The annexin lipocortin 1 is reported to mediate some anti-inflammatory effects of glucocorticoids, but the mechanisms of this mediation are incompletely understood. The involvement of lipocortin 1 in glucocorticoid inhibition of monocyte interleukin 1β (IL-1β) release has been investigated. Treatment of peripheral blood monocytes with 2 μg/ml lipopolysaccharide potently increased IL-1β release (p = 0.001) and dexamethasone (10^{-7} M) significantly reduced both resting and stimulated IL-1β release (p = 0.009). A neutralizing monoclonal antibody to lipocortin 1 (0.5–50.0 μg/ml) was unable to inhibit this effect and recombinant lipocortin 1 (2 × 10^{-6} M) and 188aa lipocortin 1 fragment (10^{-6}–10^{-5} M) had no effect. It is concluded that lipocortin 1 is not involved in the inhibition of monocyte IL-1β release by glucocorticoids.

**Key words:** Glucocorticoid, Interleukin 1, Lipocortin 1 (annexin 1), Monocyte

**Lack of involvement of lipocortin 1 in dexamethasone suppression of IL-1 release**

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**Introduction**

Lipocortin 1 (annexin 1) is a member of the annexin family of calcium–phospholipid binding proteins.1,2 The production of lipocortin 1 is induced by glucocorticoids in a number of systems3–6, including human peripheral blood mononuclear cells after in vivo exposure to glucocorticoids.7 Lipocortin 1 has been demonstrated to have a number of anti-inflammatory actions in both in vitro and in vivo systems,8–16 but the influence of this protein on cytokine production is unknown. The anti-inflammatory activity of lipocortin 1 in vivo has yet to be fully explained in terms of specific actions.

Interleukin-1 (IL-1) is a potent pro-inflammatory cytokine which is produced in a wide range of tissues including tissue macrophages, peripheral blood monocytes, brain, synovium, lung, gut, and bone.17 It is involved in the mediation of inflammation in a diverse list of conditions including rheumatoid arthritis.18,19 The production of IL-1 in inflammatory tissue sites is under the control of regulatory and counter-regulatory systems. The major inhibitors of IL-1 production are the glucocorticoids, and it is now well established that dexamethasone inhibits the induction of monocyte IL-1 release by bacterial lipopolysaccharide (LPS) in a dose dependent fashion.20 The mechanisms of this inhibition are complex and include translational, transcriptional and post-transcriptional events.21–23 An attractive explanation for some of the anti-inflammatory actions of lipocortin 1 would be the inhibition of IL-1 release or activity, and the possibility that lipocortin 1 is involved in the suppression by glucocorticoids of IL-1β release is supported by several observations. First, glucocorticoid inhibition of IL-1 release in some in vitro settings is abrogated by cycloheximide, an inhibitor of protein synthesis.24 Secondly, nuclear run-off studies suggest that glucocorticoid inhibition of the early phases of monocyte IL-1β release may occur without effects on transcription of the IL-1β gene.22 The mechanism of transport of IL-1β from the cytoplasm to the extracellular environment is not known, but IL-1β does not appear to have a signal peptide and is not transported via the Golgi apparatus.25 Annexins, often cytoskeletal associated, have been reported in preliminary studies to be implicated in cell membrane vesicle formation, exocytosis, and secretion.26,27

The role of lipocortin 1 in the inhibition by dexamethasone of IL-1β release from peripheral blood monocytes has been investigated using recombinant lipocortin 1, a bioactive lipocortin 1 fragment, and a neutralizing antibody to lipocortin 1. It is reported that none of these agents impact on LPS induced monocyte IL-1β release, or the suppression of it by glucocorticoids, and the authors conclude that lipocortin 1 is not involved in this action of glucocorticoids.

**Materials and Methods**

**Reagents:** Cells were cultured in RPMI 1640 (Gibco, UK) supplemented with penicillin, streptomycin and l-glutamine (Gibco, UK) and with 10% heat inactivated charcoal stripped foetal calf serum (Flow, ICN Laboratories, UK). Cell washes were performed with calcium and magnesium-free
phosphate buffered saline with 0.16% glucose (PBSG). Refolded recombinant human lipocortin 1 (rhLC1) and a neutralizing mouse monoclonal antibody to human LC1 (1A) were kindly provided by Dr J. Browning (Biogen, Cambridge, MA). A bioactive N-terminal 188 amino acid fragment of lipocortin 1 (1-188aa) was kindly provided by Dr F. Carey, ICI Pharmaceuticals, Cheshire, UK. IL-1β ELISA were purchased from Cascade Biochem (Reading, UK). Dexamethasone and LPS (Escherichia coli, serotype 055:B5 lipopolysaccharide) were purchased from Sigma (St. Louis, MO).

**Monocyte separation:** Peripheral venous blood was drawn from healthy volunteers into heparinized containers and diluted 1:1 with PBSG. Mononuclear cells were separated by centrifugation on a Histopaque 1077 (Sigma, St Louis, MO) density gradient for 30 min at 400 x g, washed in PBSG, and resuspended at 5 x 10^6 cells/ml in culture medium with 10% FCS. Monocytes in this suspension were allowed to adhere to 10 cm Petri dishes (Costar, Cambridge, MA) for 60 min at 37°C and 5% CO2 in a humidified incubator. After non-adherent cells were removed by vigorous pipetting with medium, adherent cells were removed by gentle scraping with a rubber 'policeman' and washing with cold PBSG. Adherent cells were <10% CD3 positive by flow cytometric analysis.

**Cell culture:** Monocytes were cultured in 1 x 10^6 cell aliquots. Neutralizing antibody to lipocortin 1 (0.5-50 µg/ml), control antibody P3 (50 µg/ml), rhLC1 (2 x 10^{-6} M) or 1-188aa fragment (2 x 10^{-6} to 2 x 10^{-8} M) were incubated with monocytes for 2 h in 96-well plates at 37°C and 5% CO2 in a humidified incubator. After non-adherent cells were removed by vigorous pipetting with medium, adherent cells were removed by gentle scraping with a rubber 'policeman' and washing with cold PBSG. Adherent cells were <10% CD3 positive by flow cytometric analysis.

**IL-1β assay:** Culture supernatants were obtained by centrifuging plates at 400 x g for 5 min and careful aspiration. Supernatants contained <1 x 10^4 cells/ml. Supernatants were stored at -70°C until assay. IL-1β ELISA were performed according to the manufacturer’s instructions and had a sensitivity of 1 pg/ml.

**Statistical analysis:** Supernatant IL-1β levels were compared using the Wilcoxon signed ranks test, or Mann Whitney U test when the number of pairs was less than six. Values of p less than 0.05 were regarded as statistically significant.

**Results**

IL-1β was detected in the supernatants of untreated monocytes (mean 623, S.E.M. 122 pg/ml, n = 13). In all experiments, LPS 2 µg/ml induced significant increases in supernatant IL-1β concentration (mean 2188, S.E.M. 298 pg/ml, p = 0.001, n = 13). Dexamethasone potently inhibited LPS induced IL-1β release in all experiments (mean 666, S.E.M. 94 pg/ml, p = 0.009, LPS vs LPS plus dexamethasone, n = 13) (Figs 1–3). Dexamethasone 10^{-7} M also inhibited the levels of IL-1β in the supernatants of non-LPS treated monocytes (mean 291, S.E.M. 85 pg/ml, p = 0.009, dexamethasone treated vs untreated, n = 7, Fig. 1 and 2). Pretreatment of monocytes with neutralizing antibody to lipocortin 1 in doses of 0.5-50.0 µg/ml had no effect on the inhibitory action of dexamethasone 10^{-7} M on IL-1β release (Fig. 1). Pretreatment of monocytes with rhLC1 2 x 10^{-6} M had no suppressive effect on non-LPS treated monocyte IL-1β release, nor on the increase in IL-1β release induced by LPS (Fig. 2). Pretreatment...
of monocytes with the 1-188aa lipocortin 1 fragment at concentrations of 2 × 10⁻⁸ M to 2 × 10⁻⁶ M similarly had no effect on untreated or LPS treated monocyte IL-1β release (Fig. 3).

Discussion

Evidence from animal models suggests that lipocortin 1, a member of the annexin family of calcium–phospholipid binding proteins, may be a mediator of some of the anti-inflammatory actions of glucocorticoids.¹,² The production of lipocortin 1 has been shown to be induced by glucocorticoids in a number of in vitro and in vivo studies.³⁻⁷ Additionally, lipocortin 1 has been demonstrated to mimic many in vitro actions of glucocorticoids, including inhibition of natural killer cell activity and antibody dependent cell-mediated cytotoxicity, inhibition of reaction oxygen species generation, and inhibition of prostaglandin and thromboxane release.⁸⁻¹⁰ Exogenous lipocortin 1 and bioactive fragments of lipocortin 1 have, furthermore, been demonstrated to exert anti-inflammatory activity in vivo in a number of animal models of inflammation.¹¹⁻¹⁶

The results reported in this paper do not support a role for lipocortin 1 in the suppression of IL-1β release by monocytes. Lipocortin 1 may, however, be involved in the mediation of glucocorticoid inhibition of the actions of IL-1, rather than its production or release. A model for this hypothesis exists in the hypothalamo–pituitary–adrenal axis. IL-1 is produced in the pituitary, IL-1 receptors have been demonstrated in pituitary cell cultures, and circulating IL-1 is active in the pituitary where it is involved in the regulation of the hypothalamo–pituitary–adrenal axis response to inflammation.²⁸⁻⁴⁰ Lipocortin 1 has been demonstrated in the rat pituitary,³¹ and intracerebroventricular (i.c.v.) infusion of lipocortin 1 or the 1-188aa peptide fragment of lipocortin 1 is associated with a reduction in the pyrogenic response to i.c.v. IL-1,¹⁴ strongly suggesting that lipocortin 1 can directly inhibit actions of IL-1. In addition, IL-1 increases phospholipase A2 (PLA2) activity and leukocyte prostaglandin release,¹⁷,³² while lipocortin 1 reduces the production of prostaglandins via inhibition of PLA2 activity, probably by binding to its substrate.³³ In contrast, prostaglandins have been reported to inhibit the production of IL-1 by monocytes, possibly as part of an autocrine feedback network.³²,³³ Potential effects of lipocortin 1 on IL-1 release or action may be reversed by its effect on prostaglandins. These suggestions of an interaction of IL-1 and lipocortin 1 are of course conjectural, and further research on the area of annexin–cytokine interactions is needed.

In summary, lipocortin 1 is a glucocorticoid induced protein whose anti-inflammatory activity remains incompletely understood. A possible mechanism of action of lipocortin 1 is the inhibition of IL-1 release, possibly through effects on its secretion. In studies with recombinant lipocortin 1, a bioactive lipocortin 1 fragment, and neutralizing antibodies to lipocortin 1, the authors have been unable to demonstrate evidence that lipocortin 1 is involved in the suppression by glucocorticoids of the release of IL-1β by human peripheral blood monocytes.

References


ACKNOWLEDGEMENTS. E.F.M. is the recipient of a Michael Mason Fellowship. Arthritis Foundation of Australia. N.J.G. thanks the Arthritis Research Council, UK for support.

Received 24 November 1992; accepted 1 December 1992