CARFAX

Mediators of Inflammation, 7, 93–98 (1998)

Reduction of nitric oxide release from alveolar macrophages by a lipocortin peptide

A. M. Kamal, T. D. Tetley, I. R. Witherden and S. F. Smith[CA]

Department of Respiratory Medicine, National Heart and Lung Institute, Imperial College School of Medicine, Charing Cross Hospital, Fulham Palace Road, London W6 8RF, UK

[CA]Corresponding Author
Tel: (+44) 181 846 1226/7173
Fax: (+44) 181 846 7170
Email: s.smith@cxwms.ac.uk

NITRIC oxide (NO), produced by alveolar macrophages (AM) is used as a marker of respiratory tract inflammation. Lipocortin 1 (Lc-1) is an anti-inflammatory, glucocorticoid-inducible protein. The current aims were to determine whether (a) an Lc-1-derived peptide, Ac2–26, inhibited lipopolysaccharide (LPS)-induced NO release by primary AM *in vitro* and (b) the inhibitory action of dexamethasone was Lc-1-dependent. LPS treatment stimulated NO release from rat AM. Ac2–26 had little effect on unstimulated release, but suppressed LPS-stimulated release at concentrations ≥320 nM (320 nM, 10 ± 3%; 3.2 μM, 15 ± 3%; 32 μM, 27 ± 4% NO inhibited, mean ± SEM, *n* = 6). Inhibition by dexamethasone of NO release was unaffected by neutralizing anti-Lc-1 indicating that this action is Lc-1-independent in primary AM. Nevertheless inhibition of NO release by Ac2–26 (80 μM) was similar to that of 1 μM dexamethasone (Ac2–26, 40 ± 6%; dexamethasone, 48 ± 6% NO inhibited, mean ± SEM, *n* = 6).

Key words: lipocortin peptide, glucocorticoid, nitric oxide, alveolar macrophage, dexamethasone

Introduction

Lipocortin-1 (Lc-1) is a 37 kDa anti-inflammatory, glucocorticoid-inducible member of the annexin superfamily of proteins. Its synthesis is upregulated by glucocorticoids both *in vitro* and *in vivo* in a number of species, including man.[1] There is considerable evidence to suggest that intact Lc-1 and synthetic peptides derived from its primary sequence possess intrinsic anti-inflammatory properties in a range of experimental models.[2–7]

Lc-1 is a potential mediator of the anti-inflammatory action of glucocorticoids. Increased levels of cellular Lc-1 protein have been observed *in vivo* in human alveolar macrophages (AM) exposed to oral glucocorticoid[8] and in human mononuclear cells following i.v. administration of hydrocortisone.[9,10] Studies on adrenalectomized rats have shown a decrease in Lc-1 mRNA and protein in the lung, spleen, liver and kidney, suggesting not only a pharmacological modulation, but endogenous physiological steroidal control on Lc-1 expression and turnover.[11] Immunoneutralization studies have confirmed that a number of the anti-inflammatory actions of exogenous glucocorticoids are, in part, dependent on Lc-1, both *in vivo*[5–7,12] and *in vitro*.[13–17]

Previous studies have demonstrated that the lung is a rich source of Lc-1[18–20] which is detectable in a number of different cell types, including human AM[21] and epithelial cells.[22] Lc-1 is present in bronchoalveolar lavage fluid,[23] in which its levels are increased by oral glucocorticoids in healthy volunteers,[24,25] patients with lung disease,[24] and control and carrageenin-treated rats.[26] Increased Lc-1 concentrations in lung lavage also occur in response to non-specific stimuli, such as cigarette smoke[27] and LPS;[28] this may reflect increased expression of Lc-1, but may also be a result of increased cell number, cell death or injury.

Inflammation in the respiratory tract is associated with the release of numerous inflammatory mediators, including cytokines and eicosanoids. Recently, nitric oxide (NO) has received much attention as a potential modulator of lung function in health and disease. Both constitutive nitric oxide synthase (cNOS) and inducible nitric oxide synthase (iNOS) are present in the respiratory tract. The former is found in endothelial cells and peripheral nerves, where it acts as a smooth muscle relaxant and neurotransmitter[29] and the latter, which is probably involved in lung defence, is localized to AM and epithelial cells[30,31] and is increased in acute and chronic inflammation.[32]

Systemic administration of LPS or generalized sepsis results in high levels of exhaled NO in the lungs of rats.[33] Wood smoke-exposed rats have elevated concentrations of NO metabolites in the plasma which correlates with increased lung epithelial permeability, a process reversible with NOS inhibitors.[34] This suggests that smoke-induced damage may be due to reactive nitrogen radicals in the lung. Intra-tracheal instillation of LPS to rats also causes an increase in epithelial leak, elevated levels of NO in respiratory tract secretions and increased NO release by AM[35] and

0962-9351/98/020093-06 $9.00

is associated with induction of iNOS mRNA.[36] Raised iNOS activity and NO release also occur in rat macrophages and epithelial cells in response to exposure to cytokines, such as IFN-γ, TNFα and IL-1.[31] Unlike cNOS, which produces NO at a constant low level, activation of iNOS results in high output NO production.[37] Thus, many pulmonary inflammatory conditions are accompanied by increased NO release. Although it is unclear whether the NO is damaging or protective, NO release is used as a marker of lung inflammation.

Glucocorticoids inhibit NOS activity in the J774.2 macrophage-like cell line,[38] an effect which is Lc-1 dependent in these cells.[15] Similarly, inhibition by glucocorticoid of LPS-induced iNOS activity in rat lung homogenates is Lc-1-dependent, the AM being suggested as a potential target for the glucocorticoid action.[15] Therefore, we hypothesized that NO release from AM *in vitro* could be inhibited by glucocorticoids in a Lc-1-dependent manner. Primary AM represent a useful *in vitro* model system for examining the relationship between glucocorticoids, Lc-1 and pulmonary NO, since they possess functional glucocorticoid receptors,[39] release NO via the activity of iNOS and express Lc-1.[21] In addition they are the predominant cell type in the airspaces with potential anti-and pro-inflammatory functions. The AM therefore encapsulates all the components under examination and, furthermore, is easily accessible and readily isolated.

Thus the aims of the current study were to determine the effect of a synthetic peptide derived from the N-terminal of Lc-1 (Ac2–26),[4,6,40,41] on NO release by primary rat AM *in vitro* and to examine whether inhibition of NO release by glucocorticoids in this system is Lc-1 dependent.

Methods

Animals and removal of lungs

Pathogen-free male Wistar rats (Charles River), weighing 200–250 g were killed by a lethal intra-peritoneal injection of pentobarbitone (1 g/kg body weight) and heparin (1000 U/kg body weight). Prior to excision, the trachea was cannulated and the lungs perfused free of blood via the right ventricle and pulmonary artery using a gravity feed of sterile 0.15 M NaCl.

Isolation and culture of alveolar macrophages (AM)

Excised lungs were fully inflated via the tracheal cannula with 10 ml aliquots of sterile 0.15 M NaCl, emptied, and lavage repeated until approximately 50 ml of fluid had been recovered. The lavage fluid was pooled and centrifuged at 300 × **g** for 10 min at 4°C. Pelleted cells were resuspended in Hanks's balanced salt solution, without Ca^{2+} or Mg^{2+} (Sigma) and centrifuged as before. BAL cells (approximately 95%AM) were resuspended in low protein hybridoma medium, containing 2% foetal calf serum, glutamine (2 mM), penicillin (100 U/ml) and streptomycin (100 µg/ml). Cells were counted and seeded in to 96-well plates (Nunc) at a density of 1×10^5 cells per well. AM were allowed to adhere for 3 h in a humidified chamber at 37°C in 5% CO_2 in air. All cell culture reagents were obtained from Gibco.

Effect of dexamethasone and peptide Ac2–26 on NO release

Following the 3-h adherence period, non-adherent cells and conditioned media were aspirated and replaced by fresh medium containing graded concentrations of Lc-1 peptide, Ac2–26 (3.2 nM–80 µM), or dexamethasone (10 nM–1 µM). Adherent cells were pre-incubated for 1 h, following which lipopolysaccharide (0.1–10 µg/ml, serotype 055:B5 from *Escherichia coli*, Sigma) was added to all wells except controls and the cells incubated for a further 24 h. Conditioned media were harvested and analysed for nitrite. The competitive NOS inhibitor N^G-monomethyl L-arginine (L-NMMA 4 mM, Sigma) was added to selected wells to determine the proportion of measured nitrite which originated from the activity of NOS.

Effect of Lc-1 immuno-neutralization on dexamethasone activity

Non-adherent cells and conditioned media were removed as above and replaced with fresh medium containing a sheep polyclonal neutralising antibody to Lc-1 (LC01, the generous gift of Professor R. J. Flower, William Harvey Research Institute, London) used at 1:50 dilution. The cells were pre-treated with this antiserum for 3 h, then incubated with graded doses of dexamethasone for 1 h and stimulated with lipopolysaccharide for 24 h as described above.

Measurement of nitrite

Spontaneous oxidation of the NO radical in aqueous solutions leads to the formation of nitrite (NO_2^-) as the predominant stable breakdown product.[29,42] Nitrite accumulation in conditioned media was therefore used as an index of NO release by AM, 24 h post-LPS. This was measured using the colorimetric Greiss reaction.[43] Nitrite concentrations were determined by comparison with standard solutions of sodium nitrite (0.78–100 µM) prepared in culture medium.

Data analysis

Data were analysed by the Wilcoxon's signed rank test for paired data; a probability of $P<0.05$ was regarded as statistically significant. Percentage inhibition of

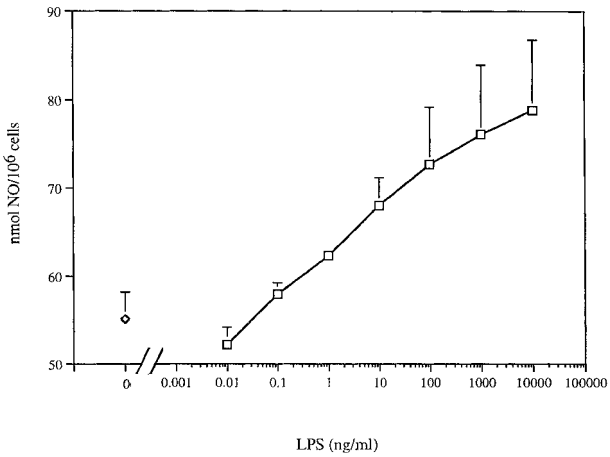

FIG. 1. Effect of increasing concentrations of LPS on NO release from rat AM *in vitro*. Data are presented as mean ± SEM, $n = 4$; Open squares represent LPS-stimulated NO release; Open diamond represents basal (unstimulated) NO release.

LPS-stimulated NO release was calculated from the data in Figure 3 as:

$$\%\,\text{Inhibition} = \frac{(\text{LPS alone} - \text{LPS} + \text{Ac2–26})}{(\text{LPS alone} - \text{LPS} + 4\,\text{mM L-NMMA})} \times 100$$

Results

Effect of lipopolysaccharide on NO release

Median basal release of NO was 56, range 47–61 nmol NO/10^6 cells/24 h in culture (Fig. 1, $n = 4$); 86% was inhibited by 4 mM L-NMMA. Stimulation of AM with LPS produced a concentration-dependent increase in nitrite accumulation in the culture medium comparable with that observed in previous studies[31,38,44] (Fig. 1). At a concentration of 10 µg LPS/ml, 85% of NO release was inhibited by L-NMMA. Based on these observations, an LPS dose range of 0.1–10 µg/ml was used in all subsequent experiments.

Effect of peptide Ac2–26 on NO release

The peptide had no significant effect on basal NO release in unstimulated cells (Fig. 2) except at the highest concentrations of Ac2–26 tested (32 µM and 80 µM) where basal release was significantly inhibited (31% and 28% respectively). Ac2–26 significantly inhibited LPS-induced NO release by AM at concentrations of 320 nM or more (Fig. 3). The maximum inhibition observed was 40% by a concentration of 80 µM Ac2–26. The action of Ac2–26 was similar irrespective of the LPS concentration used (Table 1) and therefore only data for LPS at 10 µg/ml are illustrated.

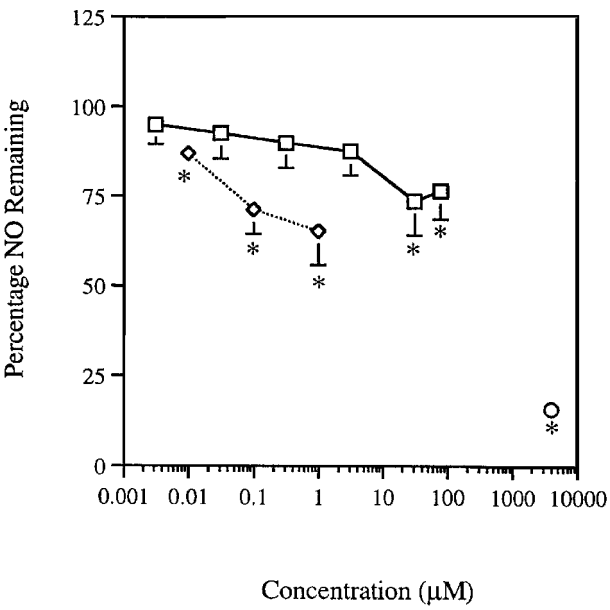

FIG. 2. Effect of Ac2–26 and dexamethasone on basal NO release by rat AM *in vitro*. Percentage NO remaining following L-NMMA, peptide or dexamethasone treatment was calculated using the following formula: (NO release by unstimulated AM + peptide/NO release by unstimulated AM) × 100 Data are presented as mean ± SEM, $n = 6$, $^*P < 0.05$, Wilcoxon's signed rank test for paired data; open squares represent NO release in the presence of Ac2–26; open diamonds represent NO release in the presence of dexamethasone; open Circle represents NO release in the presence of L-NMMA.

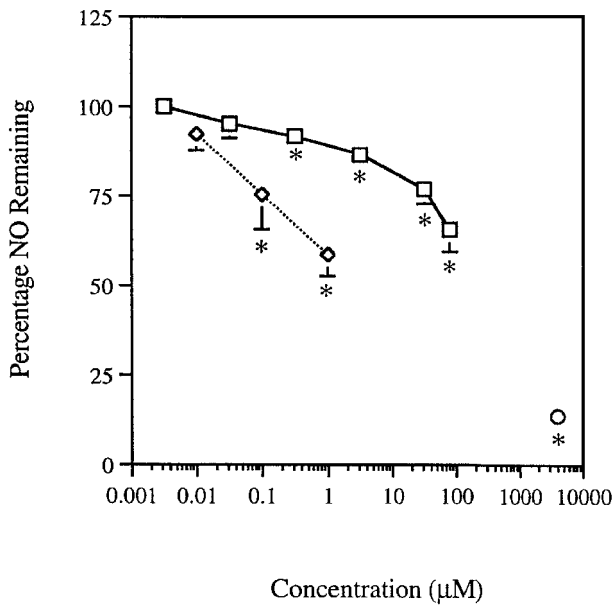

FIG. 3. Effect of Ac2–26 and dexamethasone on NO release by LPS-stimulated rat AM *in vitro* (10 µg/ml LPS). Percentage NO remaining following L-NMMA, peptide or dexamethasone treatment was calculated using the following formula: (NO release by LPS-stimulated AM + peptide/NO release by LPS-stimulated AM) × 100 Data are presented as mean ± SEM, $n = 6$, $^*P < 0.05$, Wilcoxon signed rank test for paired data; open squares represent NO release in the presence of Ac2–26; open diamonds represent NO release in the presence of dexamethasone; open Circle represents NO release in the presence of L-NMMA.

Table 1. Effect of Ac2–26 and dexamethasone on LPS-stimulated NO release. Data are presented as mean ± SEM, and calculated as described in the legend to Fig. 3

	LPS (μg/ml)			
	0	0.1	1	10
	% NO Remaining			
Ac2–26 (μM)				
0.0032	95 ± 5	100 ± 3	100 ± 2	100 ± 2
0.032	93 ± 7	99 ± 4	100 ± 3	95 ± 4
0.32	90 ± 7	94 ± 6	96 ± 3	92 ± 3*
3.2	88 ± 7	90 ± 6	95 ± 2	87 ± 3*
32	74 ± 9*	83 ± 6*	83 ± 6*	77 ± 4*
80	77 ± 8*	80 ± 7*	76 ± 6*	66 ± 6*
Dexamethasone (μM)				
0.01	87 ± 3*	89 ± 1*	90 ± 2*	92 ± 5
0.1	71 ± 7*	73 ± 5*	79 ± 11	75 ± 10*
1	65 ± 10*	61 ± 5*	62 ± 5*	59 ± 6*

*$P < 0.05$, Wilcoxon signed rank test for paired data, $n = 6$.

Effect of dexamethasone on NO release

Dexamethasone significantly inhibited basal NO release at all concentrations of drug and LPS-stimulated release at concentrations above 10 nM dexamethasone (Table 1 and Figs 2 and 3). Maximal inhibition by dexamethasone was 48%

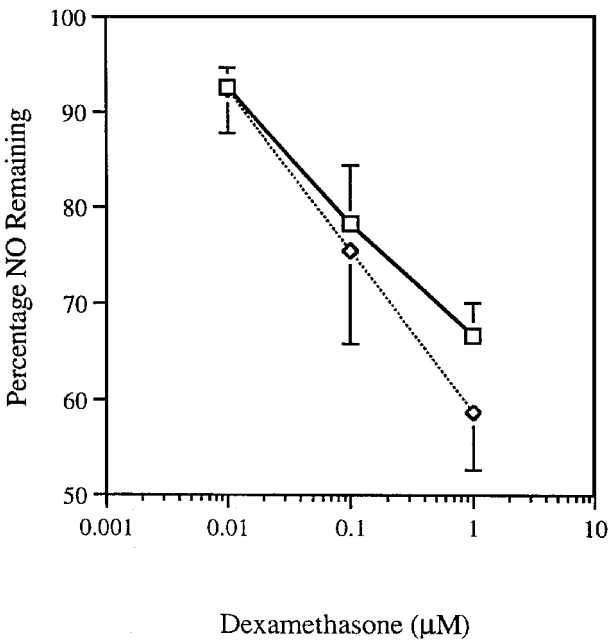

FIG. 4. Effect of Lc-1 immunoneutralization on dexamethasone-mediated inhibition of NO release by LPS-stimulated rat AM *in vitro* (10 μg/ml LPS). Data are presented as mean ± SEM, $n = 6$; open squares represent LPS-stimulated release in the presence of dexamethasone and Lc-1 neutralizing antibody (1:50 dilution); open diamonds represent LPS-stimulated release in the presence of dexamethasone alone.

Effect of anti-Lc-1 neutralizing antibody on NO release

Pre-treatment of rat AM with a neutralizing antibody to Lc-1, had no effect on dexamethasone-mediated inhibition of NO release, irrespective of the concentration of dexamethasone used (Fig. 4).

Discussion

This study demonstrates that the N-terminal peptide of Lc-1, Ac2–26, inhibits LPS-induced NO release from rat AM in a concentration-dependent manner. To our knowledge, this is the first such *in vitro* demonstration in a primary cell with a known pro-inflammatory function. By increasing the dose of Ac2–26 to 80 μM it was possible to achieve the same degree of inhibition as with 1 μM dexamethasone. Interestingly use of a neutralising anti-Lc-1 antiserum indicated that the inhibitory action of dexamethasone itself on NO release was independent of endogenous Lc-1.

Downregulation of LPS-induced NO release by a longer Lc-1 peptide, $Lc\text{-}1_{1\text{–}188}$, at a concentration of 20 μg/ml has been reported in the J774.2 cell line.[15] The observation that it was effective at a substantially lower concentration than Ac2–26 may reflect differences between the responsiveness to Lc-1 of the cell line and the primary AM used in the current study. Another possibility is that the Ac2–26 was partially degraded by AM-derived products during the long incubation period. Alternatively, there may be differences between the peptides themselves, as shown previously with murine neutrophils;[4] it is possible that the Ac2–26 does not activate the signal transduction machinery needed to trigger the cell response as effectively as does the larger molecule. The optimal size for inhibition of NO release by Lc-1-derived

peptides remains to be established. Provided that the peptides are as effective as dexamethasone and low in toxicity, the absolute concentration required to achieve inhibition is probably not a major consideration for their use *in vivo*.

Dexamethasone was an effective inhibitor of NO release by AM in this study, although interestingly, use of neutralizing antiserum indicated that its action was Lc-1-independent. Pulmonary AM treated with dexamethasone are capable of increasing their Lc-1 expression and release.[21] However, studies in our laboratory indicate that at the highest concentration of dexamethasone used in the current investigation (1 μM) the increase in cellular Lc-1 above baseline is likely to be modest in rat AM.[45] Since the anti-inflammatory actions of glucocorticoids are mediated via multiple pathways, the effect of dexamethasone on NO release is likely to be via another mechanism, perhaps downregulation of iNOS.[36] Similarly, the mode of action of dexamethasone in the lung *in vivo* is not fully known. Previous publications by us and others have demonstrated increases in cellular and extracellular pulmonary Lc-1 following oral glucocorticoid[24,25] and also after non-specific stimuli including LPS[15] and carrageenin.[26] A previous study *in vivo* by Wu *et al.*[15] showed that neutralizing antisera to Lc-1 blunted the action of dexamethasone against LPS-induced NO release from lung homogenates, suggesting that increases in endogenous Lc-1 contribute to the steroid action. However, *in vivo*, there are many other potential sources of Lc-1 in the lung which may be targets for the dexamethasone, such as epithelial and gland cells.[19] Our previous work indicates that the type II epithelial cell (TII) releases enhanced levels of Lc-1 in response to lower doses of dexamethasone than AM. Allied with their high number in the lung,[46–48] this makes TII likely cellular mediators of the Lc-1-dependent action of dexamethasone observed by Wu *et al.*[15] Similarly, *in vivo*, extracellular Lc-1 may inhibit NO release by a variety of cell types not present in our simple *in vitro* model.

In summary, we have shown that Ac2–26 causes the same degree of inhibition of LPS-induced NO release by AM as dexamethasone, but at a much higher concentration and by a different mechanism. This and other peptides derived from Lc-1 may have therapeutic potential for suppression of pulmonary inflammation, since they could be delivered topically to the target organ.

References

1. Flower RJ, Rothwell NJ. Lipocortin-1: cellular mechanisms and clinical relevance. *TiPS* 1994; **15**: 71–76.
2. Cirino G, Peers SH, Flower RJ, Browning JL, Pepinsky RB. Human recombinant lipocortin 1 has acute local anti-inflammatory properties in the rat paw oedema test. *Proc Natl Acad Sci USA* 1989; **86**: 3428–2432.
3. Maridonneau-Parini I, Errasfa M, Russo-Marie F. Inhibition of O_2^- generation by dexamethasone is mimicked by lipocortin 1 in alveolar macrophages. *J Clin Invest* 1989; **83**: 1936–1940.
4. Perretti M, Ahluwalia A, Harris JG, Goulding NJ, Flower RJ. Lipocortin-1 fragments inhibit neutrophil accumulation and neutrophil-dependent oedema in the mouse. *J Immunol* 1993; **151**: 4306–4314.
5. Perretti M, Flower RJ. Modulation of IL-1-induced neutrophil migration by dexamethasone and lipocortin 1. *J Immunol* 1993; **150**: 992–999.
6. Getting SJ, Flower RJ, Perretti M. Inhibitition of neutrophil and monocyte recruitment by endogenous and exogenous lipocortin 1. *Br J Pharm* 1997; **120**: 1075–1082.
7. Mancuso F, Flower RJ, Perretti M. Leukocyte transmigration, but not rolling or adhesion, is selectively inhibited by dexamethasone in the hamster post-capillary venule. *J Immunol* 1995; **155**: 377–386.
8. De Caterina R, Sicari, R, Giannessi D, *et al.* Macrophage-specific eicosanoid synthesis inhibition and lipocortin-1 induction by glucocorticoids. *J Appl Physiol* 1993; **75**: 2368–2375.
9. Goulding NJ, Godolphin JL, Sampson MB, Maddison PJ, Flower RJ. Hydrocortisone induces lipocortin I production by peripheral blood mononuclear cells *in vivo* in man. *Biochem Soc Trans* 1989; **18**: 306–307.
10. Goulding NJ, Godolphin JL, Sharland, *et al.* Anti-inflammatory lipocortin 1 production by peripheral blood leucocytes in response to hydrocortisone. *Lancet* 1990; **335**: 1416–1418.
11. Vishwanath BS, Frey FJ, Bradbury M, Dallman MF, Frey BM. Adrenelactomy decreases lipocortin-1 messenger ribonucleic acid and tissue protein content in rats. *Endocrinol* 1992; **130**: 585–591.
12. Perretti M, Ahluwalia, A, Harris, J, Harris, HJ, Wheller, SK, Flower RJ. Acute inflammatory response in the mouse: exacerbation by immunoneutralisation of lipocortin 1. *Br J Pharm* 1996; **117**: 1145–1154.
13. Croxtall JD, Flower RJ. Lipocortin 1 mediates dexamethasone-induced growth arrest of the A549 lung adenocarcinoma cell line. *Proc Natl Acad Sci USA* 1992; **89**: 3571–3575.
14. Croxtall JD, Choudhury Q, Tokumoto H, Flower RJ. Lipocortin-1 and the control of arachidonic acid release in cell signalling. *Biochem Pharm* 1993; **50**: 465–474.
15. Wu CC, Croxtall, JD, Perretti M, *et al.* Lipocortin 1 mediates the inhibition by dexamethasone of the induction by endotoxin of nitric oxide synthase in the rat. *Proc Natl Acad Sci USA* 1995; **92**: 3473–3477.
16. Sudlow AW, Carey F, Forder R, Rothwell N. The role of lipocortin-1 in dexamethasone-induced suppression of PGE_2 and TNFα release from human peripheral blood mononuclear cells. *Br J Pharm* 1996; **117**: 1449–1456.
17. Croxtall JD, Newman SP, Choudhury Q, Flower RJ. The concerted regulation of $cPLA_2$, COX2, and lipocortin 1 expression by IL-1β in A549 cells. *Biochem Biophys Res Comm* 1996; **220**: 491–495.
18. Pepinsky RB, Sinclair LK, Browning JL, *et al.* Purification and partial sequence analysis of a 37 kDa protein that inhibits phospholipase activity from rat peritoneal exudates. *J Biol Chem* 1986; **261**: 4239–4246.
19. Fava RA, McKanna J, Cohen S. Lipocortin 1 (p35) is abundant in a restricted number of differentiated cell types in adult organs. *J Cellul Physiol* 1989; **141**: 284–293.
20. Khanna NC, Helwig ED, Ikebuchi NW, Fitzpatrick S, Bajwa R, Waisman DM. Purification and characterisation of annexin proteins from bovine lung. *Biochem* 1990; **29**: 4852–4862.
21. Ambrose MP, Bahns CC, Hunninghake GW. Lipocortin 1 production by human alveolar macrophages. *Am J Respir Cell Mol Biol* 1992; **6**: 17–21.
22. Ambrose MP, Hunninghake GW. Corticosteroids increase lipocortin 1 in alveolar epithelial cells. *Am J Respir Cell Mol Biol* 1990; **3**: 349–353.
23. Smith SF, Tetley TD, Guz A, Flower RJ. Detection of lipocortin 1 in human lung lavage fluid: lipocortin degradation as a possible proteolytic mechanism in the control of inflammatory nmediators and inflammation. *Environmental Health Perspectives* 1990; **85**: 135–144.
24. Ambrose MP, Hunninghake GW. Corticosteroids increase lipocortin 1 in BAL fluid from normal individuals and patients with lung disease. *J Appl Physiol* 1990; **68**: 1668–1671.
25. Smith SF, Tetley TD, Datta AK, Smith T, Guz A, Flower, RJ. Lipocortin-1 distribution in bronchoalveolar lavage from healthy human lung: effect of prednisolone. *J Appl Physiol* 1995; **79**: 121–128.
26. Smith SF, Benjamin A, Dewar A, *et al.* Effect of dexamethasone on carrageenin-induced inflammation in the lung. *Mediators Inflamm* 1995; **4**: 273–281.
27. Van Hal PTW, Overbeek SE, Hoogsteden HC, *et al.* Eicosanoids and lipocortin-1 in BAL fluid in asthma: effects of smoking and inhaled glucocorticoids. *J Appl Physiol* 1996; **81**: 548–555.
28. Smith T, Smith SF, Flower RJ, Buckingham JC. Endotoxin and the expression of lipocortins in central and peripheral tissues of the rat. *Br J Pharmacol* 1991; **104**: 218P.
29. Gaston B, Drazen JM, Loscalzo J, Stamler JS. The biology of nitrogen oxides in the airways. *Am J Respir Crit Care Med* 1994; **149**: 538–551.
30. Kobzik L, Bredt DS, Lowenstein CJ, *et al.* Nitric oxide synthase in human and rat lung: immunocytochemical and histochemical localization. *Am J Respir Cell Mol Biol* 1993; **9**: 371–377.

31. Warner RL, Paine R, Christensen PJ, *et al.* Lung sources and cytokine requirements for *in vivo* expression of inducible nitric oxide synthase. *Am J Respir Cell Mol Biol* 1995; **12**: 649–661.
32. Vane JR, Mitchell JA, Appleton I, *et al.* Inducible isoforms of cyclooxygenase and nitric oxide synthase in inflammation. *Proc Natl Acad Sci USA* 1994; **91**: 2046–2050.
33. Stewart TE, Valenza F, Ribeiro SP, *et al.* Increased nitric oxide in exhaled gas as an early marker of lung inflammation in a model of sepsis. *Am J Respir Crit Care Med* 1995; **151**: 713–718.
34. Ischiropoulos H, Mendiguren I, Fisher D, Fisher AB, Thom SR. Role of neutrophils and nitric oxide in lung alveolar injury from smoke inhalation. *Am J Respir Crit Care Med* 1994; **150**: 337–341.
35. Li X, Donaldson K, MacNee W. Nitric oxide production, alveolar macrophages and type II alveolar epithelial cells in response to LPS *in vivo* and *in vitro*. *Biochem Soc Trans* 1995; **23**: 233S.
36. Liu S, Adcock IM, Old RW, Barnes PJ, Evans TW. Lipopolysaccharide treatment *in vivo* induces widespread tissue expression of inducible nitric oxide synthase mRNA. *Biochem Biophys Res Comm* 1993; **196**: 1208–1213.
37. Xie Q, Nathan C. The high output nitric oxide pathway: role and regulation. *J Leukoc Biol* 1994; **56**: 576–582.
38. Di Rosa M, Radomski M, Carnuccio R, Moncada S. Glucocorticoids inhibit the induction of nitric oxide synthase in macrophages. *Biochem Biophys Res Comm* 1990; **172**: 1246–1252.
39. Werb Z, Foler, R, Munck A. Interaction of glucocorticoids with macrophages. Identification of glucocorticoid receptors in monocytes and macrophages. *J Exp Med* 1978; **147**: 1684–1694.
40. Cirino G, Cicala C, Sorrentino L, Ciliberto G, Arpaia G, Perretti M, Flower RJ. Anti-inflammatory actions of an N-terminal peptide from human lipocortin 1. *Br J Pharm* 1993; **108**: 573–574.
41. Perretti M, Wheller SK, Choudhury Q, Croxtall JD, Flower RJ. Selective inhibition of neutrophil function by a peptide derived from lipocortin 1 N-terminus. *Biochem Pharm* 1995; **50**: 1037–1042.
42. Ignarro LJ, Fukuto JM, Griscavage JM, Rogers NE, Byrns RE. Oxidation of nitric oxide in aqueous solution to nitrite but not nitrate: comparison with enzymatically formed nitric oxide from L-arginine. *Proc Natl Acad Sci USA* 1993; **90**: 8103–8107.
43. Gross SS, Jaffe EA, Levi R, Kilbourn RG. Cytokine-activated endothelial cells express an isotype of nitric oxide synthase which is tetrahydrobiopterin-dependent, calmodulin-independent and inhibited by arginine analogs with a rank order of potency characteristic of activated macrophages. *Biochem Biophys Res Comm* 1991; **178**: 823–829.
44. Cartwright JE, Johnstone AP, Whitley GSJ. Inhibition of nitric oxide synthase by antisense techniques: investigations of the roles of NO produced by murine macrophages. *Br J Pharm* 1997; **120**: 146–152.
45. Hall SE, Smith SF, Witherden IR, Tetley TD. Relative release of lipocrtin 1 (LC1) by alveolar type II epithelial cells (TII) and alveolar macrophages (AM). *Am J Respir Crit Care Med* 1997; **155**: A617.
46. Crapo JD, Barry BE, Foscue HA, Shelburne J. Structural and biochemical changes in rat lungs occuring during exposures to lethal and adaptive doses of oxygen. *Am Rev Resp Dis* 1980; **122**: 123–143.
47. Haies DM, Gil J, Weibel ER. Morphometric study of rat lung cells I. Numerical and dimensional characteristics of parenchymal cell population. *Am Rev Resp Dis* 1981; **123**: 533–541.
48. Crapo JD, Barry BE, Gehr P, Bachofen M, Weibel ER. Cell number and cell characteristics of the normal human lung. *Am Rev Resp Dis* 1982; **125**: 332–337.

ACKNOWLEDGEMENTS. This work was supported by the Trustees Research Committe of Charing Cross &Westminster Medical School. A.M.K. thanks the Sir Jules Thorne Charitable Trust for personal support.

Received 22 December 1997; accepted 9 January 1998