0026-895X/01/5902-248–253$3.00
MOLECULAR PHARMACOLOGY
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Mol Pharmacol 59:248–253, 2001

Vol. 59, No. 2
335/880958
Printed in U.S.A.

Paclitaxel-Induced Immune Suppression Is Associated with NF-κB Activation Via Conventional PKC Isotypes in Lipopolysaccharide-Stimulated 70Z/3 Pre-B Lymphocyte Tumor Cells

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Received June 6, 2000; accepted October 20, 2000

This paper is available online at http://molpharm.aspetjournals.org

ABSTRACT

Paclitaxel, a potent antitumor agent, has been shown to be lipopolysaccharide (LPS) mimetic in mice, stimulating signaling pathways and gene expression indistinguishably from LPS. In the present study, we showed the intracellular signaling pathway of paclitaxel-induced nuclear factor-κB (NF-κB) activation and its suppressive effect on LPS-induced signaling in murine 70Z/3 pre-B cells. Stimulation of 70Z/3 cells with LPS for 30 min caused activation of NF-κB in the nuclei by detection of DNA-protein binding specific to NF-κB. Similarly, paclitaxel also produced a marked and dose-related NF-κB activation. However, pretreatment of cells with 10 μM paclitaxel for 18 h resulted in complete inhibition of LPS-mediated NF-κB activation. Interestingly, the activity of IκB kinase (IKK-β), which plays an essential role in NF-κB activation through IκB phosphorylation, was largely enhanced in paclitaxel-treated cells, detected as IκBα phosphorylation. Because protein kinase C (PKC) is implicated in the activation of NF-κB via IKK-β, the effect of paclitaxel on PKC activation was also measured. It was shown that NF-κB nuclear translocation and DNA binding in response to paclitaxel was completely blocked by the conventional PKC inhibitor, Gö 6976. Moreover, immunoblotting analysis with paclitaxel-treated cell extract demonstrated that the conventional PKC isotype PKC-α was found to be involved in the regulation of paclitaxel-induced NF-κB activation, as determined by electrophoretic mobility shift of PKC. Therefore, these data suggest that paclitaxel may activate IKK-β via conventional PKC isotypes, resulting in NF-κB activation and, finally, desensitization of LPS-inducible signaling pathway in 70Z/3 pre-B cells.

Paclitaxel (Taxol), isolated from the bark of the Pacific yew tree, is one of the more promising agents for treatment of breast cancer (Rowinsky, 1994) and is shown to block cells at the G_2/M junction of the cell cycle (Blagosklonny et al., 1996). The primary mechanism of action of paclitaxel is attributed to its ability to bind to microtubules and prevent their assembly. In addition to the blockage of mitosis, paclitaxel also triggers cellular responses that mimic those induced by LPS, a potent activator of the innate immune system, such as tyrosine phosphorylation of mitogen-activated protein kinases, translocation of NF-κB, and induction of gene expression (Perera et al., 1996; Das and White, 1997).

In particular, activation of the transcription factor NF-κB is implicated in the induction of a number of genes by LPS (Garrett et al., 1999). In unstimulated cells, this transcription factor exists in an inactive state in the cytoplasm complexed to the inhibitory protein IκB. In mammalian cells, the IκB family consists of IκBα, IκBβ, IκBε, p105, and p100 (Whiteside and Israel, 1997). Among the IκB members, IκBα and IκBβ are the most prominent and have been extensively characterized. Upon activation, IκB undergoes phosphorylation and degradation, and the NF-κB heterodimer translocates into the nucleus, where it binds to DNA and activates transcription (Rice and Ernst, 1993). Activation of NF-κB-dependent transcription has been observed consistently after stimulation by Raf-1, PKC, or protein tyrosine kinase receptors (Steffan et al., 1995; Baumann et al., 2000). In fact, however, additional kinases may be involved in LPS-mediated IκB phosphorylation (Schouten et al., 1997). A high-molecular-mass IκB kinase (IKK) complex, consisting of IKK-α, IKK-β, NF-κB-inducing kinase (NIK) and two adaptor or scaffold proteins, has been studied intensively (Woronicz et al., 1997).

Evidence has accumulated that PKC may be associated with activation of IKK (Lallena et al., 1999; Trushin et al., 1999). PKC comprises a family of related serine/threonine

ABBREVIATIONS: LPS, lipopolysaccharide; NF-κB, nuclear factor-κB; IKK, IκB kinase; PKC, protein kinase C; PAGE, polyacrylamide gel electrophoresis; MEK, mitogen-activated protein kinase kinase; GST, glutathione *S*-transferase; EMSA, electrophoretic mobility shift assay.

protein kinases implicated in the regulation of various cellular processes, including proliferation and differentiation (Liu, 1996). The PKC family is composed of at least 11 members classified into three major groups-conventional PKC, including PKC-α, -β_1, -β_2, and -γ; novel PKC, including PKC-δ, -ϵ, -ι, -θ, and -μ; and atypical PKC, including PKC-ζ and -λ (Parekh et al., 2000).

It has been thought that NF-κB activation suppresses the signals for cell death. However, here we show that NF-κB activation via conventional PKC may contribute to immune suppression by paclitaxel in LPS-stimulated pre-B cells, indicating that NF-κB can have the contrasting effects depending on the stimulus. Therefore, it is now clear that NF-κB transcription factors have a role in regulating the immune system, either as essential for immune suppression or, perhaps more commonly, as stimulators of immune response.

Experimental Procedures

Materials. Anti-Raf, anti-IKK-α, and anti-IKK-β were obtained from Santa Cruz Biotechnology (Santa Cruz, CA). The Anti-PKC antibody sampler kit was from Life Technologies (Gaithersburg, MD). Anti-phospho-IκBα was obtained from New England Biolabs (Beverly, MA). Protein A-agarose was from Roche Diagnostics (Nutley, NJ). Dulbecco's modified Eagle's medium, fetal calf serum, penicillin, and streptomycin were purchased from Life Technologies. Reagents for SDS-polyacrylamide gel electrophoresis were from Bio-Rad (Hercules, CA). [γ-^{32}P]ATP (3000 Ci/mmol) was purchased from New England Nuclear (Boston, MA). GF 109203X, Gö 6976, PD 98059, and SB 203580 were purchased from Calbiochem (San Diego, CA). Paclitaxel and LPS were obtained from Sigma (St. Louis, MO). Paclitaxel was dissolved in dimethyl sulfoxide and freshly diluted for each experiment.

Cell Line. The murine pre-B cell line, 70Z/3 (ATCC TIB 152), was obtained from the American Type Culture Collection (Manassas, VA). The cells were cultured in RPMI 1640 medium supplemented with 100 U/ml penicillin, 100 U/ml streptomycin, 2 mM L-glutamine (Life Technologies), 50 μM 2-mercaptoethanol, and 10% bovine calf serum.

Electrophoretic Mobility Shift Assay (EMSA). Nuclear extracts were prepared as described previously. (Lee and Yang, 2000a). The protein content of the nuclear extracts was determined by the Bio-Rad protein assay according to the manufacturer's instructions, and gel mobility shift assays were performed. Briefly, 5 μg of nuclear extracts were incubated with 2 μg of poly(dI-dC) (Sigma) and ^{32}P-end-labeled DNA probe (double-stranded, synthetic, 26-base-pair oligonucleotides GATCTCAGAGGGGACTTTCCGAAGAGA containing the κB enhancer of immunoglobulin-κ light chain gene). Identity of the shifted bands was confirmed by competition with unlabeled oligomer containing NF-κB site.

In Vitro c-Raf-1 Kinase Assay. The cell lysates were prepared as described previously (Ferrier et al., 1997). Immunoprecipitation was performed on the whole cell lysates using anti-Raf (Santa Cruz Biotechnology) and protein A-agarose beads. After incubation for 2 h at 4°C, immunoprecipitates were washed twice with ice-cold lysis buffer. After washing with kinase buffer (20 mM Tris, pH 7.4, 20 mM NaCl, 1 mM dithiothreitol, 10 mM $MgCl_2$), Raf kinase activity was assayed by phosphorylation of the recombinant MEK (Santa Cruz Biotechnology). The samples were resolved by SDS-PAGE, and phosphoproteins visualized by autoradiography.

IκB Kinase (IKK) Assay. Immunoprecipitation was carried out using either anti-IKK-α or IKK-β antibody and followed by the kinase assay (Nemoto et al., 1998). The kinase reaction was performed in 30 μl of kinase buffer (20 mM HEPES, pH 7.8, 10 mM $MgCl_2$, 100 μM Na_3VO_4, 20 mM β-glycerophosphate, 2 mM dithiothreitol, 50 mM NaCl) for 30 min at 30°C in the presence of 10 μM ATP/10 μCi of [γ-^{32}P]ATP (10 Ci/mmol) (NEN Life Science Products) and 500 ng of the substrate GST-IκB-α (Santa Cruz Biotechnology). The reactions were terminated with 4× Laemmli sample buffer. Proteins were analyzed on 12.5% SDS-polyacrylamide gels, dried, and visualized by autoradiography.

Cell Fractionation and Western Blot Assay. Once 70Z/3 pre-B cells reached subconfluence, the cells were incubated for additional times in the presence of LPS or paclitaxel. The cells then were washed with ice-cold PBS, lysed in lysis buffer (20 mM Tris-HCl, pH 7.4, 5 mM EGTA, 1 mM phenylmethylsulfonyl fluoride, and 20 μM leupeptin), and disrupted by Dounce homogenization. The cell homogenates were fractionated by ultracentrifugation into particulate (plasma membrane-enriched) (100,000*g* for 1 h pellet of the nucleus-free, 800*g* supernatant), and cytosolic (100,000*g* supernatant) fractions. SDS-polyacrylamide gel electrophoresis and immunoblot analysis using the anti-PKC antibodies, which recognize the isoforms of PKC-α, -ϵ, and -ζ, were performed. The enhanced chemiluminescence (Amersham Pharmacia Biotech, Piscataway, NJ) protocol was used to visualize the immunoreactive bands.

In Vitro PKC Assay. Immunoprecipitation was carried out using either PKCα or PKCϵ antibody and followed by the kinase assay. In brief, immunoprecipitates were resuspended in reaction buffer (20 mM Tris-HCl, pH 7.4, 10 mM $MgCl_2$, 1.2 mM $CaCl_2$, 20 μM ATP, 0.2 mg/ml Histone H1, 10 μM phorbol-12-myristate-13-acetate, 40 μg/ml phosphatidylserine, and 2.5 μCi of [γ-^{32}P]ATP). The reactions were carried out at 30°C for 5 min and terminated by the addition of sample buffer. Proteins were separated by SDS-PAGE and visualized by autoradiography.

Results

Paclitaxel-Induced NF-κB Activation. In agreement with other reports (Zheng et al., 1993; Garrett et al., 1999) suggesting that NF-κB plays a central role in LPS-mediated transcriptional regulation, LPS increased NF-κB binding activity in nuclear extracts of 70Z/3 pre-B cells. The intensity of NF-κB binding activity markedly increased after exposure of the cells to 1 μg/ml of LPS for 30 min (Fig. 1A). Similarly, paclitaxel also produced a marked nuclear translocation of NF-κB (Fig. 1C). This activation was dose-dependent and required $\geq$ 5 μM paclitaxel. The kinetics of NF-κB activation in 70Z/3 cells after paclitaxel exposure are shown in Fig. 1B. Paclitaxel stimulation of NF-κB reached maximum level within 30 min of treatment and was sustained for a longer time (2 h or more) after exposure.

Inhibitory Effect of Paclitaxel on LPS-Induced NF-κB Activation. Our previous results have shown that spleen cells can be induced to a state hyporesponsive to LPS stimulation by pre-exposing them to paclitaxel (Lee et al., 2000b). Therefore, to identify the effect of paclitaxel on LPS-induced NF-κB activation, 70Z/3 cells were treated with LPS for various times relative to paclitaxel treatment. In particular, pretreatment of 70Z/3 cells with paclitaxel for 18 h resulted in complete inhibition of LPS-mediated NF-κB activation, as determined by the loss of DNA binding (Fig. 2A). However, short-term pretreatment or simultaneous treatment with paclitaxel had no inhibitory effects on LPS induction (Fig. 2B).

Effect of Paclitaxel on IκB Kinase and IκBα Phosphorylation. During activation of NF-κB, IκB is phosphorylated, which serves to target it for ubiquitination and degradation. Several groups have recently characterized and cloned two IκB kinases (IKK-α and IKK-β) that phosphorylate the residues in the IκB molecule. Paclitaxel had a major

effect on IKK-β activity, which peaked at 30 min, whereas an almost negligible increase in the activity of IKK-α was observed at this time point (Fig. 3A). This result indicates that IKK-β is the principal kinase involved in paclitaxel-induced IκB phosphorylation, although IKK-α has a higher basal activity. We also determined IκB phosphorylation using a phospho-specific anti-IκBα antibody that detects IκBα only when activated by phosphorylation at Ser-32. In agreement with the results described in Fig. 3A, treatment of cells with paclitaxel led to an increase of phosphorylated IκBα, reaching a plateau between 30 and 120 min (Fig. 3B).

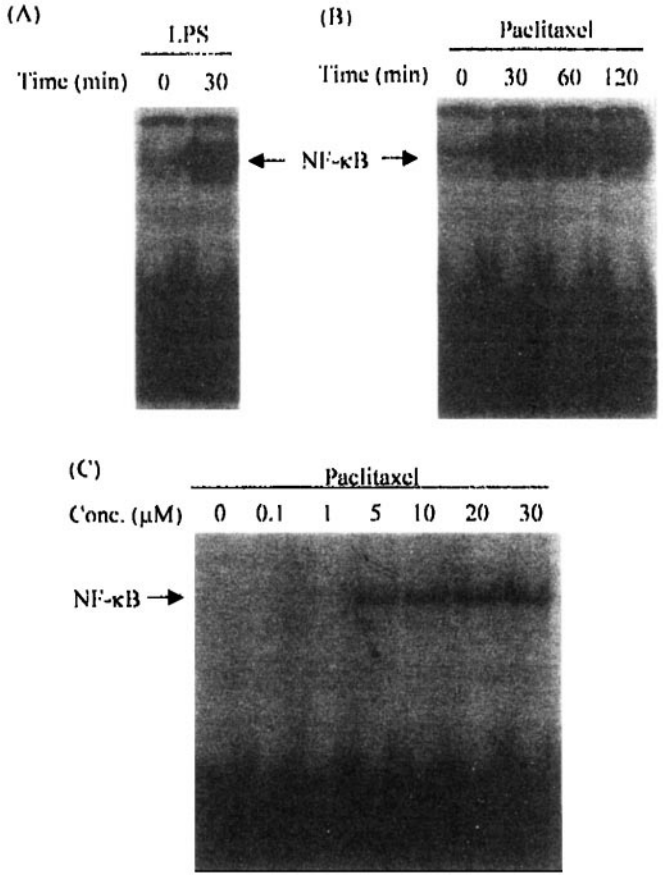

Fig. 1. NF-κB activation by LPS or paclitaxel. Murine 70Z/3 pre-B cells were stimulated with 1 μg/ml LPS (A) or 10 μM paclitaxel (B) for the indicated time. C, 70Z/3 cells were treated with paclitaxel at indicated concentrations for 30 min. Nuclear extracts were prepared, incubated with a ^{32}P-labeled κB probe, and analyzed by EMSA. The positions of NF-κB complexes are shown. The results presented are representative of three independent experiments.

Stimulation of PKC-α and -β by Paclitaxel. To further characterize the effect of paclitaxel on upstream events, a series of inhibitors of protein kinase were used (Fig. 4A). GF 109203X inhibits the conventional and novel isoforms, whereas Gö 6976 inhibits more efficiently the conventional isoforms. Pretreatment of cells for 2 h with GF 109203X did not interfere with activation of NF-κB by LPS or paclitaxel. LPS- and paclitaxel-induced NF-κB activation was, however, effectively blocked by Gö 6976, suggesting a critical role for conventional PKCs. To confirm the effect of these two PKC inhibitors, we performed dose-response analysis of both inhibitors. As shown in Fig. 4B, Gö 6976 produced a marked inhibition of NF-κB activation at concentrations as low as 0.5 μM. However, GF 109203X exhibited little inhibitory effect on LPS- and paclitaxel-induced NF-κB activation. In particular, compared with LPS-induced NF-κB activation, paclitaxel-induced NF-κB activation was more resistant to GF 109203X. On the other hand, because previous reports showed that the mitogen-activated protein kinase pathway is important in the activation of NF-κB (Nakano et al., 1998), we also investigated the effect of MEK inhibitor (PD 98059) and p38 kinase inhibitor (SB 203580) on NF-κB activation by paclitaxel or LPS. PD 98059 inhibited LPS-induced NF-κB activation, although SB 203580 was still inactive. In contrast, paclitaxel-induced NF-κB activation was not affected by either PD 98059 or SB 203580. In addition, to determine whether oncogenic Raf is required to activate NF-κB, we performed in vitro Raf-1 kinase assay by phosphorylation of MEK-1 (Fig. 4C). Contrary to the idea that Raf-1 is involved in NF-κB activation (Flory et al., 1998), neither LPS nor paclitaxel activated Raf-1 detectably.

To assess the potential specificity of PKC isotypes mediating activation of IKK complex by paclitaxel, we performed immunoblotting using the specific antibodies against a representative PKC (PKC-α, conventional isotype; PKC-ϵ, novel isotype; PKC-ζ, atypical isotype) of each isotype. We were unable to detect a characteristic translocation noted with PKC activation. Instead, the prominent LPS- and paclitaxel-induced shifts in the electrophoretic mobility of PKCs were observed when SDS-solubilized membrane and cytosolic fractions were analyzed. Exposure of the cells to 1 μg/ml LPS for 30 min induced the electrophoretic mobility shift of PKC-α,

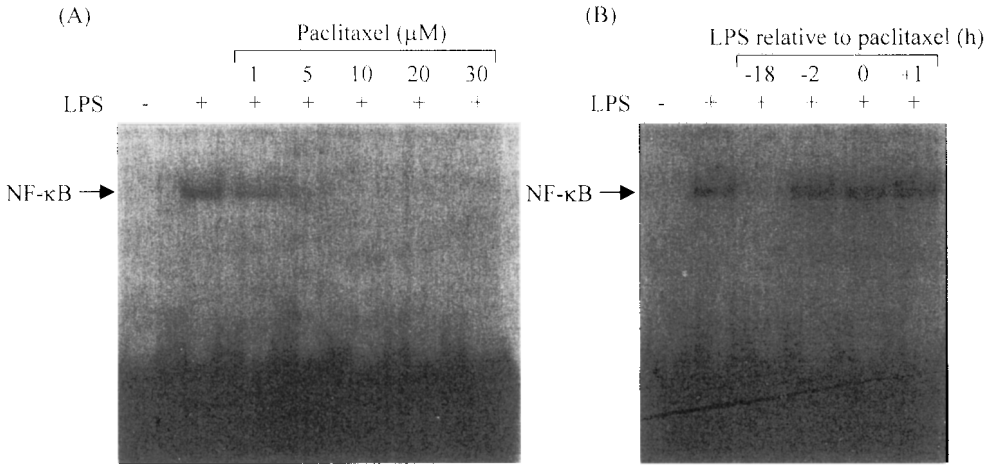

Fig. 2. Effects of paclitaxel on LPS-mediated activation of NF-κB. A, 70Z/3 cells were pretreated with paclitaxel at indicated concentrations for 18 h, washed extensively, and stimulated with LPS (1 μg/ml) for 30 min. B, 70Z/3 cells were stimulated with LPS and paclitaxel was added at the times indicated relative to LPS stimulation. EMSA revealed the NF-κB binding activity of the nuclear extracts. The results presented are representative of three independent experiments.

and -ϵ in cytosolic and membrane fraction (Fig. 5A, lane 2). In contrast, no apparent changes in gel mobility were observed with PKC-ζ of either cytosolic or membrane fraction. Paclitaxel treatment also showed a similar shift in gel mobility of PKC-α and especially PKC-ϵ (Fig. 5A, lanes 3–5). However, the kinetics of shift in gel mobility of cytosolic PKC-ϵ by paclitaxel was slower than those of conventional PKCs. The band shift of PKC-α was maximal at 30 min and then declined, whereas the band shift of PKC-ϵ was induced after 30 min through 2 h. Interestingly, paclitaxel pretreatment before LPS activation resulted in inhibition of LPS-induced shift in gel mobility of conventional PKCs but had no effect on the shift in gel mobility of PKC-ϵ (Fig. 5A, lanes 6 and 7), suggesting that PKC-ϵ is not involved in NF-κB activation by paclitaxel. To confirm the effect of paclitaxel on PKC activation, we measured the PKC activity of cytosolic and membrane fraction after immunoprecipitation using PKC-α and PKC-ϵ antibodies. Figure 5B shows that, as measured by the incorporation of [γ-^{32}P]ATP into histone, PKC activity was well correlated with the supershift of PKC. In addition, we found that treatment with paclitaxel for 18 h caused the complete down-regulation of PKCα but had little effect on PKC-ϵ. (Fig. 5C). Therefore, our results indicated that conventional PKCs, such as PKC-α, but not -ϵ and -ζ, were involved in the regulation of paclitaxel-induced NF-κB activation.

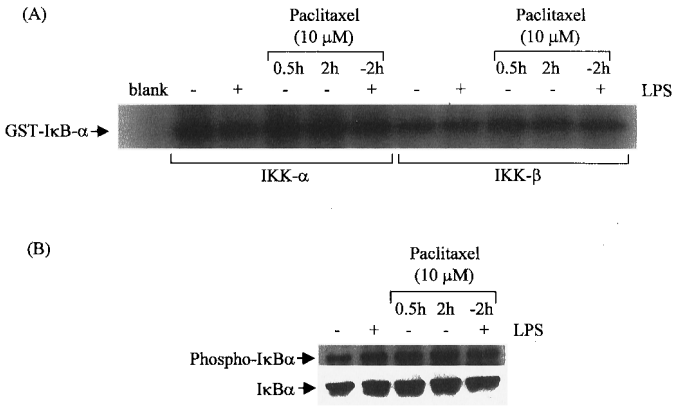

Fig. 3. Effect of paclitaxel on IκB-α phosphorylation. A, 70Z/3 pre-B cells were pretreated (+) or not (−) for the indicated times with 10 μM paclitaxel and subsequently stimulated (+) or not (−) with LPS for 30 min. Immunoprecipitates of IKK were analyzed in the in vitro kinase assay. B, the LPS-induced phosphorylation of IκB-α was investigated using an antibody that detects IκB-α only when activated by phosphorylation at Ser-32 (arrow). 70Z/3 pre-B cells were treated as indicated above, and the whole-cell lysates were analyzed by Western blot. To detect IκB phosphorylation a polyclonal phospho-specific anti-IκB-α antibody (New England Biolabs) was used that detects IκB-α only when activated by phosphorylation at Ser-32. The results presented are representative of three independent experiments.

Discussion

Paclitaxel, like LPS, was able to stimulate the translocation of primarily the 50- and 65-kDa heterodimers of NF-κB to the nucleus in 70Z/3 pre-B cells. Paclitaxel-induced NF-κB activation reached a maximal level after 30 min of treatment and maintained this levels for 2 h or more. However, LPS-induced NF-κB activation was completely inhibited by preexposing cells to paclitaxel. Many reports have recently suggested that LPS and paclitaxel share a common receptor/signaling complex (Byrd et al., 1999; Lee et al., 2000b). In addition, CD18, which participates in LPS-induced binding

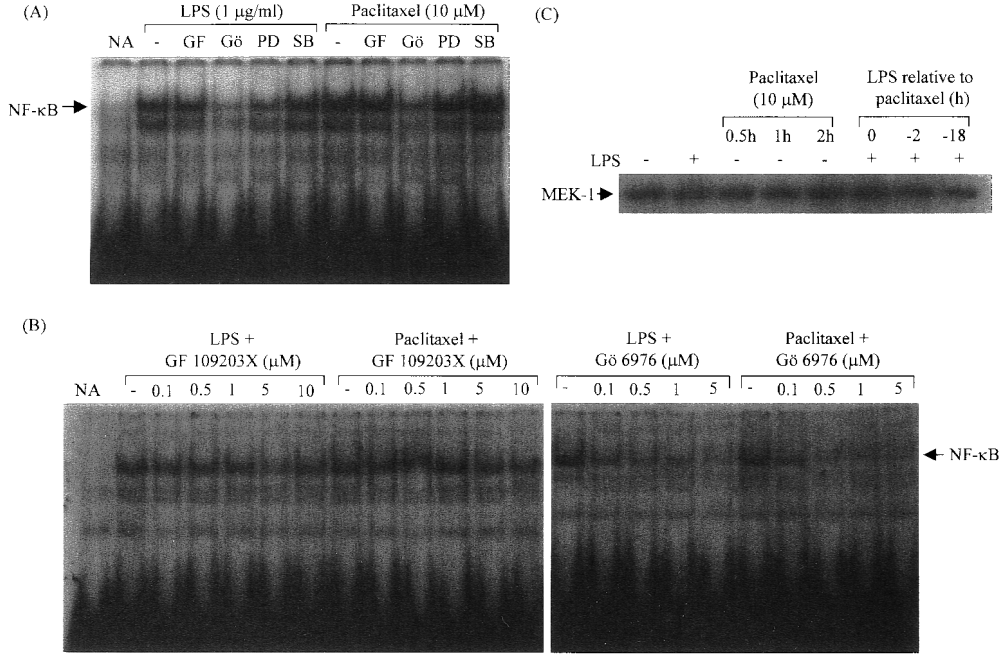

Fig. 4. Conventional PKC isoforms but not the Raf-1 pathway are required for IKK and NF-κB activation. A, 70Z/3 pre-B cells were pretreated (+) or not (−) with either GF 109203X (1 μM), Gö 6976 (1 μM), PD98059 (50 μM), or SB203580 (30 μM) for 2 h before stimulation with either LPS or paclitaxel for 30 min. EMSA was performed on nuclear extracts. Results of one representative experiment of three are shown. B, 70Z/3 cells were pretreated with GF 109203X or Gö 6976 at the indicated concentrations for 2 h before stimulation of LPS or paclitaxel for 30 min. Nuclear extracts were prepared, incubated with a ^{32}P-labeled κB probe, and analyzed by EMSA. C, for in vitro Raf-1 kinase assays, c-Raf-1 immunoprecipitates were incubated in kinase buffer containing 10 μCi of [γ-^{32}P]ATP in the presence of MEK-1 as substrate as described under *Experimental Procedures*. Samples were resolved by electrophoresis on 10% SDS-PAGE, and the radioactivity incorporated into the ^{32}P-labeled MEK-1 protein was determined by autoradiography. The results presented are representative of three independent experiments.

and signal transduction, was identified as a paclitaxel-binding protein (Bhat et al., 1999). Therefore, the suppressive effect of paclitaxel on LPS signaling seems to be attributable to the desensitization of NF-κB signaling. In addition, we can not exclude the possibility that the ability of paclitaxel to activate NF-κB may induce immunoregulatory and cytotoxic cytokines, which in turn may contribute to its immunosuppressive effects.

Paclitaxel specifically enhanced IKK-β activity but had no stimulatory effect on IKK-α activity, suggesting that IKK-β is the principal kinase involved in paclitaxel-induced IκB phosphorylation. In unstimulated cells, IKK-α inhibited the constitutive IκB kinase activity of IKK-β (O'Mahony et al., 2000). Surprisingly, LPS failed to stimulate IKK-β activity. IKK-*i* was recently shown to be an LPS-inducible IκB kinase that may play a special role in the immune response (Shimada et al., 1999). Thus, IKK-*i,* but not IKK-α or IKK-β, may be involved in stimulating the translocation of NF-κB to the nucleus or in enhancing its DNA binding activity. Because Raf-1 was initially presented as IκB kinase (Li and Sedivy, 1993) and was reported to be involved in LPS signaling (Hambleton et al., 1995; Lee et al., 1996), we analyzed the effect of paclitaxel on Raf-1 kinase. Paclitaxel has been also known to induce apoptosis that is associated with Raf-1 (Blagosklonny et al., 1996). However, in the present study, paclitaxel exhibited no effect on Raf-1 kinase activity in 70Z/3 pre-B cells. This finding is consistent with the reported results that immune complexes of Δ-Raf-1:ER did not phosphorylate a purified GST-IκB-α fusion protein (Hambleton et al., 1995). Furthermore, it has been shown recently that oncogenic Raf can activate NF-κB, not through induced nuclear translocation, but through the activation of the transcriptional function of the NF-κB RelA/p65 subunit (Wang and Baldwin, 1998). On the other hand, subsequent work has pointed to an indirect mechanism involving an autocrine pathway that ultimately uses stress-activated protein kinase/p38-dependent mechanisms (Troppmair et al., 1998). These data suggest a role for Raf-1 far more distal to the phosphorylation of IκB.

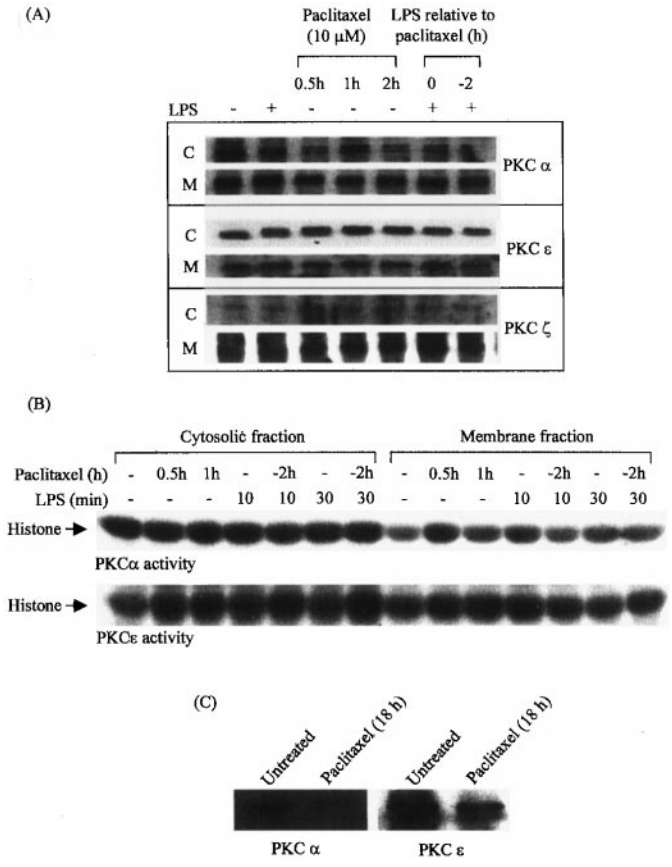

Fig. 5. The effect of paclitaxel on PKC activation. A, 70Z/3 pre-B cells were pretreated (+) or not (−) with 10 μM paclitaxel and subsequently stimulated (+) or not (−) with LPS for 30 min. Cells were disrupted by Dounce homogenization, and fractionated by centrifugation into membrane (M) and cytosolic (C) fractions as described under *Experimental Procedures*. Protein (10 μg/lane) was applied to an SDS-polyacrylamide gel, and the PKC isotypes in each fraction were detected by immunoblotting. The results presented are representative of three independent experiments. B, the fractions of cytosol and membrane were immunoprecipitated with PKC-α or PKC-ε. For in vitro PKC assays, PKC immunoprecipitates were incubated in kinase buffer containing 2.5 μCi of [γ-^{32}P]ATP in the presence of histone H1 as substrate, as described under *Experimental Procedures*. Samples were resolved by electrophoresis on 12.5% SDS-PAGE, and the radioactivity incorporated into the ^{32}P-labeled Histone protein was determined by autoradiography. C, 70Z/3 pre-B cells were treated with paclitaxel for 18 h, and whole-cell lysates were analyzed by Western blot using polyclonal anti-PKC-α and PKC-ε antibody.

Many results have demonstrated a critical role for the PKC isoforms in the NF-κB pathway at the level of IKK-β activation and IκB degradation. In particular, it has been reported that LPS-induced NF-κB activation was inhibited by antisense oligonucleotides for PKC-α, -βI, and -δ, but not -η (Chen et al., 1998). In this study, a highly selective cell-permeable conventional PKC inhibitor, Gö 6976, inhibited LPS-induced NF-κB activation, indicating that conventional PKC activation is an obligatory event in the LPS-mediated regulation of NF-κB activation in 70Z/3 cells. A similar effect, although less pronounced, is observed with the MEK inhibitor PD 98059. This result suggests that extracellular signal-regulated kinase pathway plays, in part, an important role in the LPS-induced NF-κB activation. However, the p38 mitogen-activated protein kinase inhibitor SB 203580 had no effect on LPS-induced NF-κB activation. The results suggested by others (Garrett et al., 1999), in which LPS-induced NF-κB activation was not dependent on activation of p38, showed consistency with our results. We also showed that Gö 6976 markedly suppressed NF-κB activation by paclitaxel. However, both PD 98059 and SB 203580 had little effect on paclitaxel-induced NF-κB activation, implying that paclitaxel signaling may diverge further downstream from LPS pathway despite their sharing a common receptor/signaling complex, such as the stimulation of Rho. In the case of Rho, two different signal transduction pathways exist, leading to the stimulation of Rho and subsequent stress fiber formation in Swiss 3T3 fibroblasts (Peppelenbosch et al., 1995). In Rac-dependent pathway, Rho is activated by arachidonic acid metabolites produced when Rac1 is activated by phosphatidylinositol 3-kinase, which is not necessary in Rac-independent pathways.

Interestingly, paclitaxel had no effect on the amount of distribution of PKC in cultured pre-B cells but induced a change in the mobility of PKC on SDS-PAGE. Despite few published direct demonstration, it is assumed that an increase in the phosphorylation of PKCs upon cell stimulation could be a marker PKC activation (Heidenreich et al., 1990; Acs et al., 1997). In addition, we identified that, as measured by the incorporation of [γ-^{32}P]ATP into histone, PKC activity was well correlated with the supershift of PKC. In our stud-

ies, PKC-α, but not -ϵ or -ζ, was found to be involved in the regulation of paclitaxel-induced NF-κB activation in 70Z/3 pre-B cells. This demonstrates that conventional PKCs may contribute to NF-κB activation by paclitaxel through the activation of the IKK-β. Trushin et al. (1999) have demonstrated that IKK-β is the target for PKC. Moreover, IKK-β associates in vitro with PKCα but is unable to interact with Raf-1 (Lallena et al., 1999). In addition, our result suggest that PKC-ζ is most likely not directly involved in the regulation of paclitaxel-induced NF-κB, although the recombinant PKCζ was known to directly phosphorylate IKK-β in vitro (Sanz et al., 1999). On the other hand, LPS-induced phosphorylation and degradation of IκBα and NF-κB activation were not affected by dominant negative PKC-α overexpression (St-Denis et al., 1998), suggesting the involvement of PKC isoforms different from those in paclitaxel-induced signaling.

Activation-induced cell death, a form of apoptosis, is the major mechanism by which immune cell homeostasis is maintained (Russell 1995). When pre-B cells were treated with paclitaxel, we observed the activation of nuclear NF-κB DNA binding complexes similar to those seen with LPS stimulation. However, under these conditions, cells experience growth arrest and subsequently undergo apoptosis, giving NF-κB a proapoptotic role in activation-induced cell death. Moreover, stimulation of pre-B cells with LPS concomitant with paclitaxel treatment does not rescue these cells, suggesting that the paclitaxel-induced NF-κB signal is dominant in the induction of cell death under these conditions. Lin et al. (1999) reported the paradoxical role of NF-κB in apoptosis, functioning as both a proapoptotic and antiapoptotic regulatory factor within a single cell type. Therefore, our results lead to the conclusion that the context of a NF-κB-inducing stimulus is a critical determinant in the outcome of a signal that can lead to proliferation, differentiation, or death.

In summary, we show that paclitaxel, like LPS, causes the translocation of NF-κB through classical PKC isotype-dependent IKK-β activation, which in turn might desensitize spleen cells toward incoming LPS-induced signal in murine 70Z/3 pre-B cells.

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