. J Biochem 145:87–94

Hibbs ML, Jakes S, Stacker SA et al (1991) The cytoplasmic domain of the integrin lymphocyte function-associated antigen 1 beta subunit: sites required for binding to intercellular adhesion molecule 1 and the phorbol ester-stimulated phosphorylation site. J Exp Med 174:1227–1238

Huang ZY, Barreda DR, Worth RG et al (2006) Differential kinase requirements in human and mouse Fc-gamma receptor phagocytosis and endocytosis. J Leukoc Biol 80:1553–1562

Ivetic A, Florey O, Deka J et al (2004) Mutagenesis of the ezrin-radixin-moesin binding domain of L-selectin tail affects shedding, microvillar positioning, and leukocyte tethering. J Biol Chem 279:33263–33272

Jung U, Bullard DC, Tedder TF et al (1996) Velocity differences between L- and P-selectin-dependent neutrophil rolling in venules of mouse cremaster muscle in vivo. Am J Physiol 271:H2740–H2747

Kansas GS, Ley K, Munro JM et al (1993) Regulation of leukocyte rolling and adhesion to endothelium by the cytoplasmic domain of L-selectin. J Exp Med 177:833–838

Kent JD, Sergeant S, Burns DJ et al (1996) Identification and regulation of protein kinase C-delta in human neutrophils. J Immunol 157:4641–4647

Kilian K, Dernedde J, Mueller EC et al (2004) The interaction of protein kinase C isozymes alpha, iota, and theta with the

cytoplasmic domain of L-selectin is modulated by phosphorylation of the receptor. J Biol Chem 279:34472–33480
Kilpatrick LE, Sun S, Li H et al (2010) Regulation of TNF-induced oxygen radical production in human neutrophils: role of delta-PKC. J Leukoc Biol 87:153–164
Konopatskaya O, Poole AW (2010) Protein kinase Calpha: disease regulator and therapeutic target. Trends Pharmacol Sci 31:8–14
Korchak HM, Dorsey LB, Li H et al (2007) Selective roles for alpha-PKC in positive signaling for O-(2) generation and calcium mobilization but not elastase release in differentiated HL60 cells. Biochim Biophys Acta 1773:440–449
Kunkel EJ, Ley K (1996) Distinct phenotype of E-selectin-deficient mice. E-selectin is required for slow leukocyte rolling in vivo. Circ Res 79:1196–1204
Kuwano Y, Spelten O, Zhang H et al (2010) Rolling on E- or P-selectin induces the extended, but not high affinity conformation of LFA-1 in neutrophils. Blood 116:617–624
Laudanna C, Alon R (2006) Right on the spot. Chemokine triggering of integrin-mediated arrest of rolling leukocytes. Thromb Haemost 95:5–11
Laudanna C, Mochly-Rosen D, Liron T et al (1998) Evidence of zeta protein kinase C involvement in polymorphonuclear neutrophil integrin-dependent adhesion and chemotaxis. J Biol Chem 273:30306–30315
Ley K, Laudanna C, Cybulsky MI et al (2007) Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nat Rev Immunol 7:678–689
Liu WS, Heckman CA (1998) The sevenfold way of PKC regulation. Cell Signal 10:529–542
Luo BH, Carman CV, Springer TA (2007) Structural basis of integrin regulation and signaling. Annu Rev Immunol 25:619–647
Massol P, Montcourrier P, Guillemot JC et al (1998) Fc receptor-mediated phagocytosis requires CDC42 and Rac1. EMBO J 17:6219–6229
Mayadas TN, Cullere X (2005) Neutrophil beta2 integrins: moderators of life or death decisions. Trends Immunol 26:388–395
Meier M, Menne J, Haller H (2009) Targeting the protein kinase C family in the diabetic kidney: lessons from analysis of mutant mice. Diabetologia 52:765–775
Mócsai A, Ligeti E, Lowell CA et al (1999) Adhesion-dependent degranulation of neutrophils requires the Src family kinases Fgr and Hck. J Immunol 162:1120–1126
Mócsai A, Abram CL, Jakus Z et al (2006) Integrin signaling in neutrophils and macrophages uses adaptors containing immunoreceptor tyrosine-based activation motifs. Nat Immunol 7:1326–1333
Mueller H, Stadtmann A, Van Aken H et al (2010) Tyrosine kinase Btk regulates E-selectin-mediated integrin activation and neutrophil recruitment by controlling phospholipase C (PLC) gamma2 and PI3Kgamma pathways. Blood 115:3118–3127
Nauclér C, Grinstein S, Sundler R et al (2002) Signaling to localized degranulation in neutrophils adherent to immune complexes. J Leukoc Biol 71:701–710
Nauseef WM (2007) How human neutrophils kill and degrade microbes: an integrated view. Immunol Rev 219:88–102
Ono Y, Fujii T, Ogita K et al (1988) The structure, expression, and properties of additional members of the protein kinase C family. J Biol Chem 263:6927–6932
Oseas R, Yang HH, Baehner RL et al (1981) Lactoferrin: a promoter of polymorphonuclear leukocyte adhesiveness. Blood 57:939–945
Park H, Cox D (2009) Cdc42 regulates Fc gamma receptor-mediated phagocytosis through the activation and phosphorylation of Wiskott–Aldrich syndrome protein (WASP) and neural-WASP. Mol Biol Cell 20:4500–4508
Paul R, Obermaier B, Van Ziffle J et al (2008) Myeloid Src kinases regulate phagocytosis and oxidative burst in pneumococcal meningitis by activating NADPH oxidase. J Leukoc Biol 84:1141–1150
Phillipson M, Heit B, Colarusso P et al (2006) Intraluminal crawling of neutrophils to emigration sites: a molecularly distinct process from adhesion in the recruitment cascade. J Exp Med 203:2569–2575
Piccardoni P, Manarini S, Federico L et al (2004) SRC-dependent outside-in signalling is a key step in the process of autoregulation of beta2 integrins in polymorphonuclear cells. Biochem J 380:57–65
Popa-Nita O, Proulx S, Paré G et al (2009) Crystal-induced neutrophil activation: XI. Implication and novel roles of classical protein kinase C. J Immunol 183:2104–2114
Robinson JM (2009) Phagocytic leukocytes and reactive oxygen species. Histochem Cell Biol 131:465–469
Salamanca DA, Khalil RA (2005) Protein kinase C isoforms as specific targets for modulation of vascular smooth muscle function in hypertension. Biochem Pharmacol 70:1537–1547
Sando JJ, Beals JK, Hussaini IM (2003) Subcellular translocation of protein kinase C. Methods Mol Biol 233:77–85
Schultz H, Hume J, Zhang de S et al (2007) A novel role for the bactericidal/permeability increasing protein in interactions of Gram-negative bacterial outer membrane blebs with dendritic cells. J Immunol 179:2477–2484
Sengeløv H, Kjeldsen L, Diamond MS et al (1993) Subcellular localization and dynamics of Mac-1 (alpha m beta 2) in human neutrophils. J Clin Invest 92:1467–1476
Silva MT (2010) When two is better than one: macrophages and neutrophils work in concert in innate immunity as complementary and cooperative partners of a myeloid phagocyte system. J Leukoc Biol 87:93–106
Simon SI, Cherapanov V, Nadra I et al (1999) Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and colocalization with CD18. J Immunol 163:2891–2901
Smallwood JI, Malawista SE (1992) Protein kinase C isoforms in human neutrophil cytoplasts. J Leukoc Biol 51:84–92
Smith DF, Deem TL, Bruce AC et al (2006) Leukocyte phosphoinositide-3 kinase gamma is required for chemokine-induced, sustained adhesion under flow in vivo. J Leukoc Biol 80:1491–1499
Smolen JE, Petersen TK, Koch C et al (2000) L-selectin signaling of neutrophil adhesion and degranulation involves p38 mitogen-activated protein kinase. J Biol Chem 275:15876–15884
Soehnlein O (2009) Direct and alternative antimicrobial mechanisms of neutrophil-derived granule proteins. J Mol Med 87:1157–1164
Soriani A, Moran B, de Virgilio M et al (2006) A role for PKCtheta in outside-in alpha(IIb)beta3 signaling. J Thromb Haemost 4:648–655
Steinberg SF (2008) Structural basis of protein kinase C isoform function. Physiol Rev 88:1341–1378
Urzainqui A, Serrador JM, Viedma F et al (2002) ITAM-based interaction of ERM proteins with Syk mediates signaling by the leukocyte adhesion receptor PSGL-1. Immunity 17:401–412
Witko-Sarsat V, Rieu P, Descamps-Latscha B et al (2000) Neutrophils: molecules, functions and pathophysiological aspects. Lab Invest 80:617–653
Wright SD, Meyer BC (1986) Phorbol esters cause sequential activation and deactivation of complement receptors on polymorphonuclear leukocyte. J Immunol 136:1759–1764
Xia L, Sperandio M, Yago T et al (2002) P-selectin glycoprotein ligand-1-deficient mice have impaired leukocyte tethering to E-selectin under flow. J Clin Invest 109:939–950
Xue ZH, Zhao CQ, Chua GL et al (2010) Integrin alphaMbeta2 clustering triggers phosphorylation and activation of protein

kinase C delta that regulates transcription factor Foxp1 expression in monocytes. J Immunol 184:3697–3709

Zarbock A, Lowell CA, Ley K (2007) Spleen tyrosine kinase Syk is necessary for E-selectin-induced alpha(L)beta(2) integrin-mediated rolling on intercellular adhesion molecule-1. Immunity 26:773–783

Zarbock A, Abram CL, Hundt M et al (2008) PSGL-1 engagement by E-selectin signals through Src kinase Fgr and ITAM adapters DAP12 and FcR gamma to induce slow leukocyte rolling. J Exp Med 205:2339–2347

Zhelev DV, Alteraifi A (2002) Signaling in the motility responses of the human neutrophil. Ann Biomed Eng 30:356–370