Biochemical and Biophysical Research Communications 370 (2008) 174–178

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

DPPC regulates COX-2 expression in monocytes via phosphorylation of CREB

R.H.K. Morris [a,*], A.J. Tonks [b], K.P. Jones [a], M.K. Ahluwalia [a], A.W. Thomas [a], A. Tonks [b], S.K. Jackson [c]

[a] Cardiff School of Health Sciences, University of Wales Institute Cardiff, Western Avenue, Llandaff, Cardiff, Wales CF5 2YB, UK
[b] School of Medicine, Cardiff University, Cardiff CF14 4XN, UK
[c] Centre for Research in Biomedicine, UWE, Bristol BS16 1QY, UK

ARTICLE INFO

Article history:
Received 11 March 2008
Available online 18 March 2008

Keywords:
Pulmonary surfactant
Inflammation
DPPC
COX-2
CREB

ABSTRACT

The major phospholipid in pulmonary surfactant dipalmitoyl phosphatidylcholine (DPPC) has been shown to modulate inflammatory responses. Using human monocytes, this study demonstrates that DPPC significantly increased PGE_2 ($P < 0.05$) production by 2.5-fold when compared to untreated monocyte controls. Mechanistically, this effect was concomitant with an increase in COX-2 expression which was abrogated in the presence of a COX-2 inhibitor. The regulation of COX-2 expression was independent of NF-κB activity. Further, DPPC increased the phosphorylation of the cyclic AMP response element binding protein (CREB; an important nuclear transcription factor important in regulating COX-2 expression). In addition, we also show that changing the fatty acid groups of PC (e.g. using L-α-phosphatidylcholine β-arachidonoyl-γ-palmitoyl (PAPC)) has a profound effect on the regulation of COX-2 expression and CREB activation. This study provides new evidence for the anti-inflammatory activity of DPPC and that this activity is at least in part mediated via CREB activation of COX-2.

Pulmonary surfactant plays an important immuno-modulatory role within the lung [1,2]. It has been hypothesised that pulmonary surfactant can suppress excessive inflammatory responses to inhaled stimuli preventing tissue damage within the lung and that alterations in surfactant composition are observed in several inflammatory lung diseases [3,4]. Surfactant lipids (which account for approximately 90% of surfactant by weight) [5], have been shown to modulate a number of inflammatory responses *in vitro* [4,6]. The major lipid component in surfactant is phosphatidylcholine (PC), of which 60% is in the disaturated form dipalmitoyl phosphatidylcholine (DPPC) [7]. We have previously shown that surfactant lipids plays a role in the production of a number of monocyte/macrophage inflammatory mediators including cytokines [8], reactive oxygen species, [9] and platelet activating factor [10]. Previously, we have shown that PC induced down-regulation of TNF-α in human monocytes [8] possibly via increasing the production of PGE_2 [8]. The enzyme that catalyses the conversion of arachidonic acid to PGE_2 is the inducible form of cyclo-oxygenase (COX-2) [11]. COX-2 is expressed by activated macrophages and monocytes and is over-expressed at sites of inflammation [12]. COX-2 may have both pro- and anti-inflammatory properties through the generation of different prostaglandins [13]. COX-2 belongs to a family of immediate response genes that do not require precedent protein synthesis for their expression [14] and its transcription is induced by the activation of a number of transcription factors including nuclear factor kappa B (NF-κB) and cAMP-response element binding protein (CREB). CREB exists in cells as an inactive form but is activated by protein kinase A phosphorylation on Ser-133 of its sequence [15].

The activation of monocytes/macrophages is an important step in the initialisation of the events that can lead to many inflammatory diseases, including inflammatory disease of the lung. Saturated fatty acids have previously been shown to directly induce the expression of COX-2 in a reaction mediated by the lipopolysaccharide (LPS) surface receptor toll-like receptor 4 (TLR4) [16,17]. Currently, the mechanism of action for the effects of surfactant lipid on modulating inflammatory responses in monocytes and macrophages remains largely unknown. In addition to PC (containing mixed fatty acid species), this study demonstrates that the disaturated form of PC can also increase PGE_2 production in human monocytes. This report demonstrates for the first time that this DPPC-induced PGE_2 synthesis is mediated via CREB activation and induction of COX-2 expression. Further, changing the saturation of the fatty acid group of PC has profound effects upon this mechanism. These results suggest that cellular expression of COX-2 and other inflammatory markers in monocytes and macrophages may be differentially regulated by different species of phospholipid.

* Corresponding author. Fax: +44 02920 41 698226.
E-mail address: kmorris@uwic.ac.uk (R.H.K. Morris).

0006-291X/$ - see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2008.03.052

Results

The effect of DPPC on PGE_2 synthesis and secretion in human monocytes

Previously we have shown that surfactant phospholipids modulate TNF-α production in human monocytes and at the same time increased the production of PGE_2 [8]. In order to determine whether the disaturated form of PC (DPPC; the major constituent of surfactant) also had a similar effect, we incubated DPPC (250 μg/ml) with human monocytes. When the human monocytic cell line MM6 was incubated with DPPC for up to 8 h, there was a significant production of PGE_2 (2.5-fold) when compared to untreated cells (Fig. 1A). Similarly, this effect was also observed in primary peripheral blood monocytes (PBMCs) under identical conditions (Fig. 1A). This data suggests the effects were not cell-line dependent.

Addition of the specific COX-2 inhibitor NS-398 (10 μM) prior to incubation with DPPC abrogated the release of PGE_2 from MM6 cells when compared to untreated controls (Fig. 1A). This data suggests that the effects of DPPC on PGE_2 production are mediated at least in part by COX-2. This study next investigated whether DPPC alone could increase the expression of COX-2. MM6 cells were treated with DPPC (250 μg/ml) for 0–90 min and COX-2 protein expression was determined by Western blot. Fig. 1B shows that DPPC increased COX-2 protein expression in a time dependent manner. To investigate if the disaturated acyl groups in DPPC were required for COX-2 induction, PAPC containing a polyunsaturated acyl group at position 2 of the PC was also examined. In contrast to DPPC, MM6 cells incubated with 250 μg/ml PAPC for 90 min failed to increase COX-2 protein expression (Fig. 1C). Taken together these data suggest that the surfactant lipid DPPC may modulate inflammatory responses via COX-2 and the saturation of the acyl species plays a role.

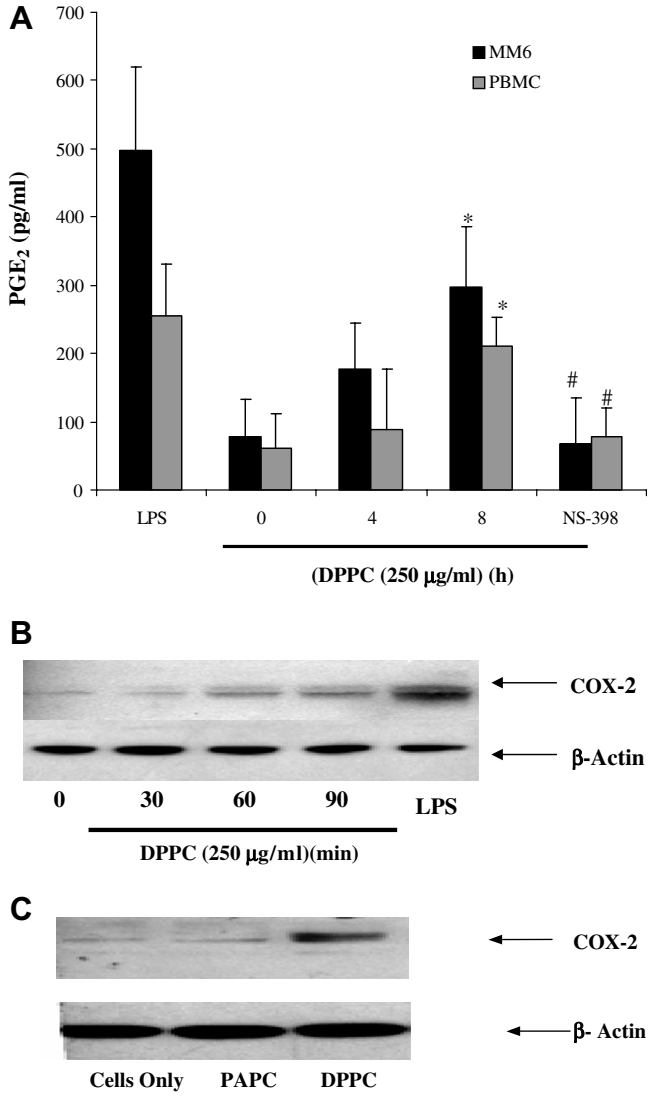

Fig. 1. DPPC induces PGE_2 production and COX-2 protein expression in human monocytes. (A) MM6 cells or PBMC were incubated with DPPC (250 μg/ml) for 0, 4 and 8 h and the PGE_2 secreted measured by standard ELISA. As a positive control for PGE_2 synthesis and secretion, cells were treated with LPS (100 ng/ml) for 8 h. The results are means ± SD of three separate experiments. *Denotes a significant increase in PGE_2 secretion compared to cells treated for 0 h (ANOVA, Tukey's pairwise analysis). #Denotes a significant reduction in PGE_2 secretion compared to cells treated for 8 h (ANOVA, Tukey's pairwise analysis). (B) MM6 cells were treated with DPPC (250 μg/ml) for 0, 30, 60 and 90 min and the COX-2 expression assessed by Western blotting. As a positive control of COX-2 activation, cells were treated with LPS (100 ng/ml) for 90 min. (C) Western blot of COX-2 expression was compared in cells treated with DPPC for 90 min with treatment with the polyunsaturated lipid, PAPC (250 μg/ml) for 90 min. Representative blots from three independent experiments are shown.

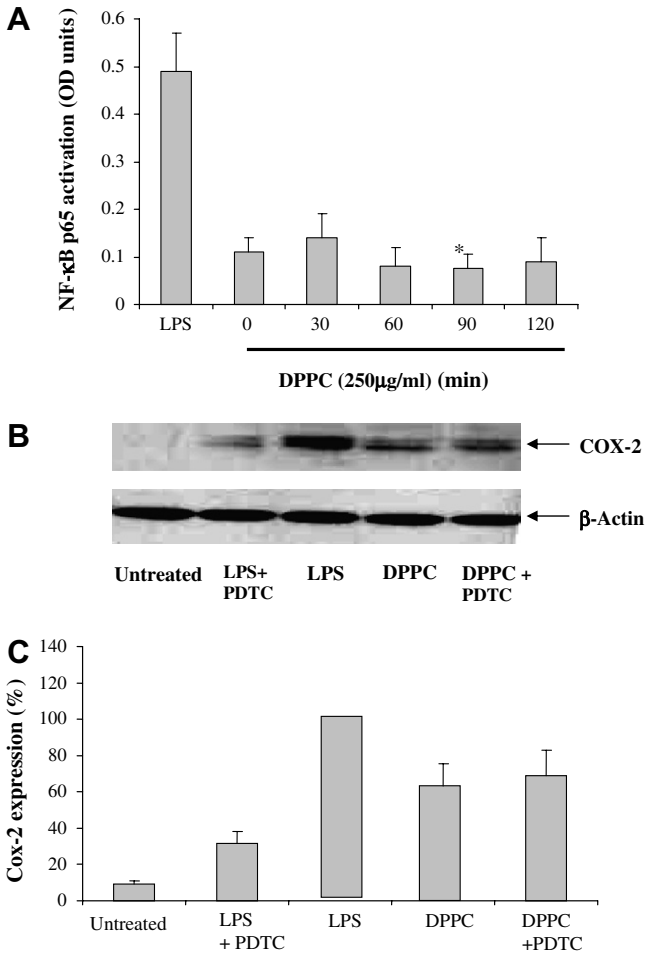

Fig. 2. DPPC does not induce NF-κB activation. (A) MM6 cells were treated with DPPC (250 μg/ml) for 0–90 min and the NF-κB p65/DNA binding activity measured. MM6 cells were treated with 100 ng/ml of LPS for 60 min as a positive control of NF-κB p65/DNA binding activity. Each result is the mean ± SD of four separate analyses *Denotes a significant decrease in NF-κB p65/DNA binding activity compared to cells treated for 0 h (ANOVA, Tukey's pairwise analysis). (B) NF-κB activation was inhibited with PDTC (10 μM) in MM6 cells treated with either DPPC or LPS for 120 min and their COX-2 expression determined by Western blot. (C) COX-2 expression as a percentage of the LPS-treated cells as determined by densitometric image analysis.

The effect of DPPC on transcription factors involved in COX-2 expression

The transcription of COX-2 is induced by the activation of a number of transcription factors including NF-κB and CREB [25,26]. NF-κB is a heterodimer of p65 and p50, which in unstimulated cells, is localised to the cytoplasm. Upon activation, p65 heterodimer translocates into the nucleus where it binds to target genes. The nuclear expression of p65 heterodimer is an indicator of NF-κB activation [18]. DPPC (under similar conditions as above) failed to induce NF-κB activation in MM6 cells (Fig. 2A), suggesting that DPPC stimulates COX-2 transcription independently of NF-κB. As a positive control, LPS induced NF-κB activity confirming that these cells were responsive to inflammatory stimuli. To support this data the effect of NF-κB inhibition on COX-2 expression was investigated. Pyrrolidine dithiocarbamate (PDTC), an inhibitor of NF-κB [19] did not abolish the production of PGE_2 (data not shown) or the expression of COX-2 in DPPC treated cells (Fig. 2B). Again, LPS and PDTC treated cells were used as positive and negative controls.

Alternatively, COX-2 expression is regulated via CREB [15]. To assess the involvement of CREB in DPPC-induced COX-2 expression, MM6 cells were treated with CREB specific siRNA. Fig. 3 shows that DPPC-induced COX-2 expression was abolished in cells treated with the specific siRNA for CREB-1. MM6 cells were also incubated without siRNA and random scrambled siRNA as positive and negative controls, respectively. This data suggests that CREB-1 is involved in this mechanism. To further substantiate this, we measured the phosphorylated form of CREB as an indicator of activity since the phosphorylation of CREB has been implicated in the activation of COX-2 in a number of cell types [15,34,35]. Incubation of MM6 cells with DPPC induced significant levels of pCREB by 4-fold when compared to untreated cells (Fig. 4). This effect was time dependent with maximal activation of the transcription factor at 45 min (Fig. 4). In contrast, PAPC failed to induce pCREB activation in MM6 cells over the same time and dose range (Fig. 4.).

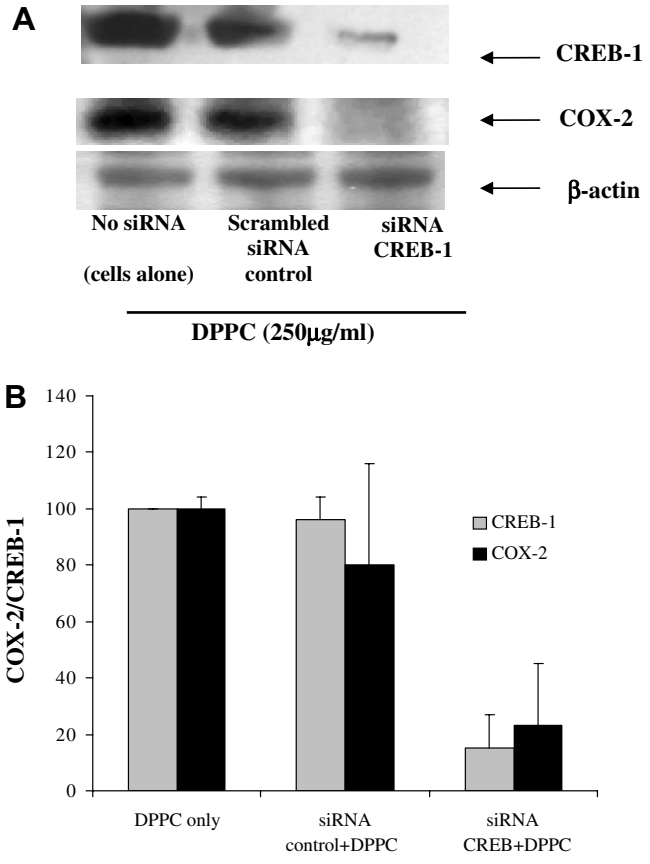

Fig. 3. Inhibition of CREB inhibits DPPC-mediated COX-2 expression. MM6 cells were treated with siRNA for CREB or scrambled control siRNA and CREB expression and DPPC-induced COX-2 expression determined by Western blotting (A). Representative blots from three separate experiments are shown. (B) Densitometric analysis.

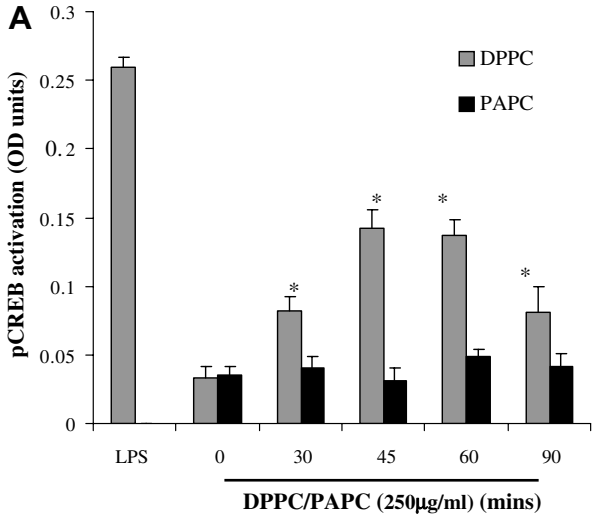

Fig. 4. DPPC enhances pCREB activity in MM6 cells. MM6 cells were incubated in the presence or absence of (A) DPPC (250 μg/ml) for 0, 30, 45, 60 and, 90 min and pCREB formation determined. As a positive control of CREB activation, cells were stimulated with LPS (100 ng/ml) for 45 min. *Denotes a significant increase in pCREB activity compared with untreated controls (ANOVA, Tukey's pairwise analysis).

Discussion

Pulmonary surfactant lipids demonstrate immuno-regulatory roles in addition to their effects on alveolar surface tension [1,2]. In particular, the major surfactant lipid, DPPC, has shown anti-inflammatory activity in a number of cell types [4,8–11]. The inflammatory response in the lungs could initiate or potentiate the development of many inflammatory lung diseases, including acute respiratory distress syndrome, cystic fibrosis, idiopathic pulmonary fibrosis, and chronic obstructive pulmonary disease (COPD) [3,20,21]. Therefore, understanding the mechanisms by which inflammation is controlled in the lung is important to the development of effective therapies for inflammatory lung disease. In this study we have shown that the surfactant phospholipid DPPC can up-regulate PGE_2 production in human monocytes via CREB-1 mediated COX-2 activation. Although expression of COX-2 and PGE_2 have been reported in disorders associated with increased levels of bronchial inflammation [22,23], it has increasingly been shown that COX-2 has anti-inflammatory effects that are partly mediated by prostaglandins such as PGE_2 [24]. The anti-inflammatory activities of PGE_2 include inhibiting the release of proinflammatory cytokines such as TNF-α and IL-1β by macrophages and T-cells and increasing IL-10 secretion by macrophages [25,26].

DPPC is the major phospholipid constituent of pulmonary surfactant and our group and others have shown they can modulate inflammatory mediator production, including TNF-α, PAF and reactive oxygen species [6,8–10]. However, the mechanisms leading to these anti-inflammatory actions remain elusive. DPPC was previously found to inhibit proinflammatory responses independently of p44/p42/p38 MAPK [27] and down-regulate the respiratory burst through a mechanism that involved modulation of PKCδ acti-

vation [9]. Monocytes rapidly ingest surfactant after the lipid components are incorporated into the plasma membrane [28]. Measurements of membrane fluidity in surfactant-treated monocytes in our laboratory revealed that DPPC incorporation significantly alters membrane fluidity [10] suggesting interference with membrane structure and function. Pinot and co-workers suggested that a phospholipid-rich surfactant preparation modulates cAMP accumulation in monocytes through a membrane-controlled mechanism [29].

The present study has demonstrated that treatment of human monocytes with DPPC (at physiologically relevant concentrations [5,8]), is able to significantly increase PGE_2 synthesis in the absence of any other stimulus. MM6 cells exhibit the phenotypic, morphological and functional characteristics of mature monocytes, the precursors of the alveolar macrophage [30] and provide an ideal model to study the regulation of inflammatory responses. The ability of DPPC to induce PGE_2 synthesis can partly be explained by COX-2 induction. The activation of COX-2 can be mediated by a number of transcriptional factors including NF-κB, NFAT, CREB and cEBPβ [15,31–33]. However, the specific factors involved in COX-2 activation depend on both the cell type and the stimulus. Thus, NF-κB contributes to COX-2 induction in some, but not all cell types. In this study we show that DPPC does not activate NF-κB and that DPPC-stimulated COX-2 expression is independent of NF-κB activity. In their study, Poligone and Baldwin [24] reported that COX-2 was capable of inhibiting NF-κB activity, which supported the enzymes anti-inflammatory features. Further we also show that DPPC initiates COX-2 expression by phosphorylation of CREB in human monocytes. CREB is phosphorylated at serine 133 by protein kinase A in response to cAMP leading to transcriptional activation of genes whose promoters contain the CRE sequence [32]. Other kinases capable of phosphorylating and activating CREB include Ca^{2+}/calmodulin-dependent kinase, Ras-dependent kinase (RSK-2)/ MAP kinases, and PKC [33–35]. Thus, there are many potential targets and pathways through which DPPC could be activating CREB. Although we previously found no effect of DPPC on MAPK phosphorylation [27] DPPC was seen to prevent PKC activation in monocytes [9] suggesting that PKC probably does not activate CREB in these cells.

Eliopoulos et al. [15] demonstrated that the phosphorylation of CREB by LPS in mouse macrophages is mediated through ERK signals that activate p90Rsk, Msk1 and the serine/threonine kinase Tpl/Cot. As LPS, like DPPC, contains a substantial saturated lipid component it is possible that the up-regulation of pCREB and hence COX-2 observed in this study is mediated by a similar mechanism. Interestingly, PAPC was unable to activate COX-2 suggesting that the ability to activate COX-2 by lipids is related to their degree of saturation. Hwang [16] and Lee et al. [17] were able to demonstrate that saturated lipids present in the lipid A moiety of LPS modulated the expression of COX-2 in a macrophage/monocyte cells. Recently, it has been shown that CREB activation may be regulated by phosphatidylinositol 3-kinase (PI3K)-mediated inhibition of glycogen synthase kinase (GSK3) via Akt [36]. Activation of CREB was shown to suppress NF-κB activation and promote anti-inflammatory responses [36]. Our study supports the anti-inflammatory bias of CREB activation in immune cells and suggests that certain saturated lipid species, such as DPPC, may also regulate CREB activation to induce anti-inflammatory mechanisms including COX-2 expression.

Materials and methods

Preparation of lipid media. L-α-Phosphatidylcholine dipalmitoyl (DPPC), L-α-phosphatidylcholine β-arachidonoyl-γ-palmitoyl (PAPC), were purchased from Sigma Chemical Co. (Dorset, UK) and prepared as previously described [9].

Human monocyte cell culture. The human monocytic cell-line MonoMac-6 (MM6) was cultured as previously described [9]. The viability of MM6 cells exposed to lipid was greater than 90% throughout. Human peripheral blood monocytes were isolated from adult non-smoking volunteers who were not receiving any medication and gave informed consent, as previously described [9].

Determination of PGE_2 release from human monocytes. Human monocytes (1×10^6/ml), were suspended in RPMI media supplemented with DPPC (250 μg/ml) for 0–8 h at 37 °C in 5% CO_2 atmosphere. Monocytes were washed in sterile PBS (3×), resuspended in supplemented RPMI and stimulated with LPS (100 ng/ml for 6 h) as a positive control. Following incubation, cell supernatants were frozen at −70 °C. PGE_2 concentration was determined by ELISA according to the manufacturer's instructions (R&D, Minneapolis, USA).

Effect of DPPC on COX-2 protein expression. MM6 cells (5×10^6/ml) were incubated with DPPC (250 μg/ml) for 0–150 min or with PAPC (250 μg/ml) for 120 min, prior to washing in PBS (3×), cell lysis and protein extraction as previously described [10,11]. Following electrophoresis and electroblotting, the nitrocellulose membrane was probed for COX-2 expression (1:500 dilution, goat anti-human COX-2, Santa Cruz Autogen Bioclear, UK) and detected as previously described [11].

Effect of NK-κB inhibition on COX-2 protein expression. MM6 cells (5×10^6) were incubated with 100 μM of pyrrolidine dithiocarbamate (PDTC, Sigma; an inhibitor of NF-κB) for 1 h followed by washing in PBS. Subsequently cells were incubated with or without DPPC (250 μg/ml) for 120 min. Cells incubated with LPS, with or without inhibitor were used as controls. COX-2 protein expression was determined as above.

Effect of DPPC and PAPC incubation on NF-κB and pCREB activation. MM6 cells (5×10^6) were incubated in the presence or absence of DPPC or PAPC (250 μg/ml) for 0–90 min. As a positive control of p65 activation 5 × 106 cells were stimulated with LPS (100 ng/ml) for 45 min. Following incubation, nuclear lysate samples were prepared according to the manufacturer's protocol (Active Motif, Rixenart, Belgium). The protein concentration of the nuclear extract was measured and nuclear activity of transcription factors quantified using TransAM enzyme-linked immunosorbent assay (ELISA)-based kits specific for human NF-κB or phosphorylated cAMP-responsive element binding protein (pCREB) (Active Motif, Europe, Rixenart, Belgium), according to the manufacturer's protocols. For NF-κB, equal amounts of nuclear extracts were pipetted on 96-well plates coated with the immobilized oligonucleotide containing the activated NF-κB consensus site (5′-GGGACTTTCC-3′) and detected through the use of antibody directed against p65 subunit followed by a secondary HRP-conjugated antibody to give a colorimetric reaction. For pCREB estimation, nuclear extracts were placed in wells coated with immobilized oligonucleotide containing the cAMP-responsive element site (5′-TGACGTCA-3′), followed by incubation with an antibody recognizing the Ser-133-phosphorylated CREB.

CREB-1 siRNA transfection in MM6 cells. CREB-1 siRNA transfection was undertaken using INTERFERin™ to deliver the siRNA duplexes. Cells were seeded the day before transfection at 30% confluence and the count was adjusted to a count of 200,000 in a volume of to 200 μl. CREB-1 siRNA is a pool of target specific 20–25 nucleotide siRNA suitable for inhibiting CREB gene expression in human cells. To optimise gene-silencing CREB-1 siRNA was added at a concentration range of 5, 10 and 20 nM for 48 and 72 h using 5 μl INTERFERin™ mixed with a 100 μl of serum free medium. The INTERFERin™ siRNA mix was added to the cells mixed and incubated at 37 °C. After 24 h, 0.7 ml of complete media was added and incubated further. The expression of CREB-1 in these cells was determined using Western blotting.

Statistical analysis. Two sample comparisons were performed using the Student's test. Comparison of more than two means was performed using ANOVA with confidence intervals for all pairwise differences between level means calculated using Tukey's method. Minitab® v14 (Havertown, PA) was used for all analyses. Results were considered significant when $P < 0.05$ throughout.

References

[1] H. Hayawaka, G. Girdhar, O.N. Myrvik, L. Kucera, Pulmonary surfactant phospholipids modulate priming of rabbit alveolar macrophages for oxidative responses, J. Leukoc. Biol. 51 (1992) 379–385.
[2] J.R. Wright, Immunomodulatory functions of surfactant, Physiol. Rev. 77 (1997) 931–962.
[3] R. Schmidt, U. Meier, M. Yabut-Perez, D. Walmrath, F. Grimminger, W. Seeger, A. Gunther, Alteration of fatty acid profiles in different pulmonary surfactant phospholipids in acute respiratory distress syndrome and severe pneumonia, Am. J. Respir. Crit. Care Med. 163 (2001) 95–100.
[4] A. Ahuja, N. Oh, W. Chao, W. Spragg, R. Smith, Inhibition of the human neutrophil respiratory burst by native and synthetic surfactant, Am. J. Respir. Cell Mol. Biol. 14 (1996) 496–503.
[5] J.L. Harwood, Lung surfactant, Prog. Lipid Res. 26 (1987) 211–256.
[6] C. Speer, B. Götze, T. Curstedt, B. Robertson, Phagocytic functions and TNF secretion of human monocytes exposed to natural porcine surfactant (Curosurf), Pediatr. Res. 30 (1991) 69–75.
[7] R. Veldhuizen, K. Nag, S. Orgeig, F. Possmayer, The role of lipids in pulmonary surfactant, Biochim. Biophys. Acta 1408 (1997) 90–108.
[8] R.H. K Morris, A.J. Price, A. Tonks, S.K. Jackson, K.P. Jones, Prostaglandin E_2 and tumour necrosis factor-α release by monocytes are modulated by phospholipids, Cytokine 12 (2000) 1717–1719.

[9] A. Tonks, J. Parton, A.J. Tonks, R.H.K. Morris, A. Finall, K.P. Jones, S.K. Jackson, Surfactant phospholipid DPPC down-regulates monocyte respiratory burst via modulation of PKC, Am. J. Physiol. Lung Cell. Mol. Physiol. 288 (2005) L1070–L1080.
[10] A.J. Tonks, A. Tonks, R.H.K. Morris, K.P. Jones, S.K. Jackson, Regulation of platelet-activating factor synthesis in human monocytes by dipalmotylphosphatidylcholine, J. Leukoc. Biol. 74 (2003) 95–101.
[11] W.L. Smith, D.L. DeWitt, R.M. Garavito, Cyclooxygenases: structural, cellular, and molecular biology, Annu. Rev. Biochem. 69 (2000) 145–182.
[12] R.N. Hinson, J.A. Williams, E. Shacter, Elevated interleukin 6 is induced by prostaglandin E_2 in a murine model of inflammation: possible role of cyclooxygenase-2, Proc. Natl. Acad. Sci. USA 93 (1996) 4885–4890.
[13] L. Ma, P. del Soldato, J.L. Wallace, Divergent effects of new cyclooxygenase inhibitors on gastric ulcer healing: shifting the angiogenic balance, Proc. Natl. Acad. Sci. USA 99 (2002) 13243–13247.
[14] H.R. Herschman, Primary response genes induced by growth factors and tumour promoters, Annu. Rev. Biochem. 60 (1991) 281–319.
[15] A.G. Eliopoulos, C.D. Dumitro, C.-C. Wang, J. Cheo, P.N. Tsichli, Induction of COX-2 by LPS in macrophages is regulated by Tpl2-dependent CREB activation signals, EMBO J. 21 (2002) 4831–4832.
[16] D. Hwang, Modulation of the expression of cyclooxygenase-2 by fatty acids mediated through toll-like receptor 4-derived pathways, FASEB J. 15 (2001) 2556–2564.
[17] J.L. Lee, K.H. Sohn, S.H. Rhee, D. Hwang, Saturated fatty acids, but not unsaturated fatty acids, induce the expression of cyclooxygenase-2 mediated through toll-like receptor 4, J. Biol. Chem. 276 (2001) 16683–16689.
[18] S. Shishodia, D. Koul, B. Aggarwal, Cyclooxygenase (COX)-2 inhibitor celecoxib abrogates TNF-induced NF-κB activation through inhibition of activation of IκBα kinase and Akt in human non-small cell lung carcinoma: correlation with suppression of COX-2 synthesis, J. Immunol. 173 (2004) 2011–2022.
[19] C. Wahl, F. Oswald, U. Simnacher, S. Weiss, R. Marre, A. Essig, Survival of *Chlamydia pneumoniae*-infected Mono Mac 6 cells is dependent on NF-kappaB binding activity, Infect. Immun. 69 (2001) 7039–7045.
[20] J.M. Hohlfeld, The role of surfactant in asthma, Respir. Res. 3 (2002) 8–16.
[21] J.M. Holfeld, H. Fabel, H. Hamm, The role of pulmonary surfactant in obstructive airways disease, Eur. Respir. J. 10 (1997) 482–492.
[22] M. Profita, A. Sala, A. Bonanno, L. Riccobono, L. Siena, M.R. Di Giorgi, F. Mirabella, M. Gjomarkaj, G. Bonsignor, A.M. Vignola, Increased prostaglandin E_2 concentrations and cyclooxygenase-2 expression in asthmatic subjects with sputum eosinophilia, J. Allergy Clin. Immunol. 112 (2001) 709–716.
[23] A.K. Bauer, L.D. Dwyer-Nield, A.M. Malkinson, High cyclooxygenase 1 (COX-1) and cyclooxygenase 2 (COX-2) contents in mouse lung tumours, Carcinogenesis 21 (2000) 543–550.
[24] B. Poligone, A.S. Baldwin, Positive and negative regulation of NF-kappaB by COX-2: roles of different prostaglandins, J. Biol. Chem. 276 (2001) 8658–38664.
[25] S. Uematsu, M. Matsumoto, K. Takeda, S. Akira, Lipopolysaccharide-dependant prostaglandin E_2 (PGE_2) production is regulated by the glutathione dependant prostaglandin E_2 synthase gene induced by the toll-like receptor 4/MyD88/NF-IL-6 pathway, J. Immunol. 168 (2002) 5811–5816.
[26] M.J. Thomassen, D.P. Meeker, J.M. Antal, M.J. Connors, H. Wiedemann, Synthetic surfactant (Exosurf) inhibits endotoxin-stimulated cytokine secretion by human alveolar macrophages, Am. J. Respir. Cell Mol. Biol. 7 (1992) 257–260.
[27] A. Tonks, R.K. Morris, A.J. Price, A.W. Thomas, K.P. Jones, S.K. Jackson, Dipalmotylphosphatidylcholine modulates inflammatory functions of monocytic cells independently of mitogen activated protein kinases, Clin. Exp. Immunol. 124 (2001) 86–94.
[28] A. Wiernik, T. Curstedt, A. Johansson, C. Jarstrand, B. Robertson, Morphology and function of blood monocytes after incubation with surfactant, Eur. J. Respir. Dis. 71 (1987) 410–418.
[29] F. Pinot, H. Walti, H.P. Haagsman, B.S. Polla, M. Bachelet, Curosurf modulates cAMP accumulation in human monocytes through a membrane-controlled mechanism, Am. J. Physiol. Lung Cell. Mol. Physiol. 278 (2000) L99–L104.
[30] M.F. Geertsma, H.R. Broos, P.H. Nibbering, R. van Furth, Lung surfactant suppresses oxygen-dependent bactericidal functions of human blood monocytes by inhibiting the assembly of the NADPH oxidase, J. Immunol. 150 (1993) 2391–2400.
[31] D.J. Wadleigh, S.T. Reddy, S. Ghosh, H.R. Herschmann, Transcriptional activation of the cycloxygenase-2 gene in endotoxin treated RAW264.7 macrophages, J. Biol. Chem. 275 (2000) 6259–6266.
[32] S.P. Persengiev, M.R. Green, The role of ATF/CREB family members in cell growth, survival and apoptosis, Apoptosis 8 (2003) 225–228.
[33] G.A. Gonzalez, M.R. Montminy, Cyclic AMP stimulates somatostatin gene transcription by phosphorylation of CREB at serine 133, Cell 59 (1989) 675–680.
[34] H. Xie, T.L. Rothstein, Protein kinase C mediates activation of nuclear cAMP response element-binding protein (CREB) in B-lymphocytes stimulated through surface immunoglobulin, J. Immunol. 154 (1995) 1717–1723.
[35] D. De Cesare, S. Jacquot, A. Hanauer, P. Sassone-Corsi, Rsk-2 activity is necessary for epidermal growth factor-induced phosphorylation of CREB protein and transcription of *c-fos* gene, Proc. Natl. Acad. Sci. USA 95 (1998) 12202–12207.
[36] M. Martin, K. Rehani, S.M. Michalek, Toll-like receptor-mediated cytokine production is differentially regulated by glycogen synthase kinase 3, Nat. Immunol. 6 (2005) 777–784.