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

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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^{-8}–10^{-6} 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

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 systems,[3–6] 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 × ***g***, washed in PBSG, and resuspended at 5×10^6 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% CO_2 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 1×10^6 cell aliquots. Neutralizing antibody to lipocortin 1 (0.5–50 μg/ml), control antibody P3 (50 μg/ml), rhLC1 (2×10^{-6} M) or 1-188aa fragment (2×10^{-6} to 2×10^{-8} M) were incubated with monocytes for 2 h in 96-well plates at 37°C and 5% CO_2 in a humidified incubator. Cells were then resuspended into 1 ml of medium in 24-well tissue culture plates (Costar, Cambridge, MA) and LPS 2 μg/ml and/or dexamethasone 10^{-7} M added. Cells were cultured for 48 h at 37°C and 5% CO_2 in a humidified incubator and viability at this time was >95%.

IL-1β assay: Culture supernatants were obtained by centrifuging plates at 400 × ***g*** for 5 min and careful aspiration. Supernatants contained $<1 \times 10^4$ 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-1$\beta$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 2×10^{-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

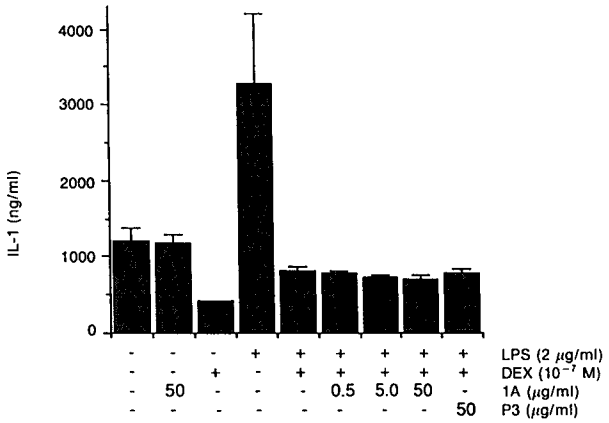

FIG. 1. Monocyte IL-1β release: effects of neutralizing antibody to lipocortin 1. Peripheral blood monocytes were cultured for 48 h in the presence of bacterial lipopolysaccharide 2 μg/ml (LPS), dexamethasone 10^{-7} M (DEX), anti-lipocortin 1 antibody 0.5–50 μg/ml (1A) and/or control antibody (P3), and the IL-1β concentration in culture supernatants measured by ELISA. LPS induced a marked increase in IL-1β release ($p = 0.001$) which was suppressed by dexamethasone ($p = 0.009$). Dexamethasone also inhibited resting (non-LPS-treated) monocyte IL-1β release ($p = 0.009$). Anti-lipocortin antibody had no effect on the ability of DEX to suppress this response.

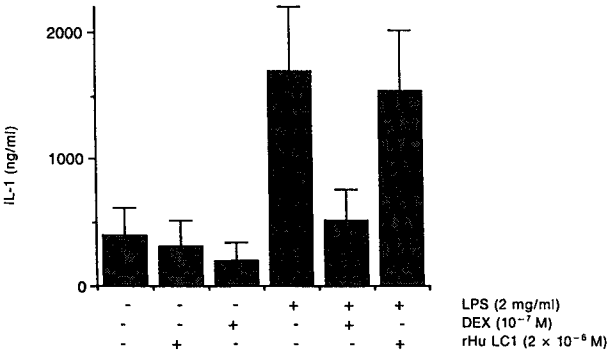

FIG. 2. Monocyte IL-1β release: effects of recombinant human lipocortin 1. Peripheral blood monocytes were cultured for 48 h in the presence of LPS 2 μg/ml, dexamethasone 10^{-7} M (DEX), and recombinant human lipocortin 1 2×10^{-6} M (rHLC-1) and the IL-1β concentration in culture supernatants measured by ELISA. rhLC-1 did not reproduce the inhibition of monocyte IL-1β release observed with dexamethasone.

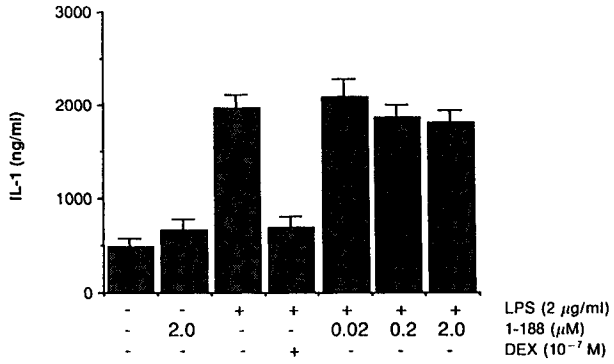

FIG. 3. Monocyte IL-1β release: effects of 188aa N-terminal fragment of lipocortin 1. Peripheral blood monocytes were cultured for 48 h in the presence of LPS 2 μg/ml, dexamethasone 10^{-7} M (DEX), and N-terminal 188 amino acid lipocortin 1 fragment 2×10^{-8}–2×10^{-6} M (1–188), and the IL-1β concentration in culture supernatants measured by ELISA. 1–188 did not reproduce the inhibition of monocyte IL-1β release observed with dexamethasone.

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.[28–30] 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.[32,34] 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.

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