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Anthrax lethal toxin down-regulates type-IIA secreted phospholipase A_2 expression through MAPK/NF-κB inactivation

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ABSTRACT

Bacillus anthracis, the etiological agent of anthrax, produces lethal toxin (LT) that displays a metalloproteolytic activity toward the *N*-terminus of the MAPK-kinases. We have previously shown that secreted type-IIA phospholipase A_2 (sPLA$_2$-IIA) exhibits potent anthracidal activity. *In vitro* expression of sPLA$_2$-IIA in guinea pig alveolar macrophages (AMs), the major source of this enzyme in lung tissues, is inhibited by LT. Here, we examined the mechanisms involved in sPLA$_2$-IIA inhibition by LT. We first showed that chemical inhibitors of p38 and ERK MAPKs reduced sPLA$_2$-IIA expression in AMs indicating that these kinases play a role in sPLA$_2$-IIA expression. LT inhibited IL-1β-induced p38 phosphorylation as well as sPLA$_2$-IIA promoter activity in CHO cells. Inhibition of sPLA$_2$-IIA promoter activity was mimicked by co-transfection with dominant negative construct of p38 (DN-p38) and reversed by the active form of p38-MAPK (AC-p38). Both LT and DN-p38 decreased IL-1β-induced NF-κB luciferase activity. This contrasted with the effect of AC-p38, which enhanced this activity. However, neither LT nor specific p-38 inhibitor interfered with LPS-induced IκBα degradation or NF-κB nuclear translocation in AMs. Subcutaneous administration of LT to guinea pig before LPS challenge reduced sPLA$_2$-IIA levels in broncho-alveolar lavages and ears. We conclude that sPLA$_2$-IIA expression is induced *via* a sequential MAPK-NF-κB activation and that LT inhibits this expression likely by interfering with the transactivation of NF-κB in the nucleus. This inhibition, which is operating both *in vitro* and *in vivo*, may represent a mechanism by which *B. anthracis* subvert host defense.

1. Introduction

Anthrax is primarily a disease of herbivores due to *Bacillus anthracis* infection, but humans are also susceptible [1]. *B. anthracis*, the etiological agent of anthrax disease, is a Gram-positive, spore-forming bacterium. Although infection by this bacterium frequently occurs *via* the cutaneous route, both gastrointestinal and pulmonary infections cause the highest mortality in human. Macrophages and/or dendritic cells take up inhaled spores leading to their germination. This is followed by the migration of these cells to the draining lymph nodes [2]. Encapsulated bacilli then enter the blood compartment and disseminate in the whole organism. Despite appropriate therapy, all these forms of infection may progress to fatal systemic anthrax, leading to shock-like symptoms and respiratory failure [3].

Studies have suggested that type-IIA secreted phospholipase A_2 (sPLA$_2$-IIA) may represent an effector involved in host defense against invading bacteria. Indeed, sPLA$_2$-IIA is a highly bactericidal enzyme, especially toward Gram-positive bacteria [4–6] and is found at high levels in airways of humans [7,8] and various animal species [9–11]. sPLA$_2$-IIA belongs to a family of enzymes that catalyzes the hydrolysis of phospholipids leading to the generation of lysophospholipids and free fatty acids [12]. We have recently shown that sPLA$_2$-IIA is highly bactericidal towards *B. anthracis*

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[13] and protects infected mice against *B. anthracis* infection [14]. These findings suggest that $sPLA_2$-IIA may represent an efficient weapon participating in host defense against anthrax.

However, a number of studies have reported the ability of *B. anthracis* to subvert immune response [15–18]. This inhibition mainly results from the action of specific *B. anthracis* toxins. Indeed, *B. anthracis* carry two plasmids, pXO1 [19] and pXO2 [20], which encode the primary virulence factors: lethal and edema toxins, and the proteins required for capsule synthesis, respectively. The toxins are composed of three secreted proteins: protective antigen (PA), lethal factor (LF) and edema factor (EF). These proteins act in pairs [21] leading to the lethal toxin (LT) and the edema toxin (ET), composed of the association of LF/PA and EF/PA respectively. Uptake of LT by the target cells is followed by the release of LF within the cytoplasm of these cells. There, LT displays its metallo-proteolytic activity toward the *N*-terminus of the MAPK-kinase (MAPKK), thereby interfering with the MAPK pathway [22]. We have recently reported that LT was able to inhibit $sPLA_2$-IIA expression by guinea pig AM [13], the main source of pulmonary $sPLA_2$-IIA in guinea pig [9]. However, the mechanisms involved in this inhibition have not been examined. The present study has been addressed to identify the signaling pathways involved in this modulation and whether LT is able to interfere with $sPLA_2$-IIA production *in vivo*.

2. Materials and methods

2.1. Animals and reagents

Male Hartley guinea pigs were purchased from Charles River Laboratories (L'Arbresle, France). RPMI 1640 cell culture medium was obtained from Invitrogen (Cergy-Pontoise, France) and Fetal calf serum (FCS) from Hyclone (Logan, USA). Chinese hamster ovary (CHO) cells were purchased from ATCC (Manassas, USA). SB 203580 and PD 98059 were purchased from Biomol (Le Perray-en-Yvelines, France). MG-132 and *Pseudomonas aeruginosa* LPS were purchased from Sigma Aldrich (St. Louis, MO, USA). Antibodies directed against, IκBα (sc-371), MEK-3 (sc-959) and β-actin (sc-81178) were from Santa Cruz Biotechnology (Santa Cruz, USA). Antibodies directed against p38-MAPK (9212), phospho-p38-MAPK (p38pT180-Y182, 9211), ERK (9102) and phospho-ERK (ERKpT202-Y204, 9101) were from Cell Signaling (Danvers, USA). Recombinant LF and PA from *B. anthracis* were purchased from List Biological Laboratory (Campbell, USA). Dominant negative and active form constructs of p38-MAPK are a gift of Dr. Jacques Pouyssegur (Institute of Developmental Biology and Cancer, CNRS, Nice, France).

2.2. Preparation of AMs and CHO cells and incubation procedures

Guinea pig broncho-alveolar lavages (BALs) were performed with PBS and AMs were isolated as previously described [23]. AMs, adjusted at 2×10^6 cells/mL in RPMI 1640 with 3% FCS, were pretreated either with LT, MAPK inhibitors or NF-κB inhibitor 1 h before LPS stimulation. Chinese hamster ovary (CHO) adjusted at 2×10^4 cells/mL (24-well plates) in HAM F12 with 10% FCS, 4 mM glutamine, 100 units/mL penicillin and 100 mg/mL streptomycin were incubated with LT for 1 h before stimulation with IL-1β. After stimulation, media and cell Lysates were collected for $sPLA_2$-IIA activity, EMSA and Western blot analyses were performed as described below.

2.3. Assay of $sPLA_2$–IIA activity

$sPLA_2$-IIA activity was assayed using [^{3}H]-oleic acid-labeled membranes of *Escherichia coli*, following the method of Franson et al. [24] modified by Paya et al. [25]. Briefly, the *E. coli* strain CECT 101 was seeded in medium containing 1% tryptone, 0.5% yeast extract, 0.5% NaCl and 0.6% sodium dihydrogen orthophosphate, pH 5.0 and grown for 6–8 h at 37 °C in the presence of 5 μCi/mL [^{3}H]-oleic acid (specific activity 10 Ci/mmol) until growth approached the end of the logarithmic phase. After centrifugation at 1800 × *g* for 10 min at 4 °C, the membranes were washed in buffer (0.7 M Tris–HCl, 10 mM $CaCl_2$, 0.1% bovine serum albumin, pH 8.0), re-suspended in saline and autoclaved for 30–45 min. The membranes were then washed, centrifuged again and frozen at −70 °C. At least, 95% of the radioactivity was incorporated into the phospholipid fraction.

Aliquots (5–20 μL) of AM supernatants, broncho-alveolar lavage fluids (BALF) or ear homogenates were incubated with 250 μL of buffer A (100 mM Tris–HCl, 1 mM $CaCl_2$, pH 7.5), for 15 min 37 °C in the presence of 10 μL autoclaved [^{3}H]-oleic acid-labeled membranes. The reactions were stopped by addition of 100 μL ice-cold stop solution (0.25% BSA in 100 mM Tris–HCl, 1 mM $CaCl_2$, pH 7.5). After centrifugation at 1800 × *g* for 10 min at 4 °C, the radioactivity in the supernatants was determined by liquid scintillation counting. BALF and ear homogenates were treated with the $sPLA_2$-IIA inhibitor, LY311727 to check that hydrolysis is due to $sPLA_2$-IIA activity. The latter was expressed as picomoles hydrolyzed substrate per minute per milligram ear homogenates or per milliter BALF or AM supernatants.

2.4. Nuclear protein extraction and electrophoretic mobility shift assays

Nuclear proteins were extracted from AMs as previously described [26]. The NF-κB double-stranded oligonucleotides (Santa Cruz Biotechnology, Santa Cruz, USA) consensus sequence 5′-AGT TGA GGG GAC TTTT CCC AGG C-3′ was γ-^{32}P-labeled with T4 polynucleotide kinase (New England Biolabs, Ipswich, USA) on the overhanging ends. Protein concentrations were determined by using Nanodrop spectrophotometer (Nyxor Biotech, Paris, France). Binding reactions were performed as follow: briefly, incubations were performed by adding 5 μg of nuclear extract, 10 μL of 2× binding buffer (40 mM HEPES, pH 7, 140 mM KCl, 4 mM DTT, 0.02% Nonidet P-40, 8% Ficoll, 200 μg/mL BSA, 1 μg of poly(dI:dC), and 1 μL of γ-^{32}P-labeled probe, in a total volume of 20 μL for 20 min at room temperature. The reaction mixtures were separated on a 5% polyacrylamide gel in 0.5% Tris/borate/EDTA buffer at 150 V for 2 h. Gels were dried and exposed for 2 to 12 h. We have previously shown, using supershift analysis, that antibodies directed against NF-κB's p50 and p65 subunits shift the NF-κB band in LPS-stimulated AMs, thus confirming that the observed complexes belong to the NF-κB family [27].

2.5. Protein extraction and Western blot analyses

Proteins from AMs and CHO cells were extracted in lysis buffer (10 mM Tris–HCl, 150 mM NaCl, 3 mM EDTA, 100 μM leupeptin, 100 μM aprotinin, 1 μM soybean trypsin inhibitor, 5 mM NEM, 1 mM PMSF, 5 mM benzamidine and 1% Triton X-100, pH 7.4) and electrophoresed under reducing conditions. Semidry transferred proteins were applied onto polyvinylidene difluoride membranes. Nonpecific binding sites were blocked overnight with 5% BSA in 20 mM Tris–HCl, pH 7.6, 140 mM NaCl and 0.1% Tween 20. Blots were probed for 1 h with indicated antibodies. After washing, the immunoreactive bands were visualized using the specifics peroxidase-conjugated anti-IgG and the ECL Plus Western Blotting Detecting System (Amersham Biosciences, Orsay, France).

2.6. Plasmid constructions and transfection

CHO cells, adjusted at 2×10^4 cells/mL (24-well plates) in HAM F12 with 10% FCS, 4 mM glutamine, 100 units/mL penicillin and

100 mg/mL streptomycin were transfected or co-transfected with the Ig-(κB) luciferase plasmid [28], [−1153; +46]-sPLA$_2$-IIA luciferase plasmid [28], dominant negative construct of p38 (DN-p38) or active form of p38-MAPK (AC-p38) using LIPOFECTAMINE™ Plus (Invitrogen, Carisbad, USA). After 24 h of transfection the cells were pretreated with LT (PA + LF) 1 h before LPS (1 μg/mL) or IL-1β (10 ng/mL) stimulation. 24 h after stimulation luciferase activity was determined in duplicate using a luciferase reporter assay kit (Promega, Madison, USA), with signal detection for 12 s by a luminometer (Berthold, Pforzheim, Germany), and normalized by dividing the relative light units by β-galactosidase activity.

2.7. In vivo experiments

Guinea pigs weighing 500–600 g were anesthetized by i.m. injection of ketamine hydrochloride (50 mg/kg body weight; Parke Davis, Courbevoie, France) and xylazine (2 mg/kg body weight; Bayer, Leverkusen, Germany). LT (PA + LF) and LPS were injected subcutaneously in each ear. A first injection (PA 10 μg + LF 5 μg in 20 μl of sterile PBS) was administrated 1 h before a second injection (PA 5 μg + LF 2.5 μg + 100 μg of LPS in 20 μL of sterile PBS). Control experiments were performed using PBS instead of LT. After overnight treatment with LPS the animals were euthanized with 40 μg/kg pentobarbital sodium (Sanofi, Libourne, France) and broncho-alveolar lavages (BALs) and ear homogenates were performed as described below.

2.8. Preparation of ear homogenates

Guinea pigs were anesthetized and then 12 mm sections of ears were punched after euthanasia. Then, frozen tissues were suspended in 0.25 mM sucrose solution containing 10 mM Tris–HCl, pH 7.4, 1 mM EDTA, 0.5 mM PMSF, 2 μg/mL leupeptin, and 2 μg/mL aprotinin to yield a 10% (w/v) homogenate. The tissues were disrupted with a mixer Ultra-Turrax T-25 (Janke and Kunkel, Staufen, Germany) at 4 °C and then centrifuged for 5 min at 1000 × *g*. The resulting supernatant was centrifuged at 20,000 × *g* for 20 min and supernatants were used for sPLA$_2$-IIA activity analyses as described above.

2.9. Control of cell viability

Cell viability was checked by the trypan blue dye exclusion test. Cell lysis was controlled by measuring the release of lactate

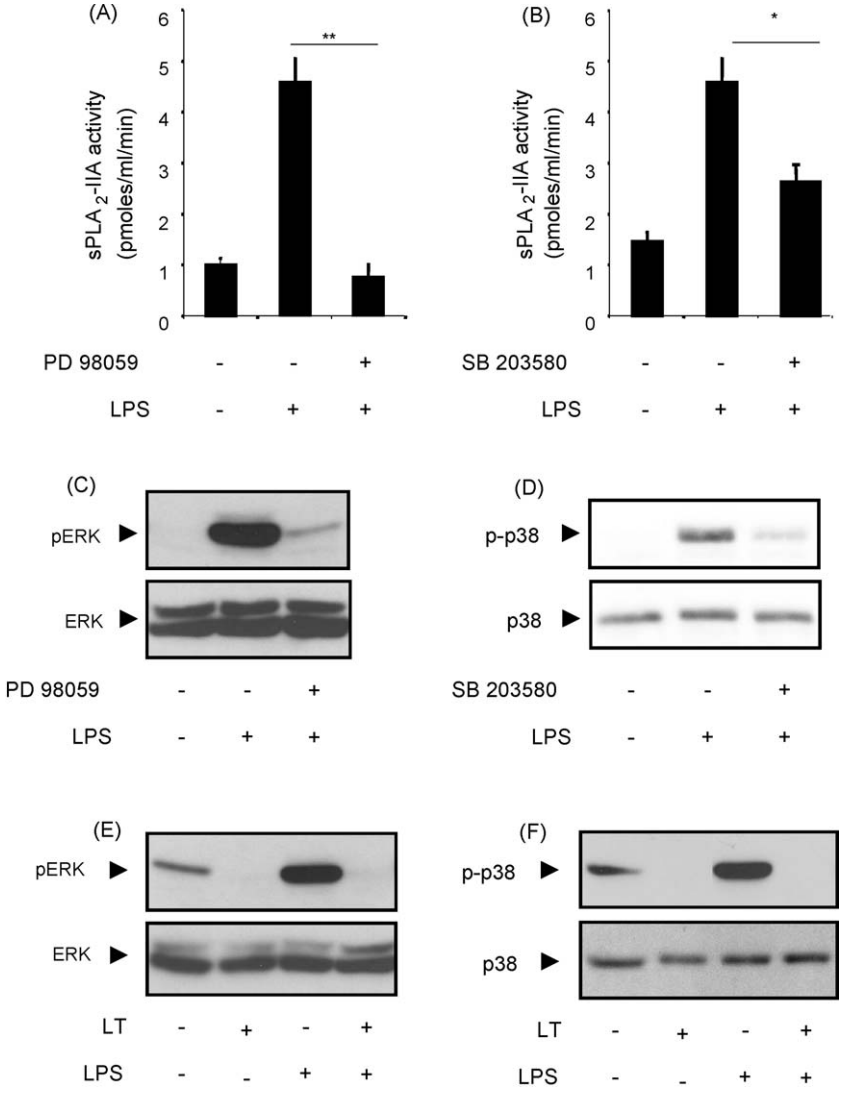

Fig. 1. MAPK inhibition reduces sPLA$_2$-IIA secretion by AMs. AMs were pretreated with (A) PD 98059 (100 μM) or (B) SB 203580 (10 μM) for 1 h, and then stimulated with LPS (50 ng/mL). After 24 h incubation, the levels of sPLA$_2$-IIA were measured in the supernatants. In (C) and (D), AMs were incubated with PD 98059 (100 μM) or SB 203580 (10 μM) for 1 h and then stimulated with LPS (50 ng/mL) for 30 min. Erk and p38 phosphorylations were analyzed by Western blot. In (E) and (F), AMs were pretreated for 1 h with LT (1 μg/mL) and then stimulated with LPS (50 ng/mL) for 30 min. Erk (E) and p38 (F) phosphorylations were analyzed by Western blot.

dehydrogenase activity using a commercial kit from Boehringer (Mannheim, Germany). No changes of cell viability or lysis were observed in AMs or CHO cells after addition of LT or LPS.

2.10. Statistical analysis

The results are presented as mean ± SEM; and are represented of at least three independent experiments. The level of statistical significance was determined by two-way analysis of variance (ANOVA) followed by Dunnett's *t*-test for multiple comparisons.

3. Results

3.1. A role for MAPK in the inhibition of sPLA$_2$-IIA expression by LT

Our previous report showed that LT inhibited LPS-induced sPLA$_2$-IIA synthesis by guinea pig AMs [13]. LT was able to cleave MEK-3 in AMs (data not shown), which is in agreement with previous reports in various cell types [22]. Thus, it is likely that inhibition of MAPK activation by LT may play a key role in the inhibition of sPLA$_2$-IIA expression in AMs. We first examined the effect of known inhibitors of MAPK cascade on sPLA$_2$-IIA production by AMs. Pre-incubation of AMs with PD 98059 and SB 203580 (respectively inhibitors of ERK and p38-MAPK), reduced LPS-induced sPLA$_2$-IIA secretion (Fig. 1A and B) and inhibited LPS-induced phosphorylation of ERK or p38-MAPK (Fig. 1C and D) as expected. We also showed that LT inhibits LPS-induced p38 and ERK phosphorylations in AMs (Fig. 1E and F).

We then investigated the impact of LT on the activation of sPLA$_2$-IIA promoter construct transfected in CHO cells. Pretreatment of CHO cells with LT inhibited IL-1β-induced sPLA$_2$-IIA promoter activity (Fig. 2A). Similar results were observed when the cells were stimulated with LPS instead of IL-1β (data not shown). Co-transfection of CHO cells with the dominant negative form of p38 (DN-p38) construct inhibited IL-1β-induced sPLA$_2$-IIA promoter activity (Fig. 2B). In contrast, co-transfection with the active form of p38-MAPK (AC-p38) construct significantly increased sPLA$_2$-IIA transcriptional activity and reversed the inhibitory effect of LT on this activity (Fig. 2C).

3.2. Inhibition of NF-κB transactivation but not on its translocation by LT: implication of MAPK

Because NF-κB is known to play a pivotal role in the induction of sPLA$_2$-IIA expression [29] we examined the effect of LT on NF-κB activation in AMs. Indeed, we have previously shown that MG-132 prevents LPS-induced NF-κB transclocation as well as sPLA2-IIA expression in AMs [27]. This compound is a proteasome inhibitor that prevents IκBα degradation which leads to inhibition of NF-κB translocation. EMSA analysis showed that LT failed to interfere with LPS-induced NF-κB nuclear translocation in AMs (Fig. 3A). The use of the p38-MAPK inhibitor SB 203580 also failed to interfere with LPS-induced NF-κB translocation in AMs. Pretreatment of AMs with LPS induced a transient degradation of the NF-κB inhibitor IκBα (Fig. 3B). LPS-induced IκBα degradation was not altered neither by LT (Fig. 3C) nor by SB 203580 (Fig. 3D). As expected, this degradation was abolished by MG-132 (Fig. 3C).

We then examined the effect of LT on NF-κB activation using CHO cells. Pretreatment of CHO cells with LT abolished the IL-1β-induced p38-MAPK phosphorylation (Fig. 4D). CHO cells were then transfected with Ig-(κB) luciferase plasmid followed by pre-incubation with LT 1 h before addition of IL-1β. As shown in

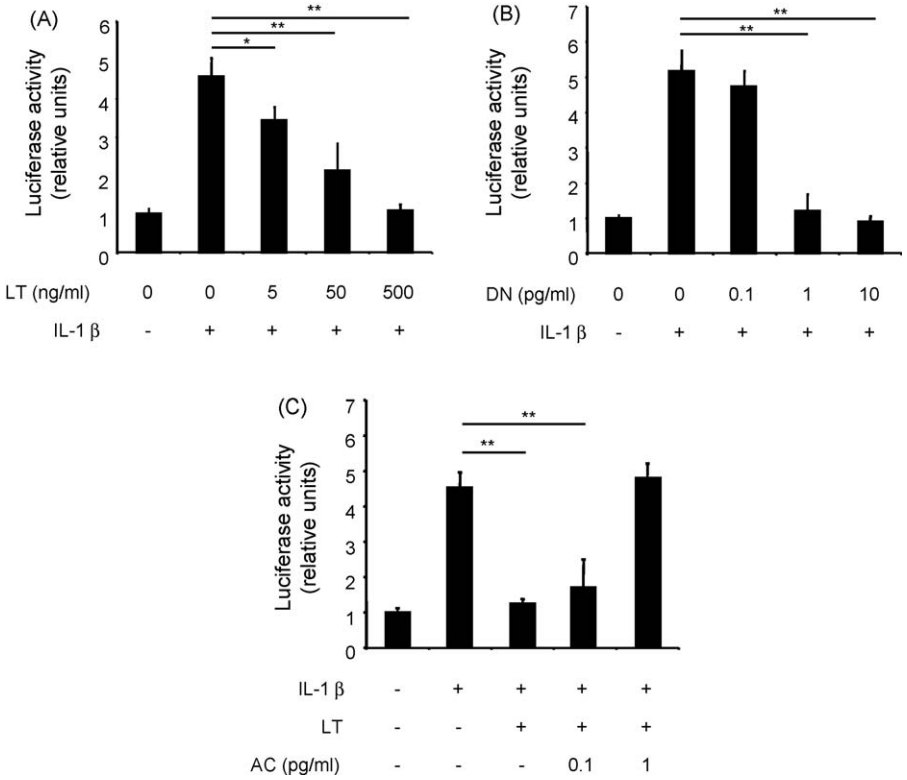

Fig. 2. LT inhibits sPLA$_2$-IIA expression in CHO cells. (A) CHO cells were transfected with the [−1153; +46]-sPLA$_2$-IIA luciferase construct. The cells were pretreated for 1 h with LT (PA 1 μg/mL + increasing concentrations of LF) and then stimulated with IL-1β (10 ng/mL). (B) Co-transfected cells with the [−1153; +46]-sPLA2-IIA luciferase construct plus DN-p38 construct were stimulated with IL-1β (10 ng/mL). (C) Co-transfected cells with the [−1153; +46]-sPLA2-IIA luciferae construct plus AC-p38 were pretreated for 1 h with LT (500 ng/mL) and then stimulated with IL-1β (10 ng/mL). Luciferase activity was measured 24 h after the addition of IL-1β.

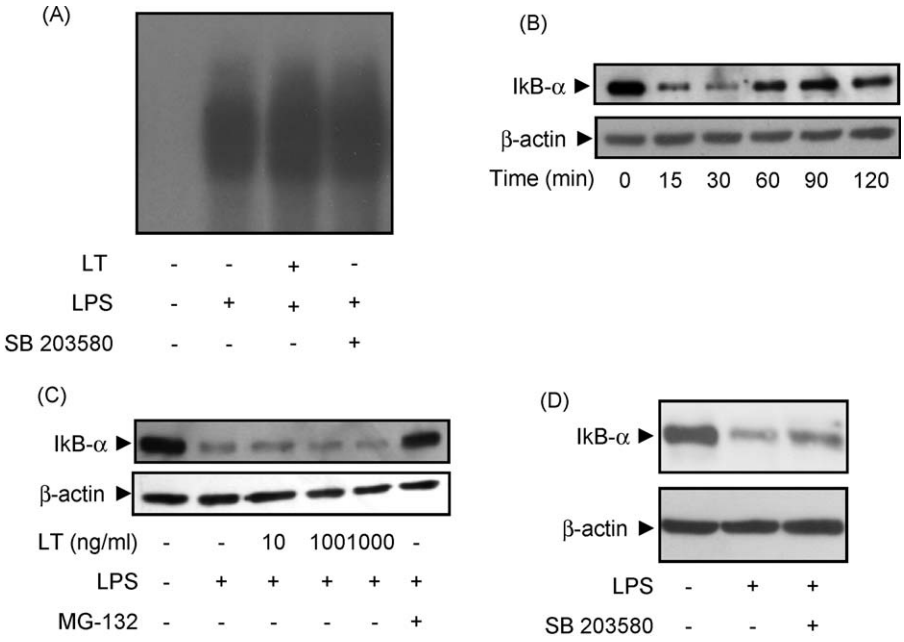

Fig. 3. LT fails to affect NF-κB translocation in AMs. (A) AMs were pretreated for 1 h with LT (1 μg/mL) and then stimulated with LPS (50 ng/mL) and EMSA were performed as indicated in Section 2. (B) AMs were stimulated with LPS (50 ng/mL) during the indicated times and IκBα levels analyzed by Western blot. (C) AMs were pretreated with LT (PA 1 μg/mL + increasing concentrations of LF) or MG-132 (1 μM) 1 h before 30 min stimulation with LPS (50 ng/mL). (D) AMs were pretreated with SB 203580 (10 μM) for 1 h and then stimulated with LPS (50 ng/mL) for 30 min. Cell extracts were subjected to IκBα analysis by Western blot.

Fig. 4A, IL-1β induced a significant increase in luciferase activity, which was abrogated by LT. Similar results were observed when cells were stimulated with LPS (data not shown). Co-transfection of CHO cells with DN-p38 reduced IL-1β-induced NF-κB luciferase activity (Fig. 4B). In contrast, co-transfection with AC-p38 significantly increased this activity (Fig. 4C).

3.3. *In vivo* effects of LT on $sPLA_2$-IIA production

These findings prompted us to examine whether the inhibitory effect of LT on $sPLA_2$-IIA expression is operating *in vivo*. Guinea pigs received ear subcutaneous administration of LT before a second inoculation of both LT and LPS. Our results showed that LPS

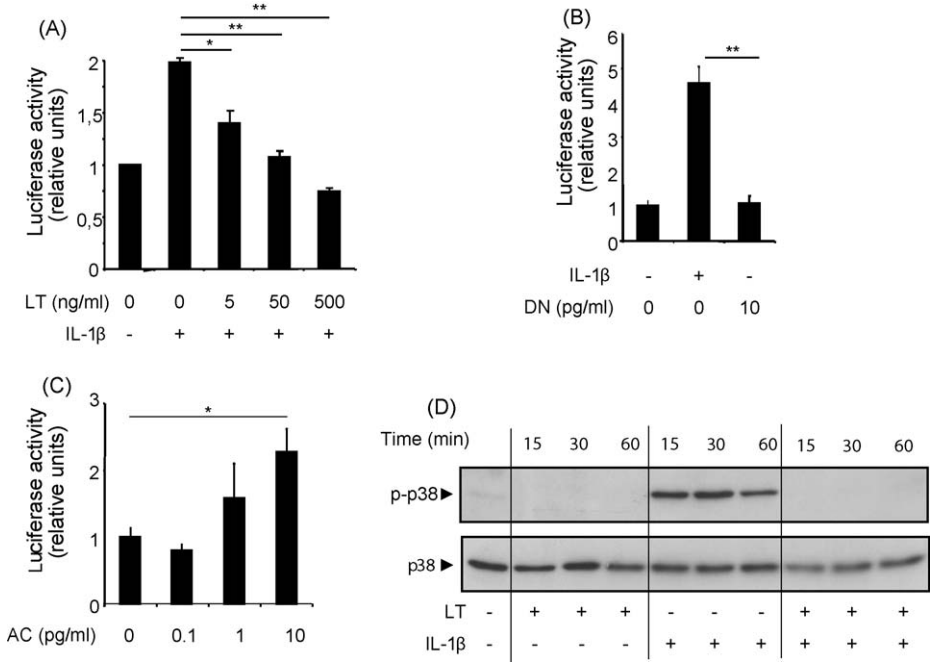

Fig. 4. LT alters NF-κB activation in CHO cells *via* MAPK-dependent process. (A) CHO were transfected with an NF-κB luciferase construct. Transfected cells were pretreated with LT (PA 1 μg/mL + indicated concentrations of LF) 1 h before IL-1β (10 ng/mL) stimulation. (B) Cells co-transfected with the NF-κB luciferase plus DN-p38 (0.1–10 pg/mL) constructs were stimulated with IL-1β (10 ng/mL). (C) Cells co-transfected with the NF-κB luciferase plus AC-p38 (0.1–10 pg/mL) constructs were pretreated with LT (500 ng/mL) 1 h before IL-1β (10 ng/mL) stimulation. Luciferase activity was measured 24 h after the addition of IL-1β. (D) Non-transfected CHO cells were pretreated with LT (1 μg/mL) for 1 h and then stimulated with IL-1β (10 ng/mL) at the indicated time intervals. p38 phosphorylation was analyzed by Western blot.

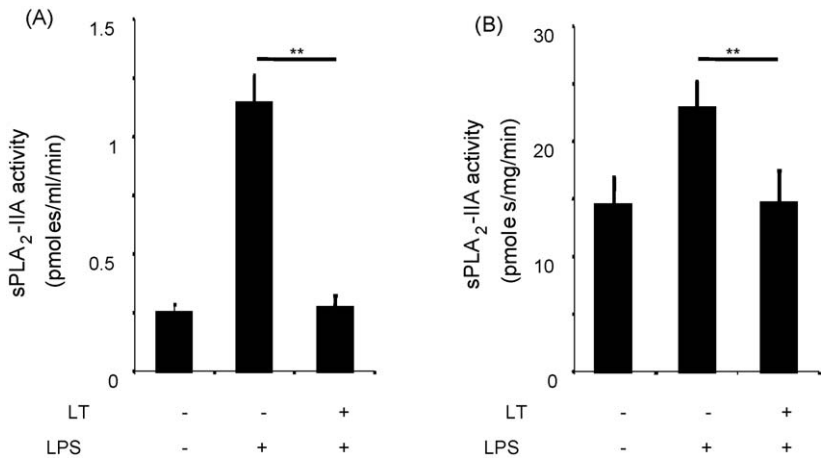

Fig. 5. LT reduces $sPLA_2$-IIA activity in BALF and guinea pig ears. Guinea pigs received subcutaneous administration of LT (PA 10 μg + LF 5 μg) 1 h before a second instillation of both LT (PA 5 μg + LF 2.5 μg) and LPS (100 μg). 14 h later, $sPLA_2$-IIA activity was measured in BALF (A) and ears (B) as detailed in Section 2.

enhanced the levels of $sPLA_2$-IIA activity, both in ears (Fig. 5B) and BALF (Fig. 5A). The levels of $sPLA_2$-IIA activity were lowered either in BALF and ears in animals pretreated with LT before LPS instillation.

4. Discussion

Our previous studies suggested that $sPLA_2$-IIA, a component of lung innate immunity present in airways of human and various animal species, plays a role in host defense against *B. anthracis* [14]. However, despite the ability of $sPLA_2$-IIA to kill this bacterium, pulmonary anthrax has been shown to cause near 100% mortality. This suggests that *B. anthracis* probably affects $sPLA_2$-IIA expression and/or activity in lung tissues. Our previous studies showed that LT was indeed able to inhibit $sPLA_2$-IIA synthesis in AMs [13], the main pulmonary source of this enzyme in guinea pig [9], but the mechanisms involved in this inhibition have not been investigated. This inhibitory effect was not due to a cytotoxic action of LT, because guinea pig AMs were found to be resistant to the lethal effect of this toxin. Thus, guinea pig AMs belong to the group of macrophages that are resistant to the lytic action of LT, but responsive to its action, as detected by the cleavage of MEK-3 (data not shown) and inhibition of $sPLA_2$-IIA production [13]. The definition of resistance and susceptibility to LT relies on its ability to exert a lethal cytotoxic effect on certain types of cells from given species. However, the PA receptor has been observed on all cell types studied to date and it has been shown that these cells are sensitive to the modifications of the intracellular pathways induced by the enzymatic activity of LF. Thus, guinea pig AMs are indeed resistant to the lethal effect of LT, but are sensitive to the MAPKK pathway disruption triggered by LF. This suggests that cleavage of MAPKK by LT and subsequent alteration of MAPK cascade have not effect on cell viability on these cells.

LT is known to cleave MAPKK thereby interfering with the activation of p38 and ERK pathways [22]. Our results clearly showed that both p38 and ERK (whose phosphorylations are inhibited by LT) play a role in the induction of $sPLA_2$-IIA expression in AMs as specific inhibitors of these kinases significantly reduced $sPLA_2$-IIA levels. This finding, *per se*, is of great interest, as it reveals an unrecognized role of these kinases in the modulation of $sPLA_2$-IIA expression. Remarkably, our studies showed that LT inhibited $sPLA_2$-IIA expression by interfering with p38-MAPK activation. We also demonstrated that this kinase mediates NF-κB activation, a major transcription factor involved in $sPLA_2$-IIA expression in guinea pig AMs [29], and that this activation is abrogated by LT. Although the mechanisms by which this kinase stimulates NF-κB activation in our cell system are still unclear, our findings indicated that this activation might not involve IκBα degradation or NF-κB nuclear translocation. It is thus likely that p38-MAPK induces NF-κB activation at a level located downstream nuclear translocation of this transcription factor. We hypothesize that p38-MAPK induces a nuclear transactivation of NF-κB through either a phosphorylation of NF-κB subunits or coactivators and that this process would lead to stimulation of $sPLA_2$-IIA expression. In this model, the cleavage of MAPKK by LT would result in the inhibition of NF-κB activation ultimately leading to alteration of $sPLA_2$-IIA expression. Our recent report showed that LT inhibit IL-8 expression in the pulmonary epithelial cells line Beas-2B in part by altering chromatin accessibility of IL-8 promoter to activated NF-κB without any effect on NF-κB activation [15]. Whether LT inhibits sPLA2-IIA expression in AMs by a similar mechanism remains to be investigated. Recent reports showed that epigenetic mechanisms play a key role the modulation of $sPLA_2$-IIA expression [30,31].

To confer a pathophysiological relevance to these studies we examined the effect of LT instillation on the production of $sPLA_2$-IIA *in vivo*. Our results showed that subcutaneous instillation of LPS in ears induced an increased production of $sPLA_2$-IIA either in ear homogenates and BALF. We suggest that in this model LPS induces locally (in ear tissues) $sPLA_2$-IIA expression the cell sources of which remains are still to be identified. In parallel, LPS may diffuse into lung tissues where it stimulates $sPLA_2$-IIA by AMs, a major source of this enzyme in lungs [9]. Moreover, a previous study showed that i.p. instillation of LPS induced an increased expression of pulmonary TNFα transcript, suggesting that LPS rapidly entered the blood stream and reached the competent cells within the lung tissues [32]. Interestingly, our data revealed that LT instillation decreased the levels of $sPLA_2$-IIA both in BALF and ears of LPS-challenged guinea pigs. Although the cell targets of LT in lung tissues have not been identified it is likely that LT target AMs leading to $sPLA_2$-IIA inhibition.

It is clearly established now that (i) $sPLA_2$-IIA exhibits potent anthracidal properties [13], (ii) this enzyme is present in airways at sufficient concentrations to kill *B. anthracis*, and (iii) LT is synthesized shortly after germination of *B. anthracis* in the host. Thus, the control of $sPLA_2$-IIA expression could represent an adaptive mechanism that allows *B. anthracis* to escape from the innate immune response. Our previous report showed that the decrease in $sPLA_2$-IIA levels was paralleled by a decrease in the anthracidal activity in LT-treated AMs [13]. The present study identifies a mechanism by which *B. anthracis* might modulate $sPLA_2$-IIA expression in AMs, thus opening a new therapeutic strategy for the treatment of this deadly infection.

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