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Role of the Protein Kinase C λ/ι Isoform in Nuclear Factor-κB Activation by Interleukin-1β or Tumor Necrosis Factor-α: Cell Type Specificities

Giuseppina Bonizzi, Jacques Piette,† Sonia Schoonbroodt,† Marie-Paule Merville* and Vincent Bours*‡*

*Laboratory of Medical Chemistry/Medical Oncology and †Laboratory of Fundamental Virology, University of Liège, Liège, Belgium

ABSTRACT. It has previously been reported that distinct signaling pathways can lead to nuclear factor (NF)-κB activation following stimulation of different cell types with inflammatory cytokines. As the role of atypical protein kinase C (PKC) isoforms in NF-κB activation remains a matter of controversy, we investigated whether this role might be cell type-dependent. Immunoblots detected atypical PKC expression in all the analyzed cell lines. The PKC inhibitor calphostin C inhibited NF-κB activation by tumor necrosis factor (TNF)-α or interleukin (IL)-1β in Jurkat or NIH3T3 cells but not in MCF7 A/Z cells. Cell transfections with a PKC λ/ι dominant negative mutant abolished TNF-α-induced NF-κB-dependent transcription in NIH3T3 and Jurkat cells but not in MCF7 A/Z cells. Similarly, the same mutant blocked NF-κB-dependent transactivation after IL-1β stimulation of NIH3T3 cells, but was ineffective after IL-1β treatment of MCF7 A/Z cells. In MCF7 A/Z cells, however, the PKC λ/ι dominant negative mutant could abolish transactivation of an AP-1-dependent reporter plasmid after stimulation with TNF-α but not with IL-1β. These data thus confirm that transduction pathways for NF-κB activation after cell stimulation with TNF-α or IL-1β are cell-type specific and that atypical PKC isoforms participate in this pathway in NIH3T3 and Jurkat cells. BIOCHEM PHARMACOL **57**;6:713–720, 1999. © 1999 Elsevier Science Inc.

KEY WORDS. protein kinase C; NF-κB; IL-1β; TNF-α; signal transduction

Atypical PKC§ isoforms are regulated by mechanisms different from the other members of the PKC family. These atypical PKC isoforms are PKC ζ and PKC λ/ι, PKC ι being the human homolog of murine PKC λ [1–3]. PKC ζ has been shown to be involved in the control of a number of cellular functions such as neuronal [4] and leukaemic cell differentiation [5]. Atypical PKCs are stimulated by micromolar concentrations of phosphatidylserine and other acidic phospholipids, but not by Ca^{2+}, phorbol ester, or diacylglycerol [6–8]. PKC ζ is also activated by intracellular lipid mediators such as phosphatidic acid [9], phosphatidylinositol 3,4,5-triphosphate [10], and ceramide [11, 12].

The NF-κB transcription factor is sequestered in the cytoplasm of resting cells by IκB proteins, the most studied of which is IκB-α [13, 14]. Following cellular stimulation by a number of agents, IκB-α is rapidly phosphorylated and then degraded by the proteasome, thus liberating active NF-κB complexes [13, 14]. Several studies indicated that PKC λ/ι was required for the activation of NF-κB in *Xenopus laevis* oocytes and murine NIH3T3 fibroblasts [15–18] and that both atypical PKCs are required for induction by TNF-α of NF-κB-dependent transcription in COS cells [1]. It was, however, shown by others in NIH3T3 cells that overexpression of PKC ζ did not modify the activity of NF-κB after cell stimulation with TNF-α [19]. Moreover, *in vitro*, purified rat PKC ζ cannot phosphorylate IκB directly, but can activate a 50 kDa IκB kinase [20]. These results, as well as the recent cloning of the IKK kinases [21–25], indicate that atypical PKC kinases do not directly phosphorylate IκB-α but could interplay with a component of the signal transduction machinery.

We recently demonstrated that IL-1β induces NF-κB activation in lymphoid cells and in epithelial transformed cells through distinct signaling pathways [26, 27]. Similar data were obtained after TNF-α stimulation of lymphoid or epithelial cells [28]. Moreover, cell stimulation by arachidonic acid also induces NF-κB in lymphoid or epithelial cells through distinct signaling pathways [27]. In the present paper, the role of PKC λ/ι in NF-κB activation following stimulation of different cell types has been investigated. In Jurkat or NIH3T3 cells, PKC λ/ι plays a key role in NF-κB activation after treatment with proinflammatory

‡ Corresponding author: Dr. Vincent Bours, Medical Oncology, CHU B35, Sart-Tilman, Université de Liège, 4000 Liège, Belgium. Tel. 32-4-3662482; FAX 32-4-3664534; E-mail: vbours@ulg.ac.be

§ *Abbreviations:* NF-κB, nuclear factor-κB; IκB, inhibitor κB; IL-1β, interleukin-1β; TNF-α, tumor necrosis factor α; PKC, protein kinase C; CAT, chloramphenicol acetyl transferase; MAPK, mitogen-activated protein kinase; CMVPKC, cytomegalovirus PKC; PMA, phorbol 12-myristate 13-acetate; and IKK, IκB kinase complex.

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cytokines, while the same kinase is not required for NF-κB induction in MCF7 A/Z breast adenocarcinoma cells.

MATERIALS AND METHODS

Cell Culture and Biological Reagents

The human ovarian carcinoma cell line OVCAR-3, the human breast cancer cell line MCF7 A/Z, the human colon carcinoma cell line HCT116, the murine fibroblast cell line NIH3T3, the human lymphoid cell lines Raji (B cells) and Jurkat (T cells), and the human monocytic cell line U937 cells were grown in RPMI 1640 medium (Life Technologies, Inc.) supplemented with 1% antibiotics, 1% glutamine, and 10% fetal bovine serum. The mouse pre-B cell line 70Z/3 was grown in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% antibiotics, 1% glutamine, and 10 mM β-mercaptoethanol. The breast cancer cell line MCF7 A/Z was a generous gift from Prof. Mareel (University of Ghent, Belgium). Jurkat, 70Z/3, and Raji cell lines were purchased from the American Tissue Type Culture Collection.

All cell lines were treated with 25 ng/mL of PMA (Sigma), 100 U/mL of TNF-α, or 50 U/mL of IL-1β (Boehringer, Mannheim). Calphostin C (Sigma) was added to the medium 60 min before TNF-α or IL-1β stimulation.

Immunoblots

Protein extracts (25 or 50 μg), obtained by SDS lysis, were separated on a 10% SDS-PAGE gel. After transfer to a nylon membrane (Immobilon-P, Millipore) and overnight blocking at 4° with Tris-buffered saline-Tween (20 mM Tris pH 7.5, 500 mM NaCl, 0.2% Tween20) plus 5% dry milk, the membranes were incubated for 1 hr with PKC ζ antipeptide antibody (Life Technologies, Inc.), washed, and then incubated with the peroxidase-conjugated secondary antibody. The reaction was revealed with the enhanced chemiluminescence detection method (ECL kit, Amersham). The MAPK antibody was purchased from Santa Cruz.

Electrophoretic Mobility Shift Assays (EMSAs)

Nuclear extracts and EMSAs were performed as previously described using a palindromic κB specific probe [26].

Plasmids

The pRcCMVPKC and pRcCMVPKC^{mut} plasmids coding for the *Xenopus* homolog of PKC λ/ι were kindly provided by Prof. J. Moscat (Centro de Biología Molecular, Consejo Superior de Investigaciones Cientificas-Universidad Autonoma de Madrid, Canto Blanco Madrid, Spain). The pRcCMVPKC^{mut} has been previously described and results from the substitution of lysine 275 for a tryptophan in the kinase domain [15].

The HIV-κB-CAT reporter plasmid contained the two κB sites from the HIV long terminal repeat sequences cloned upstream of a CAT reporter gene [29]. The AP-1 reporter plasmid contains two 12-*O*-tetra-decanoylphorbol 13-acetate response elements upstream of a CAT reporter gene (TRE2CAT) [30].

Transfections

PKC λ/ι expression vectors and the HIV-κB-CAT reporter plasmid were transfected into NIH3T3 and MCF7 A/Z cells using the DOTAP liposomal transfection reagent (Boehringer Mannheim). Jurkat cells (2×10^6) were grown for 24 hr in the medium without β-mercaptoethanol before transfection with DMRIE-reagent (Life Technologies, Inc.). After 6 hr, the DNA-containing medium was removed and replaced by fresh medium. Cells were then left untreated or were stimulated with 100 U/mL of TNF-α, 50 U/mL of IL-1β, or 25 ng/mL of PMA for 6 hr. Cellular extracts were prepared and CAT activities determined as described previously [29].

RESULTS

Expression of Atypical PKCs in Cell Lines

The expression of atypical PKCs in various cell lines was investigated. Immunoblots performed with nuclear and cytoplasmic extracts were revealed with an antibody directed against a carboxy-terminal 15 amino-acid peptide of PKC ζ. However, the same sequence is found in PKC λ/ι, with the exception of a single amino-acid [2]. Therefore, as the two proteins have a similar molecular weight, we have to consider that this antibody probably recognizes both PKC ζ and PKC λ/ι. These immunoblots detected high levels of atypical PKC expression in all the epithelial cell lines analyzed (OVCAR-3, MCF7 A/Z, and HCT116), while the fibroblastic NIH3T3 cells and lymphoid cell lines (70Z/3, Jurkat, Raji) expressed much lower levels of these proteins (Fig. 1, A and B) as did the U937 monocytic cells (data not shown). Detection of similar amounts of the ubiquitous MAPK demonstrated that the differences in atypical PKC expression were not due to differences in protein extraction (data not shown).

Inhibition of PKCs and Blocking of NF-κB Activation in Fibroblasts and Lymphoid Cells

The role of PKC kinases in NF-κB activation by proinflammatory cytokines was first evaluated by incubating different cell lines in the presence of calphostin C. This specific PKC inhibitor has been shown to inhibit NF-κB activation by antineoplastic drugs, to block NF-κB activation by IL-1β, and to inhibit PKC ζ [31–33]. The cells were stimulated with TNF-α or IL-1β, and NF-κB nuclear translocation was studied by electrophoretic mobility shift assay (EMSA). Under these conditions, calphostin C completely inhibited, in a dose-dependent manner, NF-κB induction by TNF-α or IL-1β in NIH3T3 cells or by TNF-α in Jurkat cells (Fig.

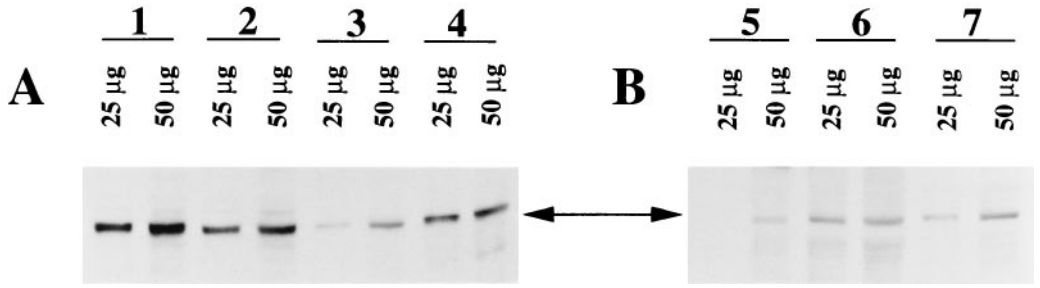

FIG. 1. Atypical PKC expression in cell lines. Immunoblots were performed with cytoplasmic extracts from various cell lines and revealed with a specific antibody directed against a peptide highly conserved between PKC ζ and PKC λ/ι. For each cell line, 25 and 50 µg of protein extract were loaded on the gel, as indicated in the figure. (A) Atypical PKC expression in epithelial and fibroblastic transformed cells. 1: OVCAR-3 ovarian carcinoma cells; 2: MCF7 A/Z breast adenocarcinoma cells; 3: NIH3T3 fibroblastic cells; 4: HCT116 colon carcinoma cells. (B) Atypical PKC expression in lymphoid cells. 5: Raji B cells; 6: 70Z/3 murine pre-B cells; 7: Jurkat human T cells. The two blots were processed simultaneously, incubated for the same time with the same antibody dilution, and exposed together.

2, A and C). However, preincubation with calphostin C did not affect NF-κB activation by the same cytokines in MCF7 A/Z cells (Fig. 2B). Similarly, other protein kinase inhibitors (H7, staurosporin, and genistein) also exerted a cell-specific action on NF-κB activation as they blocked NF-κB induction by IL-1β in lymphoid cells but not in adenocarcinoma cells (data not shown).

PKC λ/ι Involvement in TNF-α or IL-1β-Induced NF-κB Activation

To determine whether atypical PKCs are involved in κB-dependent transactivation by TNF-α, MCF7 A/Z, NIH3T3, and Jurkat cells were transfected with an NF-κB-dependent reporter plasmid together with either the control plasmid pRcCMVPKC or the plasmid pRcCMVPKC^{mut}, which encodes a PKC λ/ι kinase-defective mutant [1, 15, 16, 34]. This dominant negative mutant was derived from the *Xenopus laevis* PKC λ gene and has previously been shown to be active in mammalian cells, as expected given the very high homology between *Xenopus* and mammalian atypical PKCs [1, 11, 15]. Stimulation of NIH3T3 cells with TNF-α (100 U/mL) induced the transcription of a reporter gene under the control of the two κB sites from the HIV long terminal repeat sequences (HIV-κB-CAT) (Fig. 3A, columns 1 and 2). While expression of the PKC λ/ι enzyme did not increase basal or induced CAT activities (Fig. 3A, columns 3 and 4), TNF-α-induced κB-dependent transactivation in NIH 3T3 was dramatically inhibited by transfection of the PKC λ/ι dominant negative expression vector (Fig. 3A, columns 5 and 6). As already shown by others, PKC λ/ι is thus an essential kinase for NF-κB activation by TNF-α in NIH3T3 cells [11].

Similarly, an inhibition of κB-dependent transactivation

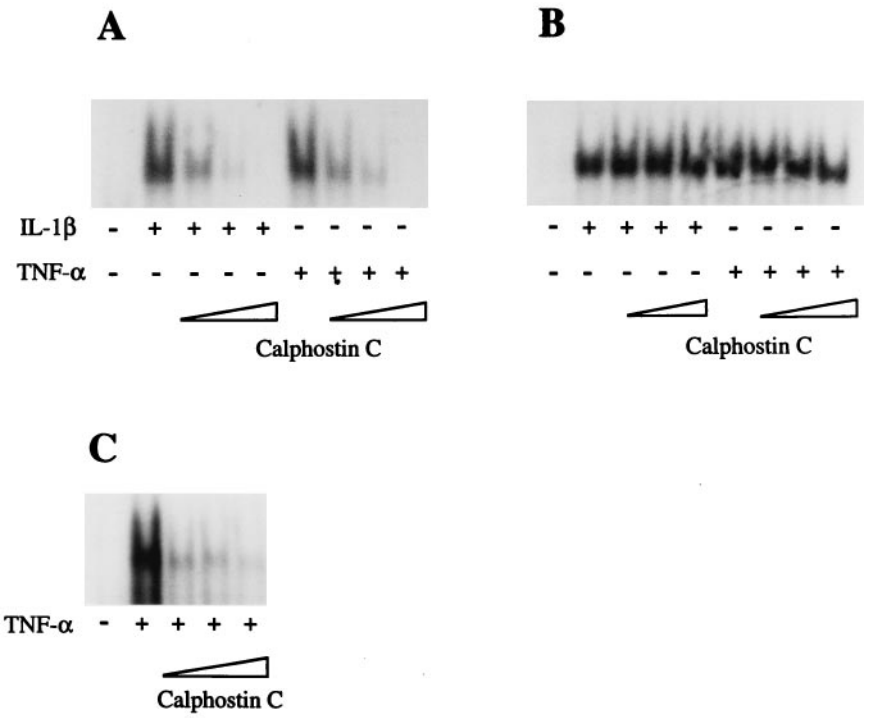

FIG. 2. Calphostin C inhibits NF-κB activation by TNF-α or IL-1β in NIH3T3 and Jurkat cells but not in MCF7 A/Z cells. NIH3T3 (A), MCF7 A/Z (B), and Jurkat (C) cells were incubated in the presence of increasing concentrations of calphostin C (0.5, 1, and 3 µM) prior to stimulation with TNF-α or IL-1β, as indicated in the figure. Nuclear extracts were prepared and analyzed on EMSAs with a specific palindromic κB probe.

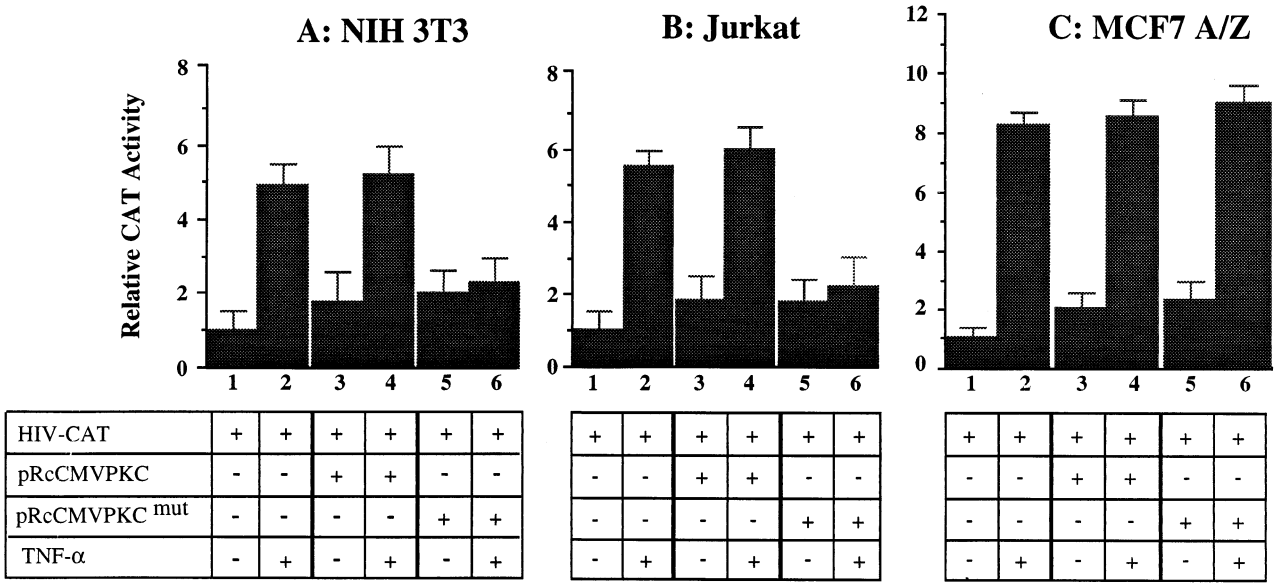

FIG. 3. The role of PKC λ/ι in TNF-α-induced κB-dependent transactivation. NIH3T3 (A), Jurkat (B), or MCF7 A/Z (C) cells were transfected with 1 μg of the HIV-κB-CAT reporter plasmid, alone or in combination with 1 μg of pRcCMVPKC or pRcCMVPKC^{mut} expression plasmid as indicated in the figure. After transfection, the cells were left untreated or were stimulated with TNF-α (100 U/mL) for 6 hr. Cellular extracts were then prepared and CAT activities measured. The figure shows the relative CAT activity over the activity observed with the CAT vector alone after normalization to the protein amounts of the extracts. Each column represents the mean of three independent experiments (± SD). The total amount of transfected DNA was kept constant throughout the experiment by adding appropriate amounts of the expression vector without insert.

by kinase inactive PKC λ/ι mutant was also observed in Jurkat cells after stimulation with TNF-α (Fig. 3B, columns 5 and 6). In these cells, transfection of the plasmid coding for the wild-type PKC λ/ι enzyme did not increase κB-dependent transactivation in either unstimulated or TNF-α-induced cells (Fig. 3B, columns 1 to 4).

Stimulation of the MCF7 A/Z breast adenocarcinoma cells with TNF-α also resulted in a strong induction of NF-κB-dependent transcription (Fig. 3C, columns 1 and 2). The expression of the PKC λ/ι enzyme did not modify basal or induced CAT activities of the reporter plasmid in these cells (Fig. 3C, columns 3 to 4). Moreover, transfection of the expression vector coding for the PKC λ/ι dominant negative mutant did not abolish TNF-α-induced κB-dependent transactivation (Fig. 3C, columns 5 and 6), demonstrating that PKC λ/ι does not play a critical role in NF-κB activation by TNF-α in these cells.

Similarly, the role of PKC λ/ι in IL-1β-induced κB-dependent transactivation was investigated in NIH3T3 and MCF7 A/Z cells. In both cell lines, stimulation with IL-1β induced a strong transactivation of the HIV-κB-CAT reporter plasmid (Fig. 4, A and B, columns 1 and 2). In NIH3T3 cells, expression of the PKC λ/ι enzyme did not modify basal or IL-1β-induced transcription of the reporter gene (Fig. 4A, columns 3 and 4), while IL-1β-induced κB-dependent transactivation was dramatically inhibited by transfection of the PKC λ/ι dominant negative mutant expression vector (Fig. 4A, columns 5 and 6). In MCF7 A/Z cells, however, expression of the PKC λ/ι dominant negative mutant left the IL-1β induction of NF-κB-dependent transactivation unchanged (Fig. 4B, columns 5 and 6), indicating that the PKC λ/ι isoform is not involved in signaling NF-κB activation by IL-1β or TNF-α in these cells. The IL-1β stimulation was not performed with Jurkat cells, as these cells lack type 1 IL-1 receptors.

PKC λ/ι Involvement in AP-1 Activation

As it has been reported that atypical PKC enzymes could be involved in AP-1 activation [34], we tested AP-1-dependent transactivation in these cells. MCF7 A/Z cell stimulation with IL-1β or TNF-α induced a strong induction of AP-1-dependent reporter gene expression (Fig. 5, A and B, columns 1 and 2). Expression of the PKC λ/ι enzyme did not modify basal or TNF-α-induced transcription of the reporter gene (Fig. 5A, columns 3 and 4), while TNF-α-induced AP-1-dependent transactivation was completely inhibited by transfection of the PKC λ/ι dominant negative expression vector (Fig. 5A, columns 5 and 6). However, both the PKC λ/ι wild-type and the PKC λ/ι mutant failed to affect IL-1β-induced AP-1-dependent transactivation (Fig. 5B, columns 3 to 6).

The Specificity of PKC λ/ι Dominant Negative Mutants

In order to check the specificity of the PKC λ/ι dominant negative mutants, their influence on NF-κB activation by the PKC activator PMA was measured. NIH3T3 and MCF7 A/Z cells were transfected with the HIV-κB-CAT reporter plasmid, alone or in combination with pRcCMVPKC or

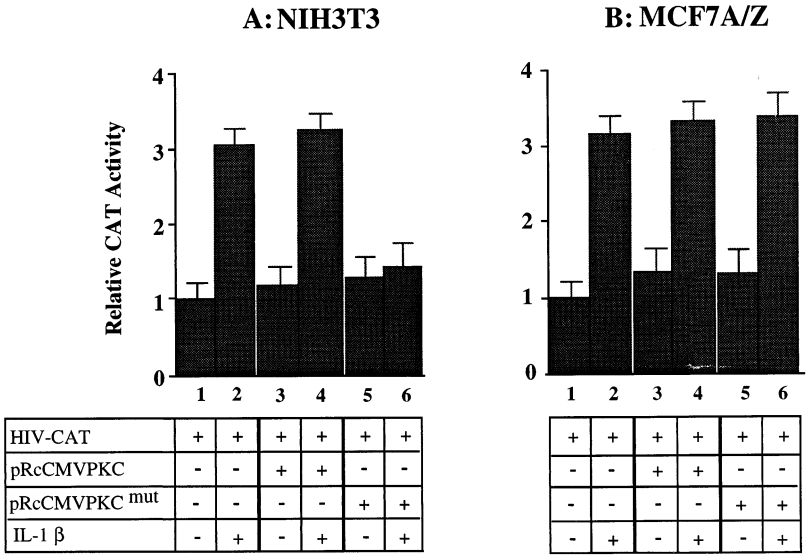

FIG. 4. PKC λ/ι involvement in IL-1β-induced κB-dependent transactivation. NIH3T3 (A) or MCF7 A/Z (B) cells were transfected with 1 μg of the HIV-κB-CAT reporter plasmid, alone or in combination with 1 μg of pRcCMVPKC or pRcCMVPKC^{mut} expression plasmid as indicated in the figure. After transfection, the cells were left untreated or were stimulated with IL-1β (50 U/mL) for 6 hr. CAT activities were determined as in Fig. 3.

pRcCMVPKC^{mut} expression plasmid, and were subsequently treated with PMA (0.2 μM for 6 hr). Under these conditions, PMA induced the CAT activity 3-fold above the control level in both cell lines (Fig. 6, A and B, compare lanes 1 and 2). Cotransfection of wild-type or dominant negative PKC λ/ι expression vectors did not modify CAT gene transcription (lanes 3 to 6), indicating that the PKC λ/ι mutant did not interfere with the function of PMA-stimulated typical PKC isoforms.

DISCUSSION

We previously demonstrated that NF-κB induction after IL-1β or TNF-α stimulation follows distinct signaling pathways in epithelial or lymphoid cells. Indeed, NF-κB activation following IL-1β or TNF-α stimulation of lymphoid cells involves and requires 5-lipoxygenase activity and the production of reactive oxygen intermediates (ROI) [26–28]. However, in adenocarcinoma cells which do not express the 5-lipoxygenase enzyme, the same stimuli activate the NF-κB transcription factor independently of any ROI production. We hypothesized that an alternate pathway in epithelial cells might induce NF-κB through the activation of the acid sphingomyelinase [27]. As the role of atypical PKCs in NF-κB activation remains a matter of controversy, we investigated whether such a function might also be cell type-specific. We therefore studied the expression of atypical PKCs in epithelial, fibroblastic, and lymphoid cells as well as the role of PKC λ/ι in NF-κB-dependent transactivation following IL-1β or TNF-α stimulation.

The expression of atypical PKCs was detected in all the cell lines analyzed, but levels of expression were higher in epithelial cells than in fibroblastic, lymphoid, or monocytic cells. Inhibition of PKCs by calphostin C blocked IL-1β- or TNF-α-induced NF-κB nuclear translocation in NIH3T3

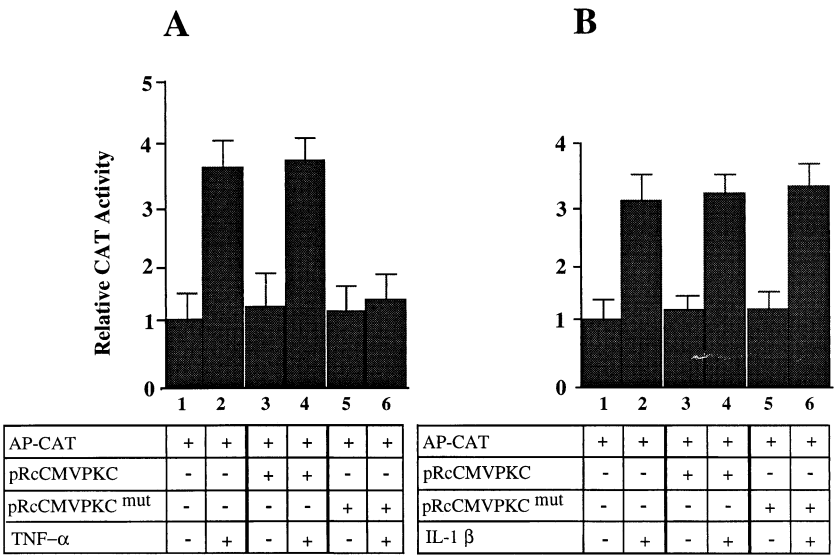

FIG. 5. The role of PKC λ/ι in TNF-α- or IL-1β-induced AP-1-dependent transactivation. MCF7 A/Z cells were transfected with 1 μg of the TRE2CAT reporter plasmid, alone or in combination with 1 μg of pRcCMVPKC or pRcCMVPKC^{mut} expression plasmid as indicated in the figure. After transfection, the cells were left untreated or were stimulated with TNF-α (100 U/mL) (A) or with IL-1β (50 U/mL) (B) for 6 hr. CAT activities were determined as in Fig. 3.

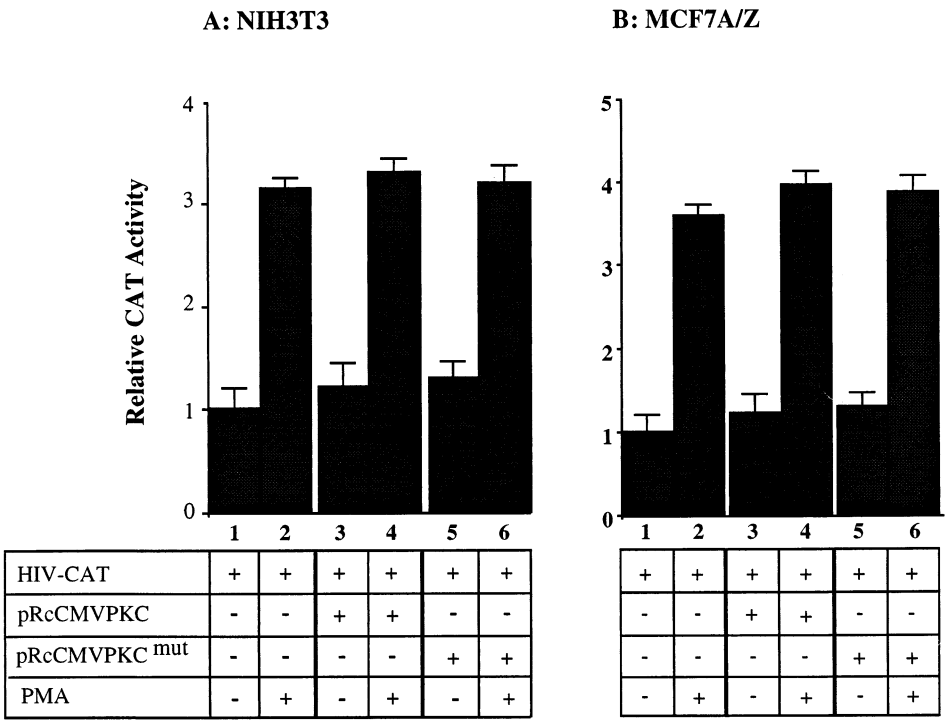

FIG. 6. PKC λ/ι does not modify NF-κB activation by PMA. NIH3T3 (A) or MCF7 A/Z (B) cells were transfected with 1 μg of the HIV-κB-CAT reporter plasmid, alone or in combination with 1 μg of pRcCMVPKC or pRcCMVPKC^{mut} expression plasmid as indicated in the figure. After transfection, the cells were left untreated or were stimulated with PMA (0.2 μM) for 6 hr. CAT activities were determined as in Fig. 3.

fibroblastic cells and in Jurkat T cells but not in MCF7 A/Z breast adenocarcinoma cells. As calphostin C is a non-specific inhibitor of all PKC isoforms, this experiment confirmed that distinct pathways prevail in different cell lines but did not strictly indicate any role for atypical PKCs.

PKC λ/ι dominant negative mutants might also interact with other PKCs or with other signaling pathways. Actually, given the high homology between PKC λ/ι and PKC ζ, it is quite possible that the dominant negative mutants blocked both atypical PKCs. However, these mutants did not inhibit PMA-dependent signal transmission, indicating that they were specific for atypical PKCs as PMA activates classical PKCs [35]. Such mutants abolished IL-1β- or TNF-α-induced transactivation of an NF-κB-dependent reporter gene in NIH3T3 fibroblastic cells. Such an observation confirmed previous data demonstrating that NF-κB activation by TNF-α was abolished by this mutant in NIH3T3 cells [11]. Similarly, PKC λ/ι was required for the TNF-α-induced NF-κB activation in Jurkat T cells. However, a completely different picture arose from the experiments performed in MCF7 A/Z breast adenocarcinoma cells. In these cells, both TNF-α and IL-1β induced a strong transactivation of the κB-dependent reporter gene, but this transactivation was independent of PKC λ/ι as transfection of the PKC λ/ι dominant negative mutant failed to abolish the induced transcription. These data confirm that distinct pathways lead to NF-κB activation following IL-1β or TNF-α stimulation of different cell types.

In order to investigate whether the PKC λ/ι isoform was functional in MCF7 A/Z cells, cytokine-induced AP-1-dependent transactivation was analyzed. It has indeed been demonstrated that the MAPK pathway is required for TNF-α-induced AP-1 activation in COS cells and that PKC ζ can activate MAPK [34]. It has also been demonstrated that dominant negative PKC λ/ι constructs inhibit AP-1-dependent transcription following serum deprivation or *ras* transformation [17]. In our experiments, AP-1 transcriptional activity is inhibited in the presence of a PKC λ/ι dominant negative mutant after TNF-α stimulation of MCF7 A/Z cells, confirming the functionality of the enzyme in these cells and suggesting that PKC λ/ι is required for AP-1 activation by TNF-α. Surprisingly, AP-1 activation by IL-1β is independent of PKC λ/ι, indicating that AP-1 activation by the two main inflammatory cytokines follows distinct pathways in MCF7 A/Z cells.

Our data thus demonstrate that atypical PKCs are involved in NF-κB activation by inflammatory cytokines in NIH3T3 and Jurkat cells. It is possible, as suggested by others, that PKC λ/ι activates the MAPK pathway, which could in turn participate in NF-κB induction [20, 34, 36]. Recently, however, a direct pathway for the degradation of IκB-α and the activation of NF-κB following TNF-α stimulation of HeLa or 293 cells was identified [21–25, 37].

This pathway involves the NIK kinase as well as the IKK kinases that directly phosphorylate IκB-α. However, the present data as well as previous reports from our group [27] and from others [11, 12, 34, 38–41] indicate that other intermediates are required for NF-κB activation by TNF-α or IL-1β. Some of these discrepancies could be explained by cell-type specificities, as we reported that IL-1β-dependent NF-κB induction follows cell-specific signaling pathways [26, 27].

One possible explanation might be that, in NIH3T3 and Jurkat cells stimulated by TNF-α or IL-1β, PKC λ/ι could induce, directly or indirectly, the activation of the IKK kinases. As NIK is a member of the MAP3K family, it is possible that PKC λ/ι phosphorylates and activates NIK. Further experiments are needed to explore this pathway.

Finally, Berra *et al.* [34] reported that a MAPK dominant negative mutant abolished NF-κB-dependent transactivation of a reporter plasmid after TNF-α stimulation of COS cells without affecting NF-κB nuclear translocation. As several NF-κB proteins are phosphorylated [42–44], it is conceivable that atypical PKCs and MAPK are not involved in IκB-α phosphorylation and degradation, but are required for functionally relevant phosphorylations of NF-κB subunits. Indeed, it has recently been demonstrated that inhibition of the p38 MAPK blocks NF-κB-dependent gene transcription following TNF-α stimulation without affecting NF-κB nuclear translocation or p65 phosphorylation [45, 46]. The identification of the PKC ζ, PKC λ/ι, and MAPK substrates in NIH3T3 and Jurkat cells should determine their role in IκB-α degradation and NF-κB activation.

It is clear from a number of studies that NF-κB plays a pivotal role in pathological processes such as inflammation and asthma. Under these conditions, proinflammatory cytokines such as TNF-α or IL-1β activate NF-κB, which in turn induces a number of genes including those coding for TNF-α or IL-1β. The identification of the signaling pathways leading to NF-κB activation is thus crucial in order to understand the molecular mechanisms at the origin of the inflammatory process and to design novel and specific therapies.

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