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Biochemical and Biophysical Research Communications 293 (2002) 252–257

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## Nuclear factor (NF)- $\kappa$ B blockade attenuates but does not abrogate LPS-mediated interleukin (IL)-1 $\beta$ biosynthesis in alveolar epithelial cells

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Received 21 March 2002

### Abstract

The role that the nuclear factor (NF)- $\kappa$ B plays in regulating the biosynthesis of interleukin (IL)-1 $\beta$ , an inflammatory cytokine, has been investigated in vitro. Irreversible inhibition of the proteasome complex by carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG-132; 1–50  $\mu$ M) had no inhibitory effect on lipopolysaccharide (LPS)-mediated IL-1 $\beta$  biosynthesis. Furthermore, selective inhibition of NF- $\kappa$ B by the action of caffeic acid phenylethyl ester (CAPE; 1–100  $\mu$ M) and sulfasalazine (SSA; 0.1–10 mM), a potent and irreversible inhibitor of NF- $\kappa$ B, partially attenuated but did not abolish LPS-dependent IL-1 $\beta$  secretion. Incorporation of a selectively permeant inhibitor of NF- $\kappa$ B, SN-50 (1–20  $\mu$ M), a peptide which contains the nuclear localization sequence (NLS) for the p50 NF- $\kappa$ B subunit and the amino-terminal sequence of Kaposi fibroblast growth factor to promote cell permeability, attenuated in a dose-dependent manner LPS-mediated release of IL-1 $\beta$ . It is concluded that the NF- $\kappa$ B pathway is partially implicated and its blockade attenuates but does not abrogate LPS-dependent IL-1 $\beta$  biosynthesis in alveolar epithelial cells. © 2002 Elsevier Science (USA). All rights reserved.

**Keywords:** Inflammation; IL-1 $\beta$ ; Lipopolysaccharide; NF- $\kappa$ B; Proteasome

Interleukin (IL)-1 $\beta$  is a potent inflammatory cytokine, which exerts its pleiotropic activities through ligand-induced cross-linking of specific receptors [1,2]. During inflammation, IL-1 $\beta$  released in the inflammatory environment transiently activates neutrophils and macrophages thereby causing them to release superoxide anion ( $O_2^{\cdot-}$ ) as a consequence of the activation of the plasma membrane NADPH oxidase [2,3]. This oxidative burst in leukocytes features a rapid but transient release of reactive oxygen species (ROS) and is a crucial part of the defense mechanism against invading microbial pathogens and tumor cell metastasis. Although IL-1 $\beta$  is primarily produced by macrophages, growing evidence suggested that other cell types, such as endothelial and epithelial cells, release IL-1 $\beta$  and other inflammatory mediators thereby amplifying and boosting the inflam-

matory reaction by activating and recruiting inflammatory cells [4,5].

Although the transcription factor nuclear factor (NF)- $\kappa$ B has been originally recognized in regulating gene expression in B-cell lymphocytes [6], subsequent studies demonstrated that it is one member of a ubiquitously expressed family of *Rel*-related transcription factors that serves as critical regulator of many genes, including those of pro-inflammatory cytokines [7]. The translocation and activation of NF- $\kappa$ B in response to various stimuli are sequentially organized at the molecular level. In its inactive state, the heterodimeric NF- $\kappa$ B, which is mainly composed of two subunits, p50 (NF- $\kappa$ B<sub>1</sub>) and p65 (RelA), is present in the cytoplasm associated with its inhibitory protein, I $\kappa$ B [8]. Upon stimulation, such as with cytokines and lipopolysaccharide (LPS), I $\kappa$ B- $\alpha$ , the major cytosolic inhibitor of NF- $\kappa$ B, undergoes phosphorylation on serine/threonine residues, ubiquitination, and subsequent proteolytic degradation, thus unmasking the nuclear localization

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signal on p65 subunit, and thereby allowing nuclear translocation of the complex. This sequential propagation of signaling ultimately results in the release of NF- $\kappa$ B subunits from I $\kappa$ B- $\alpha$  inhibitor, promoting translocation and initiation of gene transcription [7,8].

The present investigation has attempted therefore to unravel the role that NF- $\kappa$ B plays in regulating LPS-mediated IL-1 $\beta$  biosynthesis. The results could be highlighted as follows: (i) Irreversible inhibition of the proteasome complex by carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG-132), reported to inhibit NF- $\kappa$ B activation [9,10], did not abrogate LPS-induced production of IL-1 $\beta$ ; (ii) Selective inhibition of NF- $\kappa$ B by the action of caffeic acid phenylethyl ester (CAPE), a potent and specific inhibitor [11], reduced LPS-mediated IL-1 $\beta$  secretion at 100  $\mu$ M; (iii) Similarly, pre-treatment with sulfasalazine (SSA), a potent and irreversible inhibitor of NF- $\kappa$ B [12,13], reduced LPS-dependent IL-1 $\beta$  biosynthesis only at the highest dose used (10 mM); and (iv) Incorporation of a selectively permeant inhibitor of NF- $\kappa$ B, SN-50, a peptide which contains the nuclear localization sequence (NLS) for the p50 NF- $\kappa$ B subunit and the amino-terminal sequence of Kaposi fibroblast growth factor to promote cell permeability [14], attenuated LPS-mediated release of IL-1 $\beta$ . It is concluded that NF- $\kappa$ B blockade attenuates but does not abrogate LPS-dependent IL-1 $\beta$  biosynthesis in alveolar epithelial cells.

## Materials and methods

**Chemicals and reagents.** Unless indicated otherwise, chemicals of the highest analytical grade were purchased from Sigma–Aldrich (Dorset, England, UK). All experimental procedures involving the use of live animals were approved under the Animals (Scientific Procedures) Act, 1986 (UK).

**Primary cultures of alveolar epithelia.** Fetal alveolar type II (FATII) epithelial cells were isolated from lungs of fetuses, essentially as reported elsewhere [15,26]. Briefly, fetal rats were removed from pregnant *Sprague–Dawley* rats by cesarean section at day 19 of gestation (term = 22 days), the lungs excised, teased free from heart and upper airway tissue, and were finely minced, then washed free of erythrocytes using sterile, chilled  $Mg^{2+}$ - and  $Ca^{2+}$ -free Hanks' balanced salt solution (HBSS; 0.5 ml/fetus). The cleaned lung tissue was re-suspended in 1 ml/fetus HBSS containing trypsin (0.1 mg/ml), collagenase (0.06 mg/ml), and DNase I (0.012% w/v) and was agitated at 37 °C for 20 min. The solution was then centrifuged at 100g for 2 min to remove the undispersed tissue, the supernatant was saved to a fresh sterile tube, and an equal volume of Dulbecco's modified Eagle's medium (DMEM) with 10% (v/v) fetal calf serum (FCS) was added to the supernatant. After passing the supernatant through a 120  $\mu$ m pore sterile mesh, the filtrate was centrifuged at 420g for 5 min, the pellet was re-suspended in 20 ml DMEM/FCS, and the cells were placed into a T-150 culture flask for 1 h at 37 °C to enable fibroblasts and non-epithelial cells to adhere. Unattached cells were washed three times by centrifugation at 420g for 5 min each, then seeded onto 24 mm diameter Transwell-clear permeable supports (Costar; 0.4  $\mu$ m pore size) at a density of  $5 \times 10^6$  cells per filter, and allowed to adhere overnight at 152 Torr ( $\approx 21\%$   $O_2/5\%$   $CO_2$ ). DMEM/FCS was exchanged for 4 ml of serum free PC-1 media (Biowhittaker, MD, USA)

pre-equilibrated to  $pO_2 = 152$  Torr and 37 °C 24 h later and cells were maintained at this  $pO_2$  until the experiment. In each case, and under conditions of independent pre-treatments, the adenylate energy charge, an index of cell viability and competence, remained  $\geq 0.7$  and trans-epithelial monolayer resistance was monitored constantly at 250–350  $\Omega$   $cm^2$  or more [15,16].

**Drug pre-treatment and measurement of pro-inflammatory cytokine IL-1 $\beta$  by ELISA.** Cytokines have been demonstrated to induce NF- $\kappa$ B, whose activation has been implicated in mediating biological responses, including the expression of genes encoding cytokines [7,8]. Cells were pre-treated (2 h) with various inhibitors, monolayers were washed twice in pre-equilibrated PC-1 medium and subsequently challenged with LPS (1  $\mu$ g/ml) for 24 h (LPS was derived from *Escherichia coli*, serotype 026:B6). To determine whether LPS-induced release of IL-1 $\beta$  is regulated, at least in part, by NF- $\kappa$ B, we used carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG-132; Calbiochem, UK), a potent, reversible proteasome inhibitor ( $K_i = 4$  nM), reported to inhibit NF- $\kappa$ B activation [9,10]. Cells were pre-treated with MG-132 (0, 1, 10, and 50  $\mu$ M) for 2 h before exposure to LPS for further 24 h. Sulfasalazine (SSA; 0, 0.1, 1, and 10 mM; Sigma–Aldrich, UK) [12,13] and caffeic acid phenylethyl ester (CAPE; 0, 1, 10, and 100  $\mu$ M; Calbiochem, UK) [11], which are potent specific inhibitors of NF- $\kappa$ B, were also incorporated. Additionally, cells were pre-treated for 2 h with SN-50 (0, 1, 10, and 20  $\mu$ M; Calbiochem, UK), a specific permeating inhibitor of NF- $\kappa$ B nuclear translocation, followed by exposure to LPS (1  $\mu$ g/ml) for 24 h. This peptide contains the NLS for the p50 NF- $\kappa$ B subunit and the amino-terminal sequence of Kaposi fibroblast growth factor to promote cell permeability [14]. An inactive mutant control for SN-50 peptide (SN-50M; 20  $\mu$ M) corresponding to the same peptide sequence with substitutions of Lys<sup>363</sup> for Asn and Arg<sup>364</sup> for Gly in the NLS region has been used to confirm the specificity of SN-50. Cytokine (IL-1 $\beta$ ) release was assayed (24 h) following challenge.

Following exposure to LPS, cell-free supernatants were assayed for pro-inflammatory (R & D Systems, UK) cytokine (IL-1 $\beta$ ) biosynthesis by two-site, solid phase, sandwich enzyme-linked immunosorbent assay (ELISA), essentially as recounted previously [17–20]. Briefly, rabbit immunoaffinity purified polyclonal anti-rat IL-1 $\beta$  (2  $\mu$ g/ml) antibody was used to coat high-binding microtiter plates (MaxiSorp, Nunc, UK) in bicarbonate buffer (0.1 M  $NaHCO_3$  and 0.1 M  $NaCl$ , pH 8.2) [17–20]. After blocking in 3% bovine serum albumin (BSA), recombinant (standard) and biotinylated (recognition) immunoaffinity purified sheep anti-rat cytokine antibodies were employed for secondary detection. The color was developed using streptavidin-poly-HRP (Amersham Life Sciences, UK) coupled with 3,3',5,5'-tetramethyl-benzidine dihydrochloride (TMB) and 1 mM  $H_2O_2$ . The optical density was read at 450 nm against a background filter measuring at 595 nm, where the inter- and intra-assay coefficients of variations were reported at  $\leq 10\%$ . Results were extracted from the linear regression of the positive slope and cytokine concentration was expressed in pg/ml.

**Statistical analysis and data presentation.** Data are the means and the error bars are the SEM. Statistical evaluation of the difference in mean separation was performed by one-way analysis of variance (ANOVA), followed by post hoc Tukey's test, and the a priori level of significance at 95% confidence level was considered at  $P \leq 0.05$ .

## Results

### *The effect of proteasome inhibition on LPS-mediated IL-1 $\beta$ biosynthesis*

As shown in Fig. 1, exposure of epithelial cells to medium alone for 24 h had no effect on the secretion of IL-1 $\beta$ . Similarly, pre-treatment of monolayers with

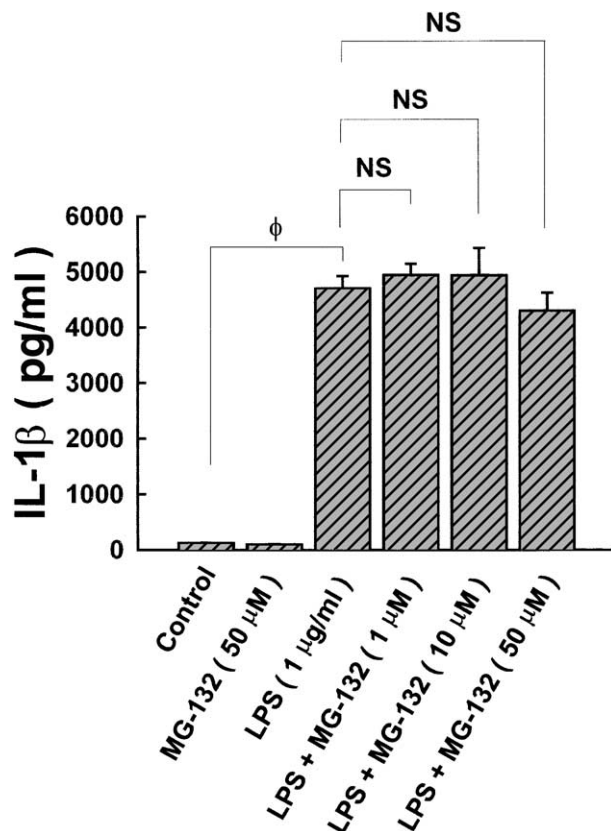


Fig. 1. The role of the proteasome complex and its inhibition in LPS-mediated IL-1 $\beta$  biosynthesis. Incubation of epithelial cells with medium alone (24 h) had no effect on IL-1 $\beta$  production, compared to pre-incubation with MG-132 (2 h), a proteasome inhibitor, followed by exposure to medium alone (24 h). Exposure to LPS (1  $\mu$ g/ml; 24 h) induced IL-1 $\beta$  secretion into the supernatant, an effect that was not affected or reversed by MG-132, at all doses tested.  $\phi P < 0.05$ , as compared with control; NS, non-specific, as compared with LPS alone;  $n = 3$ , which represents the number of independent experiments run in duplicate.

MG-132 (50  $\mu$ M), a specific inhibitor of the proteasome complex, reportedly known to blockade the NF- $\kappa$ B pathway [9,10], followed by exposure to medium alone for 24 h had no effect on IL-1 $\beta$  production either (Fig. 1). Exposure to LPS (1  $\mu$ g/ml) for 24 h induced IL-1 $\beta$  biosynthesis  $\approx$ 30–40-fold relative to medium alone measured at the same time point. Pre-treatment with MG-132 prior to challenge with LPS did not affect or reduce LPS-dependent release of IL-1 $\beta$  (Fig. 1).

#### The effect of CAPE on LPS-mediated IL-1 $\beta$ biosynthesis

As shown in Fig. 2, exposure of epithelial cells to medium alone for 24 h had no effect on the secretion of IL-1 $\beta$ . Similarly, pre-treatment of monolayers with CAPE (100  $\mu$ M), a specific inhibitor of NF- $\kappa$ B [11], followed by exposure to medium alone for 24 h had no effect on IL-1 $\beta$  production either (Fig. 2). Exposure to LPS (1  $\mu$ g/ml) for 24 h induced IL-1 $\beta$  biosynthesis

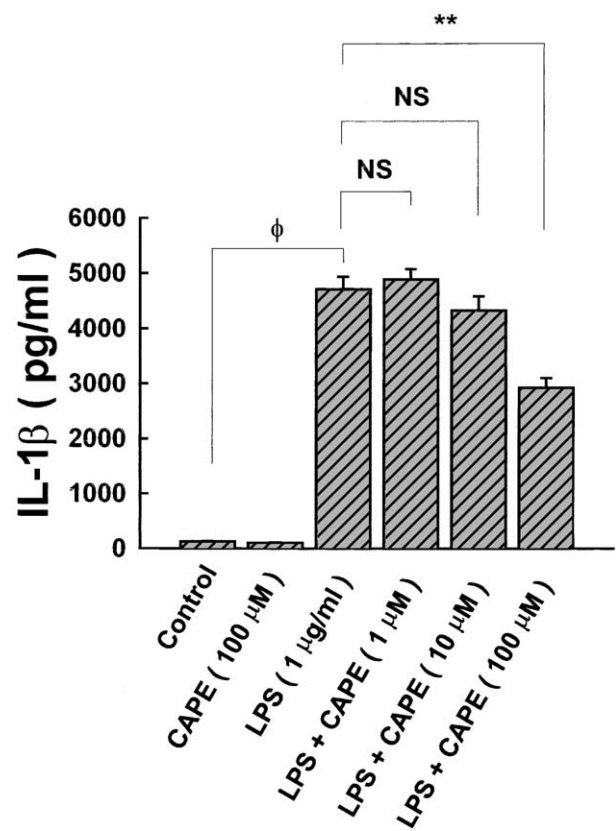


Fig. 2. The effect of CAPE on LPS-mediated IL-1 $\beta$  biosynthesis. Incubation of epithelial cells with medium alone (24 h) had no effect on IL-1 $\beta$  production, compared to pre-incubation with CAPE (2 h), an inhibitor of NF- $\kappa$ B, followed by exposure to medium alone (24 h). Exposure to LPS (1  $\mu$ g/ml; 24 h) induced IL-1 $\beta$  secretion into the supernatant, an effect that was reversed by CAPE at a dose of 100  $\mu$ M.  $\phi P < 0.05$ , as compared with control; NS, non-specific, as compared with LPS alone;  $**P < 0.01$ , as compared with LPS alone;  $n = 3$ , which represents the number of independent experiments run in duplicate.

$\approx$ 30–40-fold relative to medium alone measured at the same time point. Pre-treatment with CAPE prior to challenge with LPS reduced LPS-dependent release of IL-1 $\beta$  at a minimum effective dose of 100  $\mu$ M (Fig. 2).

#### The effect of SSA on LPS-mediated IL-1 $\beta$ biosynthesis

As shown in Fig. 3, exposure of epithelial cells to medium alone for 24 h had no effect on the secretion of IL-1 $\beta$ . Similarly, pre-treatment of monolayers with SSA (10 mM), a potent and specific inhibitor of NF- $\kappa$ B [12,13], followed by exposure to medium alone for 24 h had no effect on IL-1 $\beta$  production either (Fig. 3). Exposure to LPS (1  $\mu$ g/ml) for 24 h induced IL-1 $\beta$  biosynthesis  $\approx$ 30–40-fold relative to medium alone measured at the same time point. Pre-treatment with SSA prior to challenge with LPS reduced LPS-dependent release of IL-1 $\beta$  at the highest dose used in this study (10 mM) (Fig. 3).

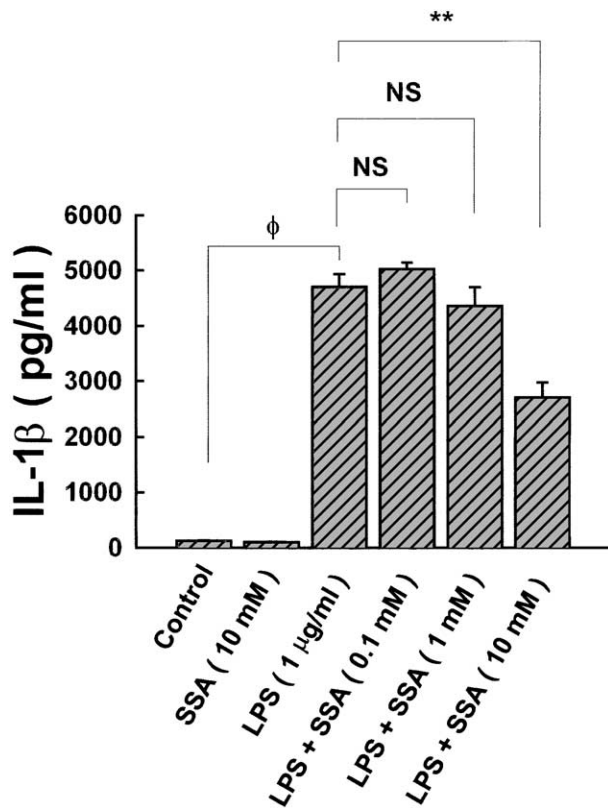


Fig. 3. The effect of SSA on LPS-mediated IL-1 $\beta$  biosynthesis. Incubation of epithelial cells with medium alone (24 h) had no effect on IL-1 $\beta$  production, compared to pre-incubation with CAPE (2 h), an inhibitor of NF- $\kappa$ B, followed by exposure to medium alone (24 h). Exposure to LPS (1  $\mu$ g/ml; 24 h) induced IL-1 $\beta$  secretion into the supernatant, an effect that was reversed by SSA only at a dose of 10 mM.  $\phi P < 0.05$ , as compared with control; NS, non-specific, as compared with LPS alone;  $**P < 0.01$ , as compared with LPS alone;  $n = 3$ , which represents the number of independent experiments run in duplicate.

#### The effect of SN-50 and its mutant, SN-50M, on LPS-mediated IL-1 $\beta$ biosynthesis

As shown in Fig. 4, exposure of epithelial cells to medium alone for 24 h had no effect on the secretion of IL-1 $\beta$ . Similarly, pre-treatment of monolayers with SN-50 (20  $\mu$ M), a potent and specific permeating inhibitor of NF- $\kappa$ B [14], followed by exposure to medium alone for 24 h had no effect on IL-1 $\beta$  production either (Fig. 4). Exposure to LPS (1  $\mu$ g/ml) for 24 h induced IL-1 $\beta$  biosynthesis  $\approx$ 30–40-fold relative to medium alone measured at the same time point. Pre-treatment with SN-50 prior to challenge with LPS reduced, in a dose-dependent manner, LPS-dependent release of IL-1 $\beta$  (Fig. 4). Mutation of the SN-50 peptide (SN-50M; 20  $\mu$ M) reversed the inhibitory effect of this peptide on LPS-mediated IL-1 $\beta$  biosynthesis (Fig. 4). The 50% minimum inhibitory effective concentration ( $IC_{50}$ ) of NF- $\kappa$ B inhibitors on LPS-mediated IL-1 $\beta$  biosynthesis is given in Table 1.

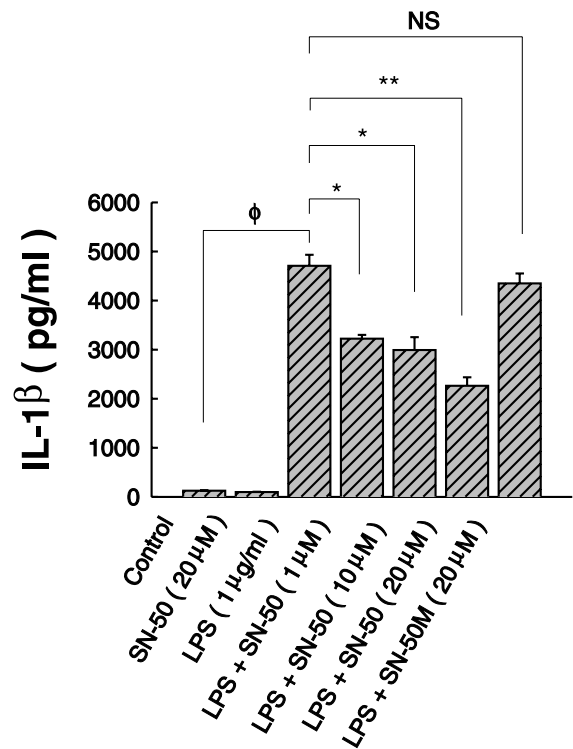


Fig. 4. The effect of SN-50 and its mutant, SN-50M, on LPS-mediated IL-1 $\beta$  biosynthesis. Incubation of epithelial cells with medium alone (24 h) had no effect on IL-1 $\beta$  production, compared to pre-incubation with SN-50 (2 h), a permeating inhibitor of NF- $\kappa$ B, followed by exposure to medium alone (24 h). Exposure to LPS (1  $\mu$ g/ml; 24 h) induced IL-1 $\beta$  secretion into the supernatant, an effect that was reversed by SN-50 in a dose-dependent manner. Mutation of the wild-type peptide (SN-50M; 20  $\mu$ M) reversed the inhibitory effect of SN-50 on LPS-dependent IL-1 $\beta$  secretion.  $\phi P < 0.05$ , as compared with control; NS, non-specific, as compared with LPS alone;  $*P < 0.05$ ,  $**P < 0.01$ , as compared with LPS alone;  $n = 3$ , which represents the number of independent experiments run in duplicate.

Table 1

Analysis of the 50% minimum effective inhibitory ( $IC_{50}$ ) concentrations of various NF- $\kappa$ B inhibitors on LPS-induced IL-1 $\beta$  biosynthesis in the alveolar epithelium

MG-132 ( $\mu$ M)	CAPE ( $\mu$ M)	SSA (mM)	SN-50 ( $\mu$ M)
–	$87.15 \pm 3.75$	$\geq 10$	$18.47 \pm 2.12$

Data are presented as means  $\pm$  SEM;  $n = 3$ , each.  $IC_{50}$  was determined from the negative slope of the linear regression curves.

#### Discussion

There is increasing evidence implicating the alveolar epithelium, in particular, as a dynamic barrier that plays an important role in regulating the inflammatory and metabolic responses to oxidative stress and the accompanying inflammatory signal, sepsis, endotoxemia, and other critical illnesses in the lung [5,13,15–23]. The respiratory epithelium is a primary target of an inflam-

matory/infectious condition at the epithelial–blood interface and is itself capable of amplifying an inflammatory signal by recruiting inflammatory cells and by producing inflammatory mediators [15–20]. Many of the side effects of LPS, derived from the cell wall of gram-negative bacteria, are secondary to the overproduction of pro-inflammatory mediators, including cytokines such as IL-1 $\beta$ . Inflammation as well as autoimmune disease is often associated with deregulated expression and biosynthesis of pro-inflammatory cytokines, which influence a plethora of cellular functions. Therefore, the down-regulation of an inflammatory signal is a major focus of the rational approach to the treatment of inflammatory diseases, such as chronic inflammation, sepsis, and rheumatoid arthritis. For instance, a novel recent study by Haskó et al. [24] reported a potential role for extracellular purines, including adenosine and ATP, and inosine, a degradation product of these purines, as potent endogenous immunomodulatory molecules that inhibit inflammatory cytokine biosynthesis and protect against endotoxin-induced shock. It has also been reported, in addition, that selective inhibition of phosphodiesterases, a family of enzymes involved in the degradation of cAMP [25,26], steroids, such as glucocorticoids [27], pyrimidylpiperazine derivatives [20,28–30], ERK, and p38/RK MAPK selective inhibitors [31,32] differentially regulate the transcription and biosynthesis of inflammatory cytokines.

The promoters of genes encoding cytokines contain multiple *cis*-acting motifs including those that bind such transcription factors as NF- $\kappa$ B [7]. Furthermore, the release of free NF- $\kappa$ B upon extracellular stimulation due to I $\kappa$ B phosphorylation and degradation by the proteasome complex leads to DNA binding to specific  $\kappa$ B moieties to initiate transcription of related genes, including immunoreceptors, cytokines and, interestingly, its own inhibitor, I $\kappa$ B [7,8,19]. Two unique features of the NF- $\kappa$ B/I $\kappa$ B complex system are deduced from its feedback regulation. The transcriptional activation of NF- $\kappa$ B triggers the synthesis of I $\kappa$ B and NF- $\kappa$ B activated transcription is maintained by continuous degradation of I $\kappa$ B, which is sustained by an extracellular stimulus [7,8]. Thus, the expression of I $\kappa$ B parallels both NF- $\kappa$ B activity and the duration of the activating extracellular stimulation, suggesting that this temporal parallelism between I $\kappa$ B accumulation/degradation and an effective external stimulation is a mechanism allowing dual regulation of NF- $\kappa$ B within the alveolar space. The selective interference with the functioning of the proteasome complex [9,10] in regulating the translocation and activation of NF- $\kappa$ B and the expression of its inhibitor I $\kappa$ B- $\alpha$  suggested that the I $\kappa$ B- $\alpha$ /NF- $\kappa$ B pathway is partially implicated in regulating LPS-mediated biosynthesis of IL-1 $\beta$ . This is rather unequivocally reinforced with the observation reported in this study that selective inhibition of the

NF- $\kappa$ B pathway attenuated but did not abrogate the inductive effect of LPS on IL-1 $\beta$  production. Therefore, the I $\kappa$ B-/NF- $\kappa$ B pathway could be partially dissociated from the presumably downstream pathway regulating IL-1 $\beta$  signaling, indicating the involvement of a possible cross-talk among several pathways working independently or in coherence to integrate signaling mechanisms governing the regulation of cytokines in the alveolar epithelium.

Many extracellular stimuli, including pro-inflammatory cytokines and other inflammatory mediators [33], elicit specific cellular responses through the activation of mitogen-activated protein kinase (MAPK) signaling pathways [31,32]. MAPKs are a proline-targeted serine-threonine kinases that transduce environmental stimuli to the nucleus and they themselves are activated by upstream MAPK kinases (MAPKKs) on both threonine and tyrosine residues within an 'activation loop' [31,32]. Once activated, MAPKs can phosphorylate and activate other kinases or nuclear proteins, including potential transcription factors and substrates. Furthermore, inflammatory mediators, such as IL-1 $\beta$ , were shown to activate the MAPK pathway in several cell types [34]. Since MAP kinase regulation has recently been implicated in regulating pro-inflammatory cytokine biosynthesis [35] and transcription [36], it is possible that this pathway is directly involved in regulating IL-1 $\beta$  biosynthesis in the alveolar epithelium. We have recent evidence from our laboratory indicating a potential role for the MAPK pathway, and in particular MAPK<sup>p38</sup> and MAPK<sup>ERK</sup>, in regulating pro-inflammatory cytokines (Haddad et al., *unpublished observations*). We are currently extending these observations to investigate the immunoregulatory role that MAPK plays in regulating inflammatory mediator transcription and biosynthesis and whether there is a potential cross-talk between MAPK and I $\kappa$ B- $\alpha$ /NF- $\kappa$ B signaling pathways.

In summary, the results could be highlighted as follows: (i) Irreversible inhibition of the proteasome complex by carbobenzoxy-L-leucyl-L-leucyl-L-leucinal (MG-132), reported to inhibit NF- $\kappa$ B activation, did not abrogate LPS-induced production of IL-1 $\beta$ ; (ii) Selective inhibition of NF- $\kappa$ B by the action of CAPE, a potent and specific inhibitor, reduced LPS-mediated IL-1 $\beta$  secretion at a dose of 100  $\mu$ M; (iii) Similarly, pre-treatment with SSA, a potent and irreversible inhibitor of NF- $\kappa$ B, reduced LPS-dependent IL-1 $\beta$  biosynthesis only at the highest dose used (10 mM); and (iv) Incorporation of a selectively permeant inhibitor of NF- $\kappa$ B, SN-50, a peptide which contains the nuclear localization sequence (NLS) for the p50 NF- $\kappa$ B subunit and the amino-terminal sequence of Kaposi fibroblast growth factor to promote cell permeability, attenuated LPS-mediated release of IL-1 $\beta$ . It is concluded that NF- $\kappa$ B blockade attenuates but does not abrogate LPS-dependent IL-1 $\beta$  biosynthesis in alveolar epithelial cells.

## Acknowledgments

This work was supported by grants from the Medical Research Council (MRC, UK), Anonymous Trust and Tenovus-Scotland. J.J.H. is a recipient of the George John Livanos prize (London). Parts of this work were presented at FASEB Experimental Biology-2001 in Orlando, Florida, USA (Dr. John J. Haddad).

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