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Regulation of lipopolysaccharide-mediated interleukin-1β release by *N*-acetylcysteine in THP-1 cells

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Regulation of lipopolysaccharide-mediated interleukin-1β release by N-*acetylcysteine in THP-1 cells. M. Parmentier, N. Hirani, I. Rahman, K. Donaldson, W. MacNee, F. Antonicelli. ©ERS Journals Ltd 2000.*

ABSTRACT: Increased levels of inflammatory cytokines such as interleukin (IL)-1 and IL-8 occur in the bronchoalveolar lavage fluid in various lung diseases. Cytokine gene expression is controlled by transcription factors such as nuclear factor-κB (NF-κB) which can be activated by a number of stimuli including the oxidants prevent. It was hypothesized that lipopolysaccharide (LPS)-induced IL-1β secretion may be modulated by the intracellular thiol redox status of the cells.

The effect of the antioxidant compound, *N*-acetyl-L-cysteine (NAC), on IL-1β release and regulation of NF-κB in a human myelo-monocytic cell line (THP-1) differentiated into macrophages was studied.

LPS (10 μg·mL^{-1}) increased IL-1β release at 24 h compared to control levels ($p<0.001$). NAC (5 mM) also enhanced LPS-induced IL-1β release from THP-1 cells ($p<0.001$). In addition, treatment of cells with cycloheximide, an inhibitor of protein synthesis, inhibited the NAC-mediated IL-1β release. Under the same conditions, NF-κB binding was activated by LPS and NAC increased this LPS-mediated effect. Western blot analysis revealed that NAC treatment leads to an increase in p50 and p65 protein synthesis.

These data indicate that *N*-acetyl-L-cysteine modulates interleukin-1β release by increasing levels of the homo- and heterodimeric forms of nuclear factor-κB.
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Multiple inflammatory events are involved in the development of lung injury. Part of this inflammatory response is the release of an array of cytokines [1–3]. The regulation of these inflammatory cytokines in response to diverse stimuli is controlled at the level of gene transcription. A crucial transcription factor that regulates the expression of several cytokines is nuclear factor-κB (NF-κB). A NF-κB consensus site is present in the promoter region of the interleukin (IL)-1β, IL-6, IL-8 and tumour necrosis factor (TNF) genes [4]. Since a large range of inflammatory genes are induced by activated NF-κB, it has been proposed that activation of this transcription factor plays a central role in inflammatory processes, and activation of NF-κB has been implicated in a wide range of inflammatory diseases such as asthma and acquired immune deficiency syndrome [5–7].

NF-κB is a member of the Rel family of proteins, a novel family of ubiquitous transcription factors sharing a common structural motif for deoxyribonucleic acid (DNA) binding [4]. NF-κB was first identified as a nuclear factor that binds the decameric DNA sequence 5′-GGGAC-TTTCC-3′. NF-κB is a cytosolic homo-heterodimer consisting of p65 and p50 subunit proteins, each having specific affinity for different decameric binding sites fitting the IκB motif [8]. Inactive NF-κB is localized in the cytoplasm due to binding of the inhibitory protein (IκB). A primary event in the activation of NF-κB is phosphorylation, ubiquitination and degradation of IκB by specific IκB kinases [9–11]. This allows translocation of activated NF-κB from the cytoplasm to the nucleus.

The levels of many pro-inflammatory cytokines are increased in lung diseases. However, the mechanism of this upregulation is currently unknown. It has been shown that cytokines such as IL-8 are transcriptionally regulated by the redox state of the cell [12]. Constitutive expression of IL-1β is kept under tight control in healthy tissues and this is an important intrapulmonary cytokine in the mediation of lipopolysaccharide (LPS)-induced effects in the lung [13, 14]. Despite numerous reports regarding the importance of IL-1β in the cytokine network, very little is known about the molecular mechanisms governing its regulation. In the present study, the molecular regulation of IL-1β was investigated. Whether this regulation is under redox control and, in particular, the effect of intracellular thiol status was also studied using the compound *N*-acetyl-L-cysteine (NAC), an antioxidant drug. Hence, this study was designed to characterize the molecular mechanism of LPS-mediated regulation of IL-1β by NAC in a human myelo-monocyte-derived macrophage cell line (THP-1).

Materials and methods

Unless otherwise stated, all of the biochemical reagents used in the present study were purchased from Sigma Chemical Co. (Poole, UK) and cell culture media from Gibco (Paisley, UK).

Cell culture

THP-1 cells were maintained in suspension in Roswell Park Memorial institute (RPMI)-1640 medium containing 10% foetal calf serum. For experiments, the cells were plated in six-well culture dishes at a density of 1×10^6 cells·mL^{-1}. Differentiation of THP-1 monocytes into macrophages was by overnight incubation with phorbol myristate acetate (PMA) at a concentration of 10 μM. Differentiated cells adhered to the flask, whereas undifferentiated monocytic cells remained in suspension and were removed by washing with prosphate-bufferred saline (PBS, pH 7.4). Adherent macrophage-like cells were incubated in serum-free RPMI-1640 medium.

Cell treatment

Cells were incubated in serum-free medium alone (control) or with LPS (10 μg·mL^{-1}). The effects of NAC (1, 5 and 10 mM) and glutathione monoethyl ester (GSHMEE; 5 mM) on IL-1β release from THP-1 cells were studied without or with coincubation with LPS (10 μg·mL^{-1}) for 24 h. Cycloheximide (CHX) (1 μg·mL^{-1}) and okadaic acid (OA) (0.1 μM) were introduced at 60 and 90 min, respectively, before addition of LPS (10 μg·mL^{-1}) and the antioxidant NAC (5 mM).

Preparation of nuclear extracts

After LPS and NAC treatment for 24 h, the medium overlying the cells was harvested for measurement of cytokine secretion and replaced with ice-cold PBS. THP-1 cells were harvested by scraping, followed by centrifugation at 1,000 × g. Nuclear extracts were prepared using the method of STAAL *et al.* [7].

Electrophoretic mobility shift assay and supershift assay

Binding reactions were established in 20 μL using 1.5 or 3 μg (4 and 24 h respectively) nuclear extract protein and 0.25 mg·mL^{-1} polydeoxyinosine-deoxycytidine in binding buffer (Promega) per reaction. In the binding reaction, the nuclear extracts were incubated with a γ-^{32}P-adenosine triphosphate end-labelled double-stranded NF-κB consensus oligonucleotide (Promega, Southampton, UK), produced using T4 polynucleotide kinase, for 20 min at room temperature. For the supershift assays, the nuclear extracts were first incubated with the appropriate antibody (rabbit antihuman NF-κB p50 and p65, AHP 287 and AHP 288 (Serotec, Oxford, UK); 2 μL) in a concentration of 1 μg·mL^{-1} for 3 h at 4°C. Preimmune rabbit serum was used as a control antibody. Samples were loaded and electrophoresed through a 6% polyacrylamide gel at a constant voltage of 180. Gels were then dried and autoradiography was performed.

Western blot analysis

THP-1 cells were lysed in buffer containing 10 mM *N*-2-hydroxyethylpiperazine–*N*-2-ethanesulphonic acid (HEPES; pH 7.8) 10 mM KCl, 2 mM $MgC1_2$, 0.1 mM ethylenediamine tetra-acetic acid (EDTA), 0.4 mM phenylmethyl sulphonyl fluoride, 0.2 mM NaF, 1 mM sodium orthovanadate, 0.3 mg·mL^{-1} leupeptin and 10% Nonidet P-40. Protein (70 μg) was loaded, using Laemmli buffer, on to a 10% polyacrylamide gel and then transferred to nitrocellulose. After saturation in Blotto (5% dry milk powder and 0.05% Tween 20 in tris(hydroxymethyl)aminomethane (Tris)-buffered saline) for 1 h, the blot was probed with different rabbit antibodies dependent on the protein being analysed (anti-p50 and -p65 and anti-IκB-α (sc-203; Santa Cruz)). A secondary goat-anti-rabbit antibody (Scottish Antibody Production Unit, Edinburgh, UK) conjugated to horseradish peroxidase was added and gels were developed using chemiluminescence and autoradiography.

Isolation of ribonucleic acid and reverse transcription

Ribonucleic acid (RNA) was isolated from THP-1 cells using TRIZOL reagent (Life Technologies, Paisley, UK). Total RNA was reverse transcribed according to the manufacturer's instructions (8025SA; Life Technologies). The resultant complementary DNA (cDNA) was stored at -20°C until required.

Analysis of interleukin-1β messenger ribonucleic acid by polymerase chain reaction

Oligonucleotide primers were chosen using the published sequence of human IL-1β cDNA [15] and β-actin [16]. The primers for IL-1β and β-actin were synthesized by MWG Biotech (Milton Keynes, UK). The sequences of the primer's used in the polymerase chain reaction (PCR) were as follows: IL-1β sense: 5′-ATGGCAGAAGTAC-CTGAGCTCGC-3′; IL-2β antisense: 5′-TAACTGACT-TCACCATGCAATTTGTG-3′; β-actin sense: 5′-CCAC-CAACTGGGACGACATG-3′; and β-actin antisense: 5′-GTCTCAAACATGATCTGGGTCATC-3′. The reverse transcribed messenger RNA (mRNA) mixture (3 μL) was added directly to the PCR mixture and used for the PCR reactions. The IL-1β PCR conditions were 10 min at 94°C followed by 35 cycles of: 45 s at 94°C, 45 s at 52°C, 120 s at 72°C, and a final extension with 1 unit of *Taq* DNA polymerase (Promega) for 10 min at 72°C. The β-actin PCR conditions were 10 min at 94°C followed by 35 cycles of: 60 s at 94°C, 60 s at 60°C, and 60 s at 72°C and a final extension with 1 unit of *Taq* DNA polymerase for 5 min at 72°C. The identity of the resulting PCR-amplified DNA fragment was confirmed by DNA sequencing. Bands were visualized and scanned using a white ultraviolet transilluminator photometer (Orme Technologies, Cambridge, UK). Levels of IL-1β mRNA (801 base pairs (bp)) were expressed as a percentage relative to the intensity of the β-actin bands (121 bp).

Enzyme-linked immunosorbent assay for interleukin-1β

The enzyme-linked immunosorbent assay (ELISA) was performed as previously described [17]. All plates were read on a microplate reader (Dynatech MR 5000, Aldermaston, Berkshire, UK) and underwent computer-assisted analysis (Assay ZAP, Blosoft, Cambridge, UK). Typically, standard curves generated with this ELISA were linear in the range 50–2,500 pg·mL^{-1} IL-1β. Only assays yielding standard curves with a calculated regression coefficient of >0.95 were used for further analysis.

Statistical analysis

Data are expressed as mean±SEM. Data comparison was carried out using analysis of variance followed by the Tukey *post hoc* test for multigroup comparisons. The software package Instat 2 (GraphPad; San Diego, CA, USA) was used for this analysis. A p-value of <0.05 was regarded as significant.

Results

Effects of lipopolysaccharide, N-acetyl-L-cysteine and glutathione monoethyl ester on interleukin-1β release from THP-1 cells

At a concentration of 10 μg·mL^{-1}, LPS caused a two-fold increase in IL-1β release after incubation for 24 h (fig. 1) but no change after 4 h (data not shown). In order to analyse the effect of antioxidant thiols on IL-1β release, THP-1 cells were incubated with NAC for 24 h. NAC alone had no effect on IL-1β release from THP-1 cells (data not shown). Coincubation of NAC, at a concentration of 5 mM, with LPS increased secretion of IL-1β into the culture medium by 300% (fig. 1). Under these conditions, IL-1β was still undetectable after incubation for 4 h, suggesting that the IL-1β release occurred at a later time point. The effect of NAC on LPS-induced IL-1β secretion was bimodal and dose-dependent. At a concentration of l mM, NAC did not enhance IL-1β secretion, whereas higher concentrations (5 and 10 mM) produced the described effect. The increase in LPS-mediated IL-1β release observed with NAC was not reproduced using the thiol compound GSHMEE (fig. 1).

Effect of cycloheximide and okadaic acid on N-acetyl-L-cysteine-induced interleukin-1β release from THP-1 cells

In order to investigate the mechanisms by which NAC enhances LPS-mediated IL-1β release, cells were pretreated with CHX (1 μg·mL^{-1}) to prevent protein synthesis.

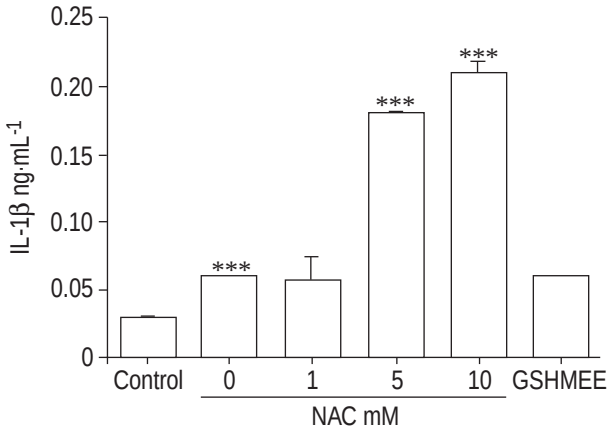

Fig. 1. – Effects of lipopolysarccharide (LPS), glutathione monoethyl ester (GSHMEE) and *N*-acetyl-L-cysteine (NAC) on interleukin-1b (IL-1β) release by THP-1 cells. Cells were incubated in serum-free medium alone (control) or with LPS (10 μg·mL^{-1}). LPS-stimulated cells were coincubated with or without NAC (1, 5 or 10 mM) or GSHMEE (5 mM) for 24 h. IL-1β accumulation in the medium was measured by enzyme-linked immunosorbent assay. Data are presented as mean±SEM (n=5). ***: p<0.001 *versus* control.

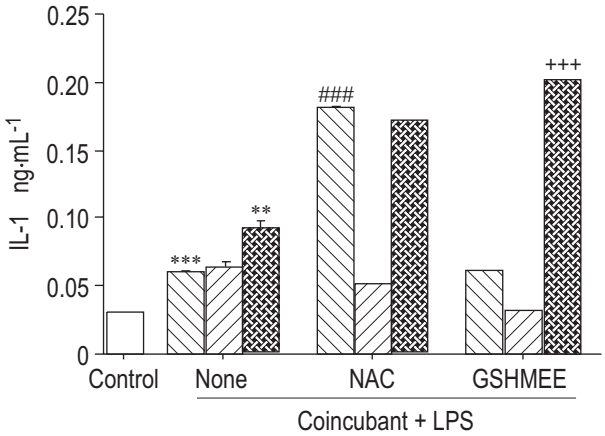

Fig. 2. – Effects of cycloheximide (CHX) or okadaic acid (OA) on *N*-acetyl-L-cysteine (NAC)-induced interleukin-1β (IL-1β) release by THP-1 cells. Cells were pretreated without (▨) or with CHX (1 μg·mL^{-1}) for 60 min (▨) or OA (0.1 μM) for 90 min (▩). The cells were then incubated in serum-free medium alone (control; no pretreatment) or with lipopolysaccharide (LPS; 10 μg·mL^{-1}). LPS-stimulated cells were coincubated with or without NAC or GSHMEE (both 5 mM) for 24 h. IL-1β accumulation in the medium was measured by enzyme-linked immunosorbent assay. Data are presented as mean±SEM (n≥3). ***: p<0.001 *versus* control; **: p<0.01 *versus* LPS only; ###: p<0.001 *versus* LPS only; +++: p<0.001 *versus* LPS plus GSHMEE.

Figure 2 shows that a 60-min CHX pretreatment had no effect on LPS-mediated IL-1β release. However, the effect of NAC on LPS-induced IL-1β release was totally abolished by inhibiting protein synthesis. One effect of NAC is to increase intracellular glutathione levels [18, 19]. Thus it was decided to investigate whether the inhibitory effect of CHX was due specifically to inhibition of glutathione. Cells pretreated with CHX were incubated in the presence of the thiol-replenishing compound GSHMEE at a concentration of 5 mM. Figure 2 shows that, under these conditions, no increase in IL-1β level was observed, demonstrating that NAC-induced increased IL-1β release cannot be restored by addition of GSHMEE alone. This result is consistent with the observation that GSHMEE had no effect on LPS-mediated IL-1β release (fig. 1). Next, it was examined whether the NAC-mediated activation of IL-1β involved a phosphorylation step. Preincubation with OA, a specific inhibitor of serine/threonine phosphatases 1 and 2A, for 90 min did not modify the effect of NAC on LPS-induced IL-1β release from THP-1 cells (fig. 2). This result suggests that the effect of NAC on IL-1β release is not through kinase activation.

Effects of lipopolysaccharide, N-acetyl-L-cysteine on interleukin-1β messenger ribonucleic acid expression

Having demonstrated that NAC enhances LPS-induced IL-1β release, it was sought to investigate this effect at the mRNA level by semiquantitative PCR. IL-1β mRNA expression after 4 and 24 h was measured using β-actin mRNA as a control (fig. 3). IL-1β mRNA levels were increased by incubation with NAC, predominantly after 4 h (250 and 125% increase compared to untreated and LPS-treated cells at 4 and 24 h respectively).

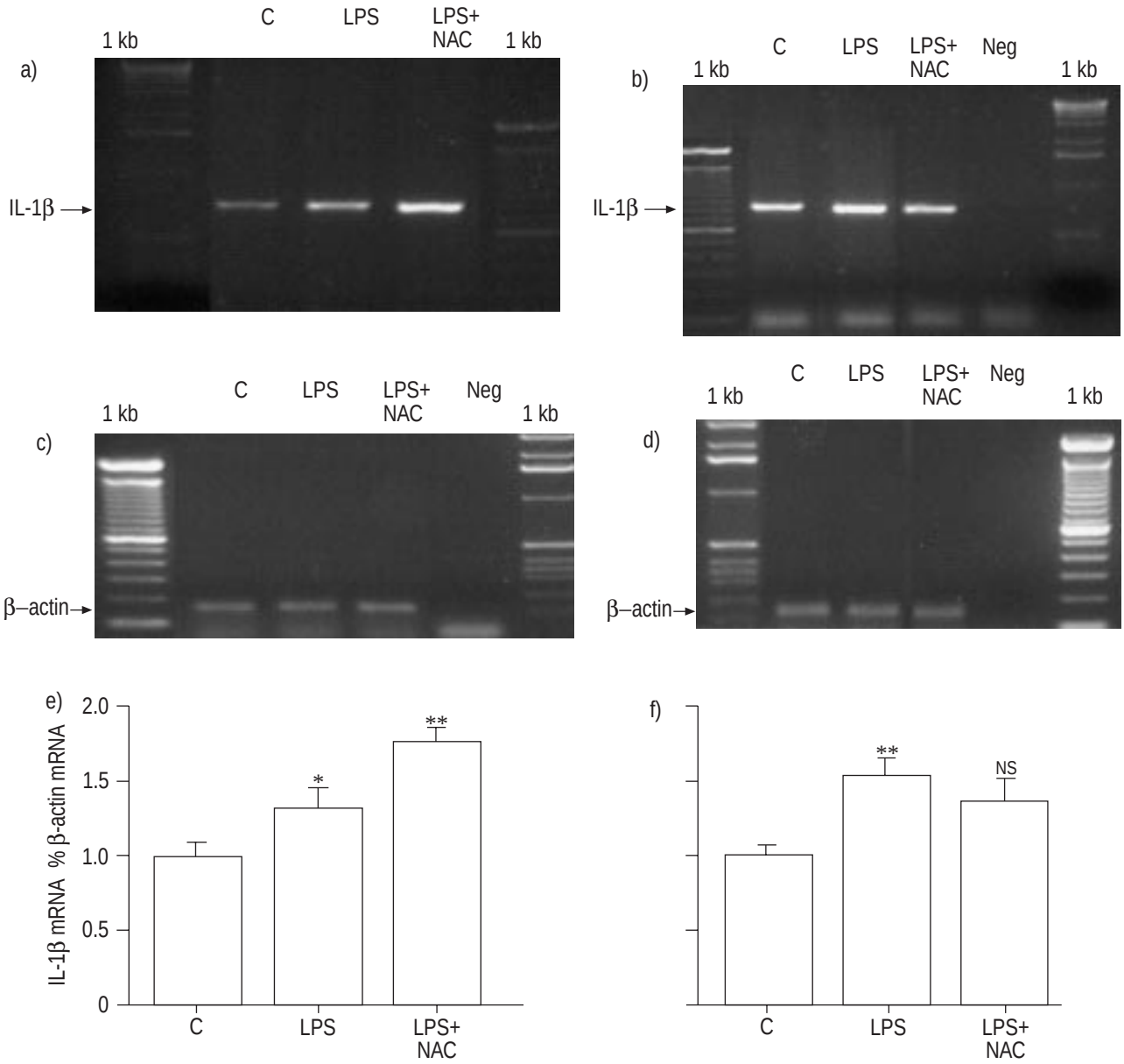

Fig. 3. – Effects of lipopolysaccharide (LPS) and *N*-acetyl-L-cysteine (NAC) in interleukin-1β (IL-1β) messenger ribonucleic acid (RNA) (mRNA) expression in THP-1 cells. Total RNA was isolated from cells incubated in serum-free medium alone (control (C)) or with LPS (10 $\mu g \cdot mL^{-1}$) with or without NAC (5 mM) for: a, c, e) 4; and b, d, f) 24 h. RNA was reverse transcribed and used for polymerase chain reaction analysis of IL-1β mRNA as described in the *Isolation of ribonucleic acid and reverse transcription* and *Analysis of interleukin-1β messenger ribonucleic acid by polymerase chain reaction sections*. e, f) Numerical estimates of IL-1β mRNA levels (a, b) compared with β-actin bands (c, d) from the same sample. Data are expressed as mean±SEM (n=3). 1 kb: S (1 kilobase ladder); Neg: negative control, distilled water; NS: nonsignificant. *: $p<0.05$; **:$p<0.01$ *versus* control.

Role of nuclear factor-κB in the N-acetyl-L-cysteine-mediated regulation of interleukin-1β expression

In order to investigate the relationship between IL-1β release and NF-κB DNA-binding, the effect of NAC and LPS on the transcription factor NF-κB was determined. The presence of LPS in the culture medium increases NF-κB DNA-binding. This effect was observed after incubation for 4 and 24 h. The presence of NAC enhanced LPS-induced NF-κB DNA-binding (fig. 4). This result was verified using human blood-derived macrophages (data not shown). Similar to THP-1 cells, NAC (5 mM) treatment increased NF-κB DNA-binding by 166% compared to control.

To further explore the associated signalling events involved in the transcriptional regulation of IL-1β, whether NAC-mediated activation of NF-κB involved a phosphorylation step, phosphorylation of IκB being a key step in NF-κB activation, was examined. Using OA, an increase was observed in NF-κB. DNA-binding (fig. 4), which followed degradation of IκB (fig. 5a-c), but produced no significant change in the NAC-mediated enhancement of LPS-stimulated activation, suggesting that a phosphorylation step is not the target of NAC.

The influence of CHX pretreatment on NAC-enhanced NF-κB nuclear binding was then assessed. The increase in NF-κB binding previously observed following treatment

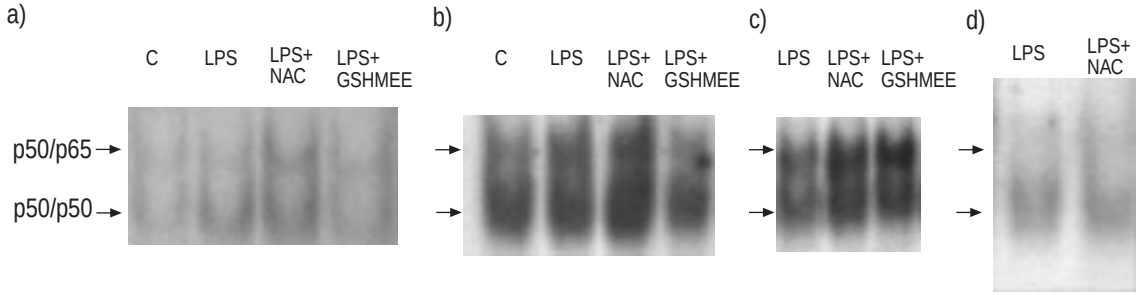

Fig. 4. – Nuclear factor-B binding in response to treatment with lipopolysaccharide (LPS) and *N*-acetyl-L-cysteine (NAC). Experiments were preformed using 3 μg of protein from the nuclear extract of THP-1 cells. Cells were pretreated without (b) or with: c) cycloheximide (1 $\mu g \cdot mL^{-1}$) for 60 min; or d) okadaic acid (0.1 μM) for 90 min. The cells were then incubated in serum-free medium alone (control (C); no pretreatment) or with LPS (10 $\mu g \cdot mL^{-1}$) with or without NAC or glutathione monoethyl ester (GSHMEE) (both 5 mM) for: a) 4; and b, c, d) 24 h. The data are representative of three separate extractions.

with NAC was totally abolished when protein synthesis was inhibited (fig. 4).

Effect of N-acetyl-L-cysteine on p50 and p65 expression

It was then determined which combination of the p50/p65 proteins are involved in the NAC effect on LPS-induced NF-κB binding. A supershift assay (fig. 6) revealed that both the p50 and p65 proteins are involved, forming the homodimer p50/p50 and the heterodimer p50/p65. In order to obtain more information as to how NAC potentiates the LPS-induced NF-κB binding, the effect of NAC on the expression of the proteins p50 and p65 was analysed. Western blot analysis (fig. 5) indicated that both p50 and p65 levels were increased in cells treated with NAC, with no change in the expression of IκB (fig. 5).

Discussion

It has been reported previously that LPS activates the human monocytic cell line THP-1 using a serum-free system [20]. In the present report, it is shown that human THP-1 cells differentiated to macrophages with PMA respond to a low concentration of LPS (10 $\mu g \cdot mL^{-1}$) in the absence of serum, leading to the production of IL-1β. A further aim of the current study was to determine whether intracellular thiol status affected the molecular mechanisms of release of pro-inflammatory cytokine IL-1β. Serum-free conditions were chosen in order to avoid any antioxidant effect due to the serum. NAC alone does not modify IL-1β release. However, the present data show that THP-1 cells respond to NAC in dose-dependent fashion. At high concentration (5 and 10 mM), NAC enhances LPS-induced IL-1β release from THP-1 cells with no effect at lower concentration (1 mM). This effect occurred at the transcriptional level and is mediated in part by activation of NF-κB.

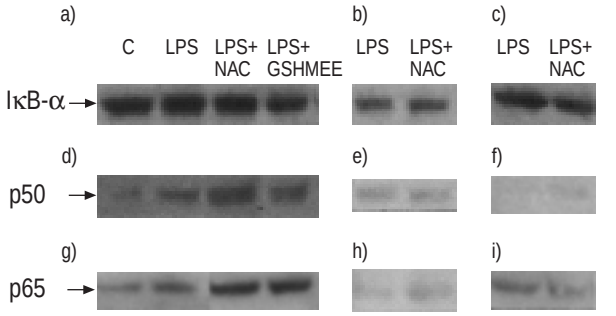

Fig. 5. – Determination of protein required for *N*-acetyl-L-cysteine (NAC)-mediated activation of nuclear factor-κB binding. Cells were pretreated without (a, d and g) or with: b, d, e, f, h) okadaic acid (0.1 μM) for 90 min; or c, f, i) cycloheximide (1 $\mu g \cdot mL^{-1}$) for 60 min. The cells were then incubated in serum-free medium alone (control (C); no pretreatment) or with lipopolysaccharide (LPS; 10 $\mu g \cdot mL^{-1}$). LPS-stimulated cells were coincubated with or without NAC or glutathione monoethyl ester (GSHMEE) for 24 h. Western blot analysis of matching extractions was performed for the determination of: a–c) the inhibitory protein (IκB-a); and d–f) p50; and p65 (g–i) protein content. Autoradiographs typical of three independent extractions are shown.

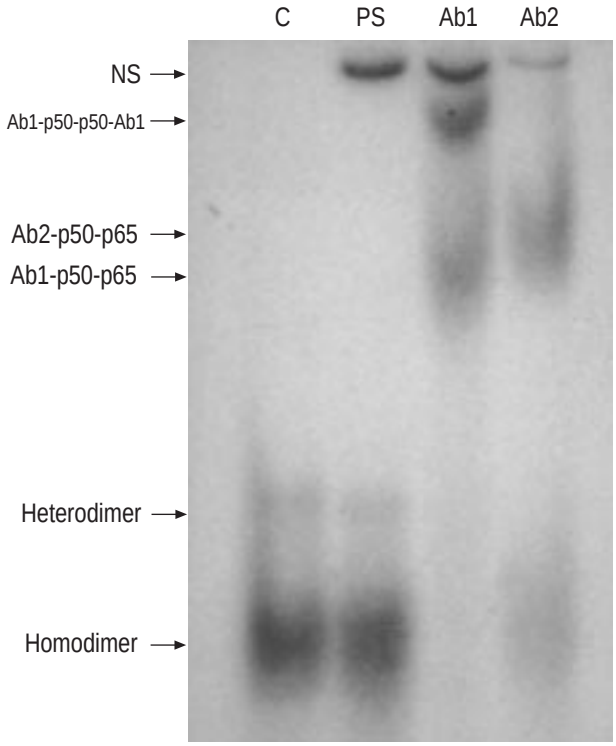

Fig. 6. – Identification of nuclear factor-κB (NF-κB) subunits by supershift assay. The addition of antibodies directed against potential components of the NF-κB complex resulted in supershift when antibodies to p50 or p65 were used. A nonspecific (NS) band was observed in the presence of preimmune serum (PS). Incubation with an antibody directed against p50 (Ab1) resulted in two supershifted bands corresponding to the homo- and heterodimer, whereas incubation with an antibody directed against p65 resulted in only one supershifted band corresponding to the heterodimer. Data are representatives of three separate extractions. C: control (LPS+NAC).

Previous studies have shown a correlation between the increase in NF-κB nuclear binding and production of IL-1β after LPS stimulation [21–24]. Other studies, in a variety of experimental systems, have shown that NAC effectively suppresses NF-κB activation induced by diverse stimuli [25, 26]. In Jurkat cells, for example, it has been shown that NAC at high concentration (20 mM) inhibits TNF-α-induced NF-κB activation, suggesting a role for reactive oxygen species (ROS) in the signalling mechanisms of NF-κB activation in these cells [27]. It appears, however, that the effect of NAC is dose-dependent such that low concentrations of NAC protect against LPS toxicity by decreasing hydrogen peroxide accumulation, whereas higher concentrations have the opposite effect [26]. In THP-1 cells, the dose-dependent effect of NAC on LPS-induced IL-1β showed a threshold between 1 and 5 mM. At a concentration of 5 mM, NAC enhances LPS-induced NF-κB binding. This is in accordance with the results of SUZUKI *et al.* [28] and BRENNAN *et al.* [27] who showed that NAC, at similar concentrations, did not inhibit the activation of NF-κB induced by stimulation with calyculin A in Jurkat cells or IL-1β in KB and Elu. NOB1 cells. Thus the effect of NAC on NF-κB activation is critically dependent upon concentration, cell type and stimulus. This is in agreement with the fact that the mode of action of NAC on signalling events is unclear. FOX and LEINGANG [29] showed that TNF-induced NF-κB activation was modified by NAC through an intermediate event.

In order to test the hypothesis that the effect of NAC might require an intermediate event, experiments were performed using OA, a potent inhibitor of serine/threonine phosphatases, and CHX, an inhibitor of protein synthesis. Phosphorylation is an important element in signal transduction for NF-κB [9, 10]. IκB dissociation involves its phosphorylation-controlled proteolytic degradation. ROS appear to play a role in regulating IκB phosphorylation through activation of the IκB kinase or inhibition of phosphatases. However, after 24-h incubation, OA did not modify the effect of NAC on LPS-induced IL-1β release from THP-1 cells and no changes were observed in IκB degradation. These results suggest that phosphorylation is not an intermediate event in NAC-induced NF-κB activation at 24 h and are consistent with the fact that IκB degradation is generally observed at an early time point. However, the same IL-1β level as that found in NAC-stimulated cells was reached in THP-1 cells cotreated with OA and GSHMEE, suggesting a possible inhibitory effect of NAC on the phosphatase.

By contrast, although it is clear that protein synthesis is not required for LPS-mediated release of IL-lβ, the present study shows, for the first time, that CHX prevents NAC-enhanced IL-1β secretion, demonstrating the requirement for new protein synthesis in this phenomenon. MIHM *et al.* [30] showed modulation of the activation of the transcription factor NF-κB by intracellular reduced glutathione levels and by variations in extracellular cysteine supply. In THP-1 cells, it was found, in the present study, that 5mM NAC enhanced LPS-induced IL-1β release and NF-κB DNA-binding. However, the authors have also shown that NAC inhibits IL-8 release in THP-1 cells, confirming the potent antioxidant effect of this drug (unpublished data). Supplementation of cells with GSHMEE did not suppress the CHX inhibition of the NAC effect, suggesting that glutathione synthesis is not involved in the NAC activation of either IL-1β release or NF-κB binding. Furthermore, it was demonstrated that NAC increases both p50 and p65 expression and thus this is the probable mechanism of increased NF-κB activation. It could be argued that the NF-κB activation is not a direct effect of NAC but a secondary effect through IL-1β, since IL-1β itself is a strong inducer of NF-κB in monocytic cells [21]. However, the present finding of an increase in NF-κB binding after only a 4-h incubation with NAC, a time point at which IL-1β secretion was still undetectable, argues strongly in favour of a direct effect of NAC on NF-κB activation. Indeed, this effect was also observed after only 1 h with NAC (data not shown). This is in accordance with the observation that NAC has no effect on IL-1β-induced NF-κB activation in human vascular smooth muscle cells [31]. Furthermore, ZHANG *et al.* [32] showed a difference between signals transduced by IL-1β and LPS at the receptor level. Toll-like receptors (TLRs) 2 and 4 are present at the THP-1 cell membrane surface and are thought to transduce the LPS signal [32], whereas IL-1β binds to the IL^{-1} receptor. Thus NAC could interfere at a step between the TLR and MyD88 (an adaptor myeloid differentiation protein), which is a convergent point between the IL^{-1} and LPS signalling cascades. The resulting increase in IL-1β levels may then act in an autocrine loop mechanism, amplifying the effect.

Interleukin-1β is often considered to be an important pro-inflammatory cytokine, and, in this context, the present observation that *N*-acetyl-L-cysteine enhances interleukin-1β secretion may be interpreted as a potentially detrimental effect in patients with inflammatory diseases. However, this should be balanced against the known potent anti-oxidant, and hence anti-inflammatory, actions of *N*-acetyl-L-cysteine [27, 33, 34]. In other experiments, for example, it was demonstrated that, although the interleukin-1β level is enhanced, the interleukin-8 level is reduced by *N*-acetyl-L-cysteine (data not shown). Furthermore, under certain conditions, interleukin-1β itself may play an anti-inflammatory role. For example, in rats, an increased level of cytokine-induced chemoattractant (the interleukin-8 equivalent) was detected in lung lavage fluid, which was associated with a neutrophil-dependent acute oedematous lung leak. Under these conditions, pretreatment with interleukin-1 prevented lung leak in rats [35]. This balance between the potential pro-inflammatory and anti-inflammatory effects of *N*-acetyl-L-cysteine may be of direct relevance for patients with chronic inflammatory lung diseases.

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