Atypical λ1PKC Conveys 5-Lipoxygenase/Leukotriene B4-mediated Cross-talk between Phospholipase A2’s Regulating NF-κB Activation in Response to Tumor Necrosis Factor-α and Interleukin-1β*

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Marit W. Anthonsen‡, Sonja Andersen, Anita Solhaug, and Berit Johansen

From the UNIGEN Center for Molecular Biology, Faculty of Chemistry and Biology, Norwegian University of Science and Technology, N-7491 Trondheim, Norway

The transcription factor nuclear factor κB (NF-κB) plays crucial roles in a wide variety of biological functions such as inflammation, stress, and immune responses. We have shown previously that secretory non-pancreatic (snp) and cytosolic (c) phospholipase A2 (PLA2) regulate NF-κB activation in response to tumor necrosis factor (TNF)-α or interleukin (IL)-1β activation and that a functional coupling mediated by the 5-lipoxygenase (5-LO) metabolite leukotriene B4 (LTB4) exists between snpPLA2 and cPLA2 in human keratinocytes. In this study, we have further investigated the mechanisms of PLA2-modulated NF-κB activation with respect to specific kinases involved in TNF-α/IL-1β-stimulated cPLA2 phosphorylation and NF-κB activation. The protein kinase C (PKC) inhibitors RO 31-8220, Go6976, and a pseudosubstrate peptide inhibitor of atypical PKCs attenuated arachidonic acid release, cPLA2 phosphorylation, and NF-κB activation induced by TNF-α or IL-1β, thus indicating atypical PKCs in cPLA2 regulation and transcription factor activation. Transfection of a kinase-inactive mutant of λ1PKC in NIH-3T3 fibroblasts completely abolished TNF-α/IL-1β-stimulated cellular arachidonic acid release and cPLA2 activation assayed in vitro, confirming the role of λ1PKC in cPLA2 regulation. Furthermore, λ1PKC and cPLA2 phosphorylation was attenuated by phosphatidyinositol 3-kinase (PI3-kinase) inhibitors, which also reduced NF-κB activation in response to TNF-α and IL-1β, indicating a role for PI3-kinase in these processes in human keratinocytes. TNF-α and IL-1β-induced phosphorylation of λ1PKC was attenuated by inhibitors toward snpPLA2 and 5-LO and by an LTB4 receptor antagonist, suggesting λ1PKC as a downstream effector of snpPLA2 and 5-LO/LTB4 the LTB4 receptor. Hence, λ1PKC regulates snpPLA2/LTB4-mediated cPLA2 activation, cellular arachidonic acid release, and NF-κB activation induced by TNF-α and IL-1β. In addition, our results demonstrate that PI3-kinase and λ1PKC are involved in cytokine-induced cPLA2 and NF-κB activation, thus identifying λ1PKC as a novel regulator of cPLA2.

Phospholipase A2 (PLA2) enzymes play a crucial role in liberating free fatty acids and lysophospholipids from membrane phospholipids, thereby initiating the production of biologically active lipids, which mediate inflammatory reactions. PLA2 cleave the fatty acid substituent from the sn-2 position of membrane phospholipids leading to production of platelet-activating factor, lysophosphatidic acid, and arachidonic acid (AA). AA is metabolized to the bioactive eicosanoids, including prostaglandins, hydroxy fatty acids, leukotrienes, and thromboxanes, whose action is in part mediated by cell surface G-protein-coupled receptors (1, 2). Numerous types of mammalian PLA2s having distinct enzymatic properties, including substrate specificity and Ca2+ requirements, have been identified and classified into several groups (3, 4). Secretory low molecular weight PLA2s (14–16 kDa) are characterized by an absolute requirement for millimolar concentrations of Ca2+ and a broad specificity for phospholipids with different polar head groups and fatty acyl chains (5). Several distinct secretory nonpancreatic PLA2s (snpPLA2), including group IIA, IID, IIE, IIF, III, V, and X, have been identified, and the roles of different snpPLA2s in regulation of cellular AA release and lipid mediator generation are extensively studied (4, 6, 7). Increased levels of group IIA snpPLA2 in circulation and affected tissues have been found in association with various pathological conditions, including rheumatoid arthritis, sepsis, acute pancreatitis, psoriasis, and in endotoxic shock (8–11). Group IV cytosolic PLA2 (cPLA2) consists of three human paralogs (12), of which cPLA2 is, herein denoted cPLA2, is ubiquitously expressed. cPLA2 is essential for prostaglandin, leukotriene, and platelet-activating factor formation, as determined, e.g., in cPLA2 knockout mice (13). Activation of cPLA2 upon cellular stimulation is mediated by Ca2+-dependent translocation to membranes and by phosphorylations catalyzed by MAP kinase cascades (14, 15). Many genes that are implicated in the initiation of inflammatory processes are regulated by the transcription factor NF-κB, e.g., inflammatory cytokines (IL-1β, TNF-α), chemokines (IL-8), and the adhesion molecules ICAM-1 and VCAM-1 (16). In resting cells, NF-κB is sequestered in an inactive form in cytosol bound to its inhibitor, IκB. Following cellular activation

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‡ To whom correspondence should be addressed. Tel.: 47 73 551278; Fax: 47 73 596100.

1 The abbreviations used are: PLA2, phospholipase A2; cPLA2, cytosolic phospholipase A2; snpPLA2, secretory nonpancreatic phospholipase A2; MAP, mitogen-activated protein kinase; PKC, protein kinase C; dm/λ1PKC, dominant negative λ1PKC; TRAF, TNF receptor-associated factor; PMA, phorbol 12-myristate 13-acetate; PI3, phosphatidylinositol 3; AA, arachidonic acid; BSA, bovine serum albumin; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; PAGE, polyacrylamide gel electrophoresis.
by inflammatory cytokines, e.g. TNF-α and IL-1β, UV irradiation, or bacterial products, isB is phosphorylated and proteolytically degraded, leading to nuclear translocation of NF-κB. The signaling cascades emerging from TNF-α and IL-1 receptor 1 leading to NF-κB activation are in part understood and involve receptor-associated proteins of the TNF-α receptor-associated protein family (TRAF) (17, 18) and receptor-interacting protein (19) in the TNF-α-induced pathway. The IL-1β-induced pathway is initiated by recruitment of TRAF6 (20) and the kinase IL-1 receptor-associated kinase (21) to the IL-1β receptor. Both TNF-α and IL-1β activate the recently identified kinases, NF-κB-inducing kinase (22), IKKα (23), and IKKβ (24), which leads to phosphorylation and degradation of the NF-κB inhibitor IκB. Other identified components in cytokine-induced NF-κB activation include phosphatidylcholine-specific phospholipase C, sphingomyelinase, and protein kinase C enzymes, but their mechanisms in NF-κB activation are not fully understood (25-27).

We have recently reported the involvement of snpPLA2, LTβ, and cPLA2 in TNF-α and IL-1β-induced NF-κB activation in human keratinocytes (28, 29). We also found that a functional coupling between snpPLA2 and cPLA2 in which cPLA2 phosphorylation and activation was preceded by snpPLA2/5-LO/LTB4 activity, was of importance for cytokine-induced NF-κB activation (29). In this study, we have addressed the signaling mechanisms and kinases mediating cytokine-stimulated cPLA2 and NF-κB activation. We present herein novel results showing that atypical λ/ιPKC, in a PI3-kinase-dependent manner, acts downstream of snpPLA2 and 5-LO/LTβ and regulates cPLA2 phosphorylation, leading to cellular AA mobilization and PLAc-mediated NF-κB activation in response to TNF-α and IL-1β.

EXPERIMENTAL PROCEDURES

Materials—Human interleukin-1β (IL-1β; 5 × 10^6 units/mg), leupeptin, and pepstatin were from Roche Molecular Biochemicals. TNF-α (specific activity 7.6 × 10^6 units/mg protein, Genentech Inc.) was a generous gift from Prof. Terje Espesvik, Norwegian University of Science and Technology Trondheim, Norway. Fatty acid-free bovine serum albumin (BSA), phorbol 12-myristate 13-acetate (PMA), phosphatidylcholine, and phosphatidylserine were from Sigma. Methyl arachidonylfluorophosphate (MAFP) and arachidonic acid (AA) were obtained from Cayman Chemicals. 12-Epi-secalarubin, α-pentyl-4-(2-quinoilinylmethoxy)-benzene methanol (L-655,238), and MR-886 were obtained from Maxymeeting, PA. Goat anti-λ/ιPKC (1:1000), rabbit anti-cPLA2 (1:2000). Protein levels were determined using the Luciferase Reporter Assay System (Promega) and Turner Luminometer model TD-20/20 (Turner Design) as described by the manufacturer.

Measurement of [3H]Arachidonic Acid Release—NIH-3T3 and HaCaT cells were cultured in DMEM (with 4.5 g or 1 g of glucose/liter for NIH-3T3 and HaCaT, respectively) and 10% FCS to 50% confluency (NIH-3T3) or to 2 days post-confluency (HaCaT). Cells were labeled for 16 h with [3H]AA (0.4 μCi/ml) in DMEM culture medium containing 0.5% FCS. About 90% of the added [3H]AA was incorporated by this procedure. After labeling, the cells were washed two times with phosphate-buffered saline containing fatty acid-free BSA (2 mg/ml) in order to remove unincorporated radioactivity. Cells were then allowed to equilibrate at 37 °C before addition of indicated reagents (10 min). The release of [3H]AA from the cells was assessed by liquid scintillation counting. Adherent cells were dissolved in 1 ml NaOH in order to determine incorporated [3H]AA in the cells by liquid scintillation counting. The results are given as released [3H]AA in the supernatant relative to total [3H]AA incorporated into the cells.

Assay of cPLA2 Activity—NIH-3T3 cells were cultured in DMEM with 4.5 g of glucose/liter and 10% FCS to 50% confluency in 6-round multiwell plates and starved in 0.5% FCS-containing DMEM for 24 h. Stimulated NIH-3T3 cells were scraped into lysis buffer (10 mM Hepes, pH 7.5, 0.5% sucrose, 0.8 mM Triton X-100, 1 mM EDTA, 1 mM EGTA, 10 μg/ml leupeptin, 1 mM phenylmethylsulfonyl fluoride, 1 μg pepstatin), homogenized by 10 passes through a 26-gauge needle, and centrifuged (14,000 rpm, 10 min, 4 °C). For each sample, 70 μl of total protein of the supernatant was assayed for cPLA2 activity using sonicated vesicles of 1-palmitoyl-2-arachidonoyl-sn-glycerol-3-phosphorylcholine (100 μM) containing 100,000 cpm of 1-palmitoyl-2-[1-14C]arachidonoyl-sn-glycerol-3-phosphorylcholine (specific activity, 40-60 mCi/mmol) in order to equilibrate at 37 °C before addition of indicated reagents (10 min). The release of [3H]AA in the cells by liquid scintillation counting. The results are given as released [3H]AA in the supernatant relative to total [3H]AA incorporated into the cells.

Membrane Preparation—NIH-3T3 or HaCaT cells were homogenized in 100,000 cpm of 1-palmitoyl-2-[1-14C]arachidonoyl-sn-glycerol-3-phosphorylcholine (specific activity, 40-60 mCi/mmol) and lysed for 20 min at 4 °C. The reaction was terminated by liquid scintillation counting. Adherent cells were dissolved in 1 ml NaOH in order to determine incorporated [3H]AA in the cells by liquid scintillation counting. The results are given as released [3H]AA in the supernatant relative to total [3H]AA incorporated into the cells.

Protein Analysis—Protein levels were determined using theBCA Assay (Pierce) and the Bio-Rad Protein Assay (Bio-Rad) using BSA as standards.
results indicate that atypical PKCs and p38 MAP kinase are involved in TNF-α/IL-1β-induced NF-κB activation in human HaCaT keratinocytes.

Cellular AA Release and cPLA₂ Phosphorylation in Response to IL-1β Are Mediated by PKC—To examine if PKCs regulate PLA₂ enzymes in HaCaT cells, we examined the effects of RO 31-8220 and the atypical PKC pseudosubstrate peptide inhibitor on cellular AA mobilization. Both inhibitors dose-dependently impaired IL-1β-elicited AA release (Fig. 2A), indicating that atypical PKCs act upstream of AA releasing PLA₂ enzymes. We have previously shown that TNF-α- and IL-1β-stimulated AA release from HaCaT cells is accompanied by cPLA₂ phosphorylation (29), and phosphorylation of cPLA₂ is important for its activation and AA releasing activity in response to cytokines (45–47). To determine if the inhibitory effect of RO 31-8220 on AA mobilization correlated with reduced phosphorylation of cPLA₂, we analyzed cPLA₂ phosphorylation levels by in vivo phosphorylation experiments. HaCaT cells were metabolically labeled with [32P]orthophosphate and pretreated with RO 31-8220 before addition of IL-1β. Treat-
ment with TNF-α or IL-1β for 1 h resulted in 2–4-fold increase in cPLA2 phosphorylation as reported previously (29), and IL-1β-stimulated phosphorylation of cPLA2 was reduced in the presence of RO 31-8220 (1 μM; Fig. 2B). Similar results were obtained with TNF-α (data not shown). These results indicate that atypical PKCs (since the specific pseudosubstrate inhibitor attenuates AA liberation) are upstream mediators of TNF-α/IL-1β-induced cPLA2 phosphorylation and AA mobilization in HaCaT keratinocytes.

Transfection of a Kinase-inactive λ/ιPKC Abrogates TNF-α-stimulated AA Mobilization and cPLA2 Activation—To investigate further the participation of the atypical PKC isoform λ/ιPKC in cPLA2 activation and AA release, we examined TNF-α-stimulated AA release in NIH-3T3 fibroblasts transfected with v-ras or doubly-transfected with v-ras and a kinase-inactive mutant of λ/ιPKC previously shown to act in a dominant negative manner (48). Transfection of the kinase-inactive λ/ιPKC abolished TNF-α-elicited AA release compared with v-ras-transfected cells, reducing AA liberation in v-ras-dn/λ/ιPKC-transfected cells to similar levels as in wild type NIH-3T3 cells (Fig. 3A). Also, TNF-α-induced AA liberation in v-ras-transfected cells was completely blocked by the PKC inhibitor RO 31-8220 (10 μM) and stimulated with TNF-α (20 ng/ml; 2 h) was determined as described under “Experimental Procedures.” The results are normalized to show fold induction relative to untreated cells. The data are representative of three independent experiments and are expressed as mean ± S.D. of triplicate (A) or duplicate (B) determinations. * and ** indicate that values are statistically different from untreated (*) or TNF-α-stimulated cells (**).
treated from 30 min to 10 h, whereas cPLA₂ activity in lysates from v-ras-transfected cells was reproducibly increased 1.5–2-fold (Fig. 3B). The TNF-α-induced cPLA₂ activity was inhibited by the cPLA₂ inhibitor MAFP (Fig. 3B). These results confirm results shown in Fig. 2, thus suggesting that λ/ιPKC is an upstream mediator of TNF-α-elicted cPLA₂ activation.

**TNF-α- and IL-1β-induced Phosphorylation of Atypical PKCs**

**Is PI3-Kinase-dependent—**Atypical PKCs have been reported to be phosphorylated in a PI3-kinase dependent manner in response to different stimuli (49–51). The PI3-kinase inhibitor LY294002 dose-dependently inhibited TNF-α- and IL-1β-stimulated NF-κB activation in HaCaT keratinocytes (Fig. 5A). Also, wortmannin, a PI3-kinase inhibitor structurally unrelated to LY294002, resulted in complete inhibition of cytokine-stimulated NF-κB activation at 100 nM (data not shown), indicating that PI3-kinase contributes to NF-κB activation. To investigate if PI3-kinase is involved in cytokine-elicited λ/ιPKC and cPLA₂ phosphorylation in HaCaT keratinocytes, we pre-treated [³²P]orthophosphate-labeled cells with the PI3-kinase inhibitor LY294002 before addition of TNF-α or IL-1β. The PI3-kinase and 5-LO/LTB₄ inhibitors were involved in cytokine-induced NF-κB activation, cellular AA release, and cPLA₂ phosphorylation. To examine the sequential involvement of 5-LO/LTB₄ and λ/ιPKC, we studied the effect of inhibitors of the 5-LO pathway on cPLA₂ phosphorylation and AA release in cytokine-treated HaCaT keratinocytes (29). Hence, from our previous and present results, we propose that both atypical λ/ιPKC and 5LO/LTB₄ are involved in cytokine-induced NF-κB activation, cellular AA release, and cPLA₂ phosphorylation. To examine the sequential involvement of 5-LO/LTB₄ and λ/ιPKC, we studied the effect of inhibitors of the 5-LO pathway on cPLA₂ phosphorylation and AA release in cytokine-treated HaCaT keratinocytes (29). Hence, from our previous and present results, we propose that both atypical λ/ιPKC and 5LO/LTB₄ are involved in cytokine-induced NF-κB activation, cellular AA release, and cPLA₂ phosphorylation. To examine the sequential involvement of 5-LO/LTB₄ and λ/ιPKC, we studied the effect of inhibitors of the 5-LO pathway on cPLA₂ phosphorylation and AA release in cytokine-treated HaCaT keratinocytes (29). Hence, from our previous and present results, we propose that both atypical λ/ιPKC and 5LO/LTB₄ are involved in cytokine-induced NF-κB activation, cellular AA release, and cPLA₂ phosphorylation.

**snpPLA₂ Activity Precedes λ/ιPKC Phosphorylation Induced by TNF-α and IL-1β—**We have demonstrated recently that catalytic activity of snpPLA₂ and 5-LO is necessary for TNF-α/IL-1β-induced cPLA₂ phosphorylation and NF-κB activation (29). Therefore, we next examined if snpPLA₂ activity also precedes λ/ιPKC phosphorylation, by preincubating [³²P]orthophosphate-labeled HaCaT keratinocytes with snpPLA₂ inhibitors before cytokine treatment. The group IIa snpPLA₂ active site-directed inhibitor SB203347 (54, 55) and 12-epi-scalaradial (56) significantly reduced λ/ιPKC phosphorylation induced by IL-1β and TNF-α (Fig. 6). To elucidate whether the inhibitory effect of snpPLA₂ inhibitors on λ/ιPKC phosphorylation was due to reduced AA levels, we examined the influence of AA addition on λ/ιPKC phosphorylation in the presence of snpPLA₂ inhibitors. Exogenously added AA was able to relieve the inhibitory effect of 12-epi-scalaradial on IL-1β- and TNF-α-induced phosphorylation of λ/ιPKC (Fig. 6A; data not shown). Similar results with addition of AA were obtained for λ/ιPKC phosphorylation inhibited by the active site-directed snpPLA₂ inhibitor SB203347 (data not shown). Hence, we may suggest that snpPLA₂ activity and AA...
contribute to PKC activation in TNF- and IL-1-stimulated HaCaT keratinocytes.

**DISCUSSION**

AA metabolites regulate a variety of inflammatory responses, including chemoattractant properties, integrin activa-
Atypical λ/ιPKC and PLA2-mediated NF-κB Activation

We have recently shown that both spleen PLAPα and cPLA2, through AA-derived 5-LO metabolites as LTB4 acting through its G-protein-coupled receptor in an autocrine feedback mechanism, contribute to activation of the immunomodulatory transcription factor NF-κB in response to TNF-α and IL-1β (29). Other authors (62, 63) have also described that snpPLAPα and LTB4 (acting in an autocrine fashion) increase AA release, cPLA2 phosphorylation, and cPLA2 activation. However, the mechanisms explaining how snpPLAPα/LTB4 regulate cPLA2 activation is not understood in detail. In the present study we show that atypical λ/ιPKC in a PI3-kinase-dependent manner conveys snpPLAPα/LTB4-mediated cPLA2 phosphorylation and cellular AA mobilization in response to TNF-α and IL-1β, promoting NF-κB activation. Several lines of evidence support the sequential involvement of snpPLAPα/LTB4, λ/ιPKC, and cPLA2 as follows. 1) TNF-α/IL-1β-stimulated AA liberation and cPLA2 phosphorylation are reduced by PKC inhibitors. 2) TNF-α-induced AA release and cPLA2 activation are abrogated by transfection of a kinase-inactive mutant of λ/ιPKC kinase. 3) PI3-kinase is activated by cytokines and λ/ιPKC, and cPLA2 phosphorylation is inhibited in a PI3-kinase-dependent manner. 4) Inhibitors of 5-LO/LTB4 functionality attenuate cytokine-elicited λ/ιPKC phosphorylation. 5) TNF-α/IL-1β-stimulated λ/ιPKC phosphorylation is reduced by snpPLAPα inhibitors and relieved by exogenously added AA. Additional information on the sequential involvement of PLAPα-s and LTB4 is given by our previous results showing that snpPLAPα/5-LO/LTB4 regulates AA release and cPLA2 phosphorylation (29), also illustrating the positive feedback mechanism exerted by LTB4 in HaCaT keratinocytes, as suggested in other cell types.

Previously, only phorbol ester-sensitive PKCs have been implicated in cPLA2 phosphorylation and activation (64–66). In contrast, our results demonstrate for the first time that PMA-insensitive atypical λ/ιPKC regulates cPLA2 activity and AA liberation. It has been shown previously that PMA induces cPLA2 phosphorylation in human platelets, but PKC inhibitors fail to reduce cPLA2 phosphorylation and AA release, thus illustrating that PMA-stimulated cPLA2 phosphorylation is non-physiological (67). Although Ser-727 in cPLA2 is located in a consensus motif that can be phosphorylated by PKC or PKA (14), and the enzyme is phosphorylated by PKC in vitro (68, 69), it is uncertain if λ/ιPKC directly phosphorylates cPLA2 in our model. Perhaps more probable is that cPLA2 phosphorylation conveyed by λ/ιPKC is brought about in a p38 MAP kinase-dependent manner causing phosphorylation on Ser-505 as reported (70, 71) and possibly on Ser-727, recently shown to be phosphorylated by the p38 MAP kinase-activated MAP kinase interacting kinase 1 or a closely related kinase (15). MAP kinases are known to be activated downstream of atypical PKCs (33, 72–75), and the involvement of p38 or p42/44 MAP kinases in TNF-α/IL-1β/sPLA2/LTB4-mediated cPLA2 activation has recently been suggested (46, 63, 76, 77). Also, p38 MAP kinase and λ/ιPKC have been implicated in TNF-α/IL-1β-stimulated NF-κB activation (37–39, 79–81). We find that TNF-α and IL-1β activates p38 and p42/44 MAP kinases in HaCaT keratinocytes, but only p38 MAP kinase inhibition abrogates NF-κB activation (Fig. 1). Furthermore, inhibition of p38 MAP kinase reduces TNF-α-induced cPLA2 phosphorylation, suggesting that p38 MAP kinase participates in cPLA2 and NF-κB regulation. However, it is uncertain whether there is a direct sequential relationship between p38 MAP kinase and λ/ιPKC in HaCaT keratinocytes or if they are parts of parallel signaling pathways.

Inhibition of PI3-kinase attenuated cytokine-induced phosphorylation of λ/ιPKC/cPLA2 and NF-κB activation (Fig. 5), thus suggesting that PI3-kinase acts upstream of λ/ιPKC and cPLA2 in NF-κB activation. Indeed, PI3-kinase is known as an upstream regulator of atypical PKCs, which are subject to regulation by both lipid binding and phosphorylation in vivo (49–51). Also, our results corroborate recent findings showing that PI3-kinase mediates cytokine-elicited NF-κB activation (82, 83). Interestingly, we observed that both p85-dependent class IA and G-protein activated class IB PI3-kinases were activated in response to TNF-α (with slower activation of the G-protein-activated PI3-kinase isofrom; Fig. 5). However, the precise location in the signaling pathway and the contribution of either PI3-kinase isofrom to λ/ιPKC/cPLA2 phosphorylation and NF-κB activation in HaCaT cells is unknown and should be determined in future experiments. Class IB PI3-kinase has been reported to bind to IL1β-induced tyrosine-phosphorylated IL-1 receptor 1 through binding of SH2 domains in the p85 subunit, whereas class IA PI3-kinase is activated downstream of G-protein-coupled receptors, coupling those to c-Jun N-terminal kinase/stress-activated protein kinase and MAP kinase cascades (84–86). Of note, lysophosphatidic acid and LTB4 acting via G-protein-coupled receptors induce PI3-kinase activity (75, 87), of relevance to our results.

Atypical PKCs and PLA2 enzymes/AA signaling contribute to signaling pathways controlling cell growth, differentiation, and survival (67, 78, 88–91). Hence, our results linking atypical PKCs and PLA2 in NF-κB signaling may provide a molecular explanation to the participation of both atypical PKCs and PLA2 in similar cellular functions. A mechanistic explanation to the roles of atypical PKCs in NF-κB activation has been provided by the findings that atypical PKCs bind to the scaffold protein p62 via protein complex formation with receptor interacting protein or TRAF6 in TNF-α- or IL-1β-signaling, respectively (39, 81). Importantly, these protein interactions serve to link atypical PKCs to IKKβ and NF-κB activation. The presence or identities of intermediary steps between the PLA2/LTB4-mediated autocrine feedback loop and the known NF-κB signaling components are at present unknown. However, the PLA2-mediated activity contributing to NF-κB activation could reside in a signaling pathway parallel to the classical MAP3K-IKK-IκBα pathway. Alternatively, PLA2-promoted activity could couple upstream the NF-κB-inducing kinase or IκB kinases (IKKαs). The coupling to IKKαs could possibly be mediated through atypical PKC, acting in the PLA2-mediated positive feedback loop, e.g. by interaction of atypical PKC with p62 adapter protein as described (39, 81). Thus, PLA2-mediated activity may contribute to NF-κB activation by enhancing phosphorylation-induced events increasing kinase activities, e.g. of atypical PKC, resulting in enhanced NF-κB-dependent transcription.

In conclusion, our results elucidate the mechanisms underlying the functional coupling and positive feedback mechanisms between spleen PLAPα/LTB4 and cPLA2, identifying novel signaling pathways involving atypical λ/ιPKC as a downstream effector of 5-LO/LTB4 promoting cPLA2 phosphorylation, cellular AA mobilization, and NF-κB activation in TNF-α/IL-1β-treated human keratinocytes.

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