LIPOCORTIN 1 (Lcl) is an anti-inflammatory protein, which, given systemically, inhibits polymorphonuclear neutrophil (PMN) emigration from the circulation to sites of inflammation; delivery of Lcl to the inflamed site is ineffective. We have examined the effect of Lcl on changes in PMN deformability, and observed a consistent improvement in the deformability of unstimulated PMN; N-formyl-methionyl-leucyl-phenylalanine (fMLP)-activated cell deformability was unaltered. A Lcl-induced increase in cell deformability may reduce PMN sequestration so contributing to the anti-migratory effects of systemic Lcl previously demonstrated in vivo.

**Key words:** Deformability, Lipocortin 1, Polymorphonuclear neutrophil

---

**Introduction**

Glucocorticoids are powerful anti-inflammatory drugs capable of modulating both cellular and soluble components of the inflammatory response. In this investigation we have studied an anti-inflammatory, glucocorticoid-inducible protein, lipocortin 1 (Lcl, annexin 1), which mediates a number of glucocorticoid actions including inhibition of PMN emigration to sites of inflammation. The mechanism of this action has not been fully elucidated, but could include effects on PMN rolling adhesion, expression of adhesion molecules, or diapedesis, since, to reach a site of inflammation, circulating PMN must slow down, adhere to the endothelium and migrate between the endothelial cells.

Most previous studies suggest that glucocorticoids and Lcl do not directly alter expression of PMN or endothelial cell adhesion molecules, an exception being Cronstein and colleagues who demonstrated that glucocorticoids diminish ELAM-1 and ICAM-1 expression on LPS-stimulated endothelium, although these changes appear to be both time and stimulus-dependent.

Although the details of extravasation are thought to vary between the systemic and pulmonary circulations, a reduction in cell deformability can prolong the transit time of PMN through the microvasculature, thus increasing opportunities for selectin-mediated rolling adhesion of PMN to endothelial cells, this being a prerequisite under shear conditions for the leucocyte firm (CD11/CD18-mediated) adhesion which is required for PMN emigration. Therefore, the aim of the present study was to investigate the effect of Lcl on PMN deformability.

**The effect of lipocortin 1 on neutrophil deformability**

E. M. Drost, W. MacNee and S. F. Smith

1Department of Medicine, Rayne Laboratory, University of Edinburgh, Medical School, Teviot Place, Edinburgh EH8 9AG, UK; and 2Department of Medicine, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, UK

---

**Materials and Methods**

Recombinant human Lcl (rhLcl) was the generous gift of Dr J. L Browning, Biogen Inc., Cambridge, MA. PMN populations (n = 9; > 96.9 ± 2.2% pure) were harvested from peripheral venous blood as described previously. Cell suspensions were pre-incubated with rhLcl dissolved in PBS containing 1 mg human serum albumin/ml for 30 min at 37°C at a concentration of 2 μg/10⁶ cells/ml after which the cells remained > 98% viable. Lcl binds to human peripheral blood leukocytes in a dose-dependent manner; the cell surface binding sites on PMN are approximately 80% saturated at the concentration of Lcl and the temperature used in the present study. Biologically inactive rhLcl was used as a control.

Cell deformability was assessed in the presence and absence of 10⁻⁷ M N-formyl-methionyl-leucyl-phenylalanine (fMLP), by filtration of PMN suspensions (1 x 10⁵/ml PBS containing 5% albumin) at constant flow through a Nucleopore polycarbonate membrane (5 μm diameter pores). The more rigid the cells, the longer they take to enter and pass through the pores resulting in a higher pressure. Hence, the pressure developed by a cell suspension over 6 min filtration compared to the pressure of PBS alone was measured as an indicator of cell deformability.

**Statistics:** Differences between treatments were assessed using a one-way analysis of variance
Effect of lipocortin 1 on neutrophil deformability

**Results**

Following pre-incubation with functional rhLC1, passive PMN from every subject (n = 9) showed increased deformability compared to control cells at each time point examined up to 6 min filtration (Fig. 1). However, no change in PMN deformability was observed in the presence of biologically inactive rhLC1 (6 min pressure; control, 3.09 ± 0.68 cm H2O; inactive rhLC1, 3.00 ± 0.24 cm H2O; n = 5; mean ± S.E.M.; p > 0.05 ANOVA).

When PMN were stimulated with fMLP (6 min pressure; fMLP, 8.03 ± 1.33 cm H2O) their deformability was significantly reduced compared to unstimulated cells (6 min pressure; control, 3.09 ± 0.68 cm H2O). Neither pre-incubation with functional nor inactive rhLC1 had a consistent or significant effect on PMN deformability in the presence of fMLP (6 min pressure; fMLP + rhLC1, 8.49 ± 1.0 cm H2O; fMLP + inactive rhLC1, 10.12 ± 0.7 cm H2O; n = 5; p > 0.05 ANOVA).

Examination of PMN by light microscopy confirmed that no cell aggregation occurred with any treatment regimen, and thus the pressures generated were indeed a reflection of cell deformability.

**Discussion**

In this study we observed that incubation of passive PMN with rhLC1 *in vitro* resulted in a consistent increase in cell deformability, which occurred only when the protein was in its correct, three-dimensional conformation. The dimensions of the microvasculature impose a restraint on PMN passage, particularly in the pulmonary capillaries.7,10 The disparity between the diameters of circulating PMN (6–8 μm) and the systemic (6 μm) or pulmonary (5 μm) capillaries require that the cells must deform during capillary transit (reviewed in Reference 10). Thus, the more deformable the cell, the more rapid the transit through the capillary beds, the briefer the contact with the endothelium and the fewer the opportunities for the PMN to adhere prior to migrating out of the circulation. Hence, a LC1-induced increase in cell deformability may decrease sequestration of PMN in the capillary bed; this mechanism could contribute to the reduction by intravenous LC1 of experimentally induced neutropenia in vivo.11

Interestingly, when PMN were activated with fMLP, LC1 had no effect on cell deformability.

This is consistent with observations *in vivo* which have shown that whilst systemic LC1 reduces the migration of (passive) circulating neutrophils, LC1 administered directly to a site of inflammation has no effect on (activated) migrated PMN.11

How LC1 causes the observed change in the mechanical properties of PMN is currently unknown. PMN deformability is determined mainly by the intracellular levels, conformation and distribution of filamentous (F) actin. LC1 can bind F-actin12 and thus might directly modify the conformation and/or distribution of this cytoskeletal protein. However, the effect of LC1 on PMN deformability may potentially be mediated by changes in signal transduction since studies on A549 cells demonstrate that LC1 can inhibit G-protein-mediated activation of arachidonic acid release.13 However, as LC1 treatment did not affect the deformability of activated PMN in our study, this mechanism seems less likely to explain the observation reported here. We plan to investigate the effect of LC1 on F-actin in PMN more fully.

**References**


ACKNOWLEDGEMENTS. We thank Dr T. Tetley for helpful comments on the manuscript. A Wellcome Travel Grant made to S.F.S. facilitated the writing up of the study.

Received 12 December 1995; accepted 22 February 1996
Submit your manuscripts at http://www.hindawi.com