CYTOKINES, GLUCOCORTICOIDS AND LIPOCORTINS IN THE CONTROL OF NEUTROPHIL MIGRATION

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SUMMARY

By using a simple murine air-pouch technique we were able to measure an intense neutrophil infiltration in response to the local application of specific pro-inflammatory stimuli such as interleukin-1 and interleukin-8. The role of endogenous and exogenous lipocortin 1 on this cellular migratory response was evaluated. Exogenously i.v. administration of human recombinant lipocortin 1 and its N-terminus peptide Ac2-26 dose-dependently inhibited IL-1-induced neutrophil migration with calculated ED$_{50}$ values of 5.18±0.74 µg and of 88.0±13.1 µg per mouse, respectively. Direct injection of these agents into the pouch was ineffective. In keeping with these observations, the inhibitory action exerted by dexamethasone, in both IL-1- and IL-8-induced cell infiltration, was abrogated by passive immunisation of mice with specific anti-lipocortin 1 antibodies. In conclusion, lipocortin 1 is not only a mediator of some of the anti-inflammatory actions of glucocorticoid hormones, but can also modulate the effect that specific cytokines have on the trafficking of highly specialised cells like the neutrophils.

KEY WORDS: lipocortin 1, interleukin-1, inflammation, migration, interleukin-8.

INTRODUCTION

The anti-inflammatory effect of glucocorticoid hormones is the overall result of a series of actions which affect the release and/or the action of pivotal mediators as well as the cell component which characterizes the acute inflammatory process. In recent years the nature of a so-called ‘second mediator’ of glucocorticoids action has been identified, cloned and termed lipocortin 1 (LC1) [1]. LC1 is induced and released from proper target cells, i.e. monocytes and macrophages, following in vivo treatment with glucocorticoid hormones in both animals and humans [2,3] and local and systemic treatment with human recombinant LC1 mimics the inhibitory property exerted by steroids in the rat carrageenan–oedema model [4,5]. The observation that local injection of an anti-LC1 polyclonal antibody partially reverses the anti-oedema effect of dexamethasone given directly into the paw [6]
substantiates the concept that LC1 mediates at least some of the anti-inflammatory effects of glucocorticoids.

Cytokines are endogenous proteins with paracrine and endocrine functions. Some of these cytokines play a pivotal role in inducing and sustaining the inflammatory process by affecting both the humoral and cellular responses. Interleukin-1 (IL-1) has a key role as a coordinator of the inflammatory and immune response [7]. Among the many actions of this cytokine, its ability to attract polymorphonuclear leukocyte (PMN) migration when injected into specific tissue sites is certainly of great importance in the development of acute inflammation. This property is likely to be primarily due to endothelial cell activation through a type I receptor with subsequent PMN sequestration and migration [8]. In contrast to IL-1, interleukin-8 (IL-8) has been described as a direct PMN chemoattractant causing in vivo accumulation of this cell type in an actinomycin-D-insensitive way [9], and indeed, IL-8 induces a classical pattern of PMN activation in vitro [10].

The object of this study has been to ascertain the effect of the glucocorticoid hormone dexamethasone (DEX) on IL-1- and IL-8-induced PMN accumulation in a murine air-pouch model and to investigate the effect of exogenous LC1 under these experimental conditions.

MATERIALS AND METHODS

Materials

Human recombinant IL-1β and IL-8 (72 aa form) were a generous gift of Dr L. Parente (IRIS, Siena, Italy) and Dr I. Lindley (Sandoz Forschuninstitut, Vienna, Austria), respectively. Re-folded human recombinant LC1 was a generous gift of Dr J. L. Browning (Biogen Research Corp., Cambridge, MA, USA). The protein was stored frozen in aliquots until required and freshly prepared solutions in phosphate-buffered solution (PBS) were used in each experiment. The LC1 N-terminus peptide, peptide Ac2-26 (N-acetyl-AMVSEFLQAWFIENEE QEYVQTVK), was purchased from Bachem, Saffron Walden, Essex, UK, while Dexamethasone (DEX, sodium salt) from David Ball Laboratories, Warwick, UK. Anti-LC1 monoclonal antibodies 1A and 1B were from Biogen Research Corp. whereas the sheep anti-LC1 polyclonal serum was prepared in house. All other reagents were from Sigma, Poole, Dorset, UK.

Mouse air-pouch model

Male Swiss Albino mice (24–26 g) received a dorsal s.c. injection of 2.5 ml air on day 0 and day 3. Six days after the first injection of air, animals received either IL-1β (20 ng) or IL-8 (1 μg) in a volume of 0.5 ml of carboxymethylcellulose (CMC) 0.5% in PBS directly into the pouch. Control mice received the vehicle only. Four hours after challenge with the cytokines, air-pouches were thoroughly washed with 2 ml PBS containing 50 U ml⁻¹ heparin. Total and differential cell counting was performed following staining in Turk’s solution using an improved Neubauer haemocytometer. The number of PMN recovered from each animal was then calculated.
Drug treatment

DEX was given i.v. 2 h prior to local challenge with the cytokine. Human recombinant LC1 and its N-terminus peptide Ac2-26 were given i.v. 20 min before the cytokines. In all cases the 4 h PMN accumulation was evaluated. In a separate set of experiments, mice were passively immunised with the anti-LC1 antibodies by s.c. injection of either 50 μl serum or 100 μg monoclonal antibodies. DEX was then given i.v. 24 h after the immunisation, and the cytokines applied locally 2 h later. Cell infiltration at 4 h was measured as described above. The pretreatment time of 24 h with the antibodies was chosen on the basis of preliminary experiments. In a separate set of experiments, DEX, LC1 and peptide Ac2-26 were given locally concomitantly with IL-1β and PMN migration evaluated 4 h later as described above.

Statistics

Statistical differences between the numbers of PMN migrating were assessed by an analysis of variance followed by the Bonferroni test for intergroup comparison. Values of probability less than 0.05 were taken as significant.

RESULTS

Direct injection of either 20 ng IL-1β or 1 μg IL-8 greatly increased the number of PMN that accumulated in the air-pouch compared to the modest infiltration caused by the vehicle, CMC, alone: 1.40±0.14×10⁶ per mouse for CMC, n=40; 7.74±0.28×10⁶ per mouse, n=88, P<0.01 for IL-1β and 5.84±0.28×10⁶ per mouse, n=70.

Fig. 1. Dose-dependent inhibition of IL-1-induced PMN accumulation into the mouse air-pouch by systemic treatment with dexamethasone. Mice received either PBS or dexamethasone 2 h prior to the local challenge with IL-1β (20 ng in 0.5 ml CMC) PMN infiltration into the air-pouches was assessed 4 h later. Results are mean ± st of 6–12 mice per group. The dotted line represents the migration obtained with CMC alone. *P<0.01 vs control group.
Intravenous treatment with DEX resulted in a dose-dependent inhibition of IL-1β-induced PMN accumulation with as reported in Fig. 1. The glucocorticoid hormone had an identical effect on IL-8-induced PMN migration with an approximate ED50 of 5 μg per mouse (~0.15 mg kg⁻¹ i.v.) and a maximal inhibition of ~90% at 50 μg dose. Human recombinant LC1 and its N-terminus peptide Ac2-26 mimicked the steroid action with a dose-dependent inhibition of IL-1β-induced PMN migration reaching a maximal effect of 80–90%. When given i.v. 20 min prior to IL-1β the ED50 values were 5.18±0.74 μg for LC1 and of 88.0±13.1 μg for peptide Ac2-26. The peptide was also tested on IL-8-induced PMN migration giving 46% inhibition (P<0.05) at a 700-μg i.v. dose.

Passive immunisation of mice with specific anti-LC1 antibodies did not modify cell migration in response to the cytokine alone. However, treatment of animals with the anti-LC1 sheep polyclonal serum or with the specific monoclonal antibody 1B prevented the inhibitory action of DEX tested at its ED50 dose of 5 μg per mouse (Fig. 2). Control non-immune serum and IgG were ineffective. Similar results were obtained with IL-8-induced PMN migration where passive immunisation of mice with the anti-LC1 sheep serum prevented the inhibitory action of DEX (5 μg i.v.) (not shown).

Different data were obtained with the effect of locally injected agents was evaluated. DEX, at a dose which was ineffective when given systemically (1 μg at time 0), significantly reduced IL-1-induced 4-h PMN migration. However, both LC1 and peptide Ac2-26 were inactive (Fig. 3). Consistent with this observation were the data obtained with the passive immunisation protocol in that local DEX inhibition was not prevented by the anti-LC1 antibodies (not shown) thus excluding a role for LC1 in the local action of the steroids in this particular model.

**DISCUSSION**

Two main conclusions may be drawn from the present study: (i) cytokine-induced PMN migration is exquisitely sensitive to the hormone DEX and the acute inhibitory action observed following systemic treatment with the steroid is LC1-mediated; (ii) the PMN seems an optimal target cell for LC1 and peptide Ac2-26.

Both IL-1β and IL-8 induced a consistent and intense PMN accumulation in the mouse air-pouch. The glucocorticoid hormone DEX was a potent inhibitor of this migration with an approximate ED50 of 0.15 mg kg⁻¹ i.v. This effect was clearly LC1 dependent as assessed with the passive immunisation experiments where specific anti-LC1 antibodies abrogated the inhibition exerted by systemic DEX. Moreover, i.v. treatment with LC1 and the active fragment Ac2-26 mimicked the action of the steroid. It is important to highlight the observation that LC1 was extremely active in reducing PMN infiltration being almost 70 times more potent than DEX on a molar basis (approximate ED50 of 0.14 nmol and 9 nmol for LC1 and DEX, respectively). In this context, the efficacy of the N-terminus peptide acquires particular importance since its ED50 was around 30 nmol per mouse, not much less than the ED50 of DEX. Some other mechanism(s) apart from LC1 may underline DEX inhibition following local treatment in the pouch, since anti-LC1 antibodies did not alter steroid effectiveness and, consistently, both LC1 and
Fig. 2. Effect of local treatment with dexamethasone (DEX) lipocortin 1 (LC1) and its N-terminus peptide Ac2-26 on IL-1β-induced PMN accumulation into the mouse air-pouch. The different agents were injected directly into the air-pouches on the same time as IL-1β (20 ng in 0.5 ml CMC). PMN migration was evaluated at 4 h time-point. Values are mean ± se of n=7–10 mice per group. *P<0.01 vs control group (IL-1β alone).

peptide Ac2-26 were inactive when given locally. IL-1-induced PMN migration is an actinomycin D sensitive phenomenon [11]: interference with adhesion molecules expression and/or release of chemotactic agents such as a murine IL-8 analogue or PAF may be responsible for the effect of local DEX.
Human recombinant LC1 was originally reported to possess anti-inflammatory activity in vivo following both local and systemic treatment in the rat carrageenin–oedema model [4,5]. We have now demonstrated that this protein can potently affect cell migration elicited by proinflammatory cytokines. This effect was evident only following i.v. treatment whereas LC1 was inactive when given directly in the pouch: this suggests that LC1 has to interact with the neutrophils in order to exert its effect. The recent description of a specific and saturable LC1 binding to human and murine PMN strengthens this possibility [12,13]. Moreover, PMN taken from an inflammatory site lose LC1 binding capacity [13]; this may be a mechanism for allowing them to escape from the inhibitory action of this protein.

The investigation of LC1 pharmacology has long been partially hampered by the problems of stability reviewed by Browning et al. [14]. Because of this the possibility of finding the pharmacophore(s) site(s) within the protein has been studied. The first successful attempt was described a few years ago [15], however the effectiveness of the identified nonapeptide has been differently reported (reviewed in [16]). We have focused upon the N-terminus region which is unique to each member of the LC family and which has therefore been proposed to account for the selectivity of action of the different members of the family [17]. Indeed, peptide Ac2-26 fully mimicked LC1 action being effective after i.v. treatment and not when given locally. This peptide also showed inhibitory property in two models of rat paw oedema [18]. From all these data it appears that peptide Ac2-26 can be considered a LC1 substitute with powerful action on PMN functions.

In conclusion, this investigation describes endogenous and exogenous LC1 as a powerful functional antagonist of the PMN elicitation induced by proinflammatory cytokines. This effect may be brought about by an interaction with this specific cell type which may consequently be regarded as a major target for this anti-inflammatory protein.

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REFERENCES


