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SELECTIVE INHIBITION OF NEUTROPHIL FUNCTION BY A PEPTIDE DERIVED FROM LIPOCORTIN 1 N-TERMINUS

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Abstract—A multi-faceted approach was used to investigate the effect of an anti-inflammatory peptide derived from human lipocortin 1 N-terminus region (amino acid 2-26; termed human Ac2-26) on human neutrophil activation *in vitro*. When incubated with purified human neutrophils. human Ac2-26 produced a concentration-dependent inhibition of elastase release stimulated by formyl-Met-Leu-Phe (fMLP), platelet-activating factor, or leukotriene B_4, with an approximate EC_{50} of 33 μM (100 μg/ml). At this concentration, human Ac2-26 also inhibited (77%) the release of [^{3}H]-arachidonic acid from neutrophils stimulated with fMLP. The peptide, however, did not inhibit the up-regulation of the β_2-integrin CD11b and the concomitant shedding of L-selectin from neutrophil plasma membrane induced by fMLP. In adhesion experiments, human Ac2-26 inhibited neutrophil adhesion to endothelial monolayers when this was stimulated with fMLP, but not when this followed endothelial cell activation with histamine or platelet-activating factor. Again, the effect of the peptide was concentration-dependent, and an approximate EC_{50} of 33 μM was calculated. When a preparation of ^{125}I-labeled human Ac2-26 was incubated with the neutrophils, the peptide was internalised in an energy-dependent fashion. All together, these observations lead us to propose a model in which this peptide derived from the N-terminus of human lipocortin 1 alters a common cellular mechanism producing a selective inhibition of neutrophil activation.

Key words: arachidonic acid; elastase; cell adhesion; adhesion molecules; F-actin; inflammation

Several of the anti-inflammatory effects that glucocorticoid hormones exert when administered *in vivo* are mediated by an endogenous protein, lipocortin 1 (LC1)[2] [1]. Structurally, LC1 belongs to a family of calcium- and phospholipid-binding proteins, termed either lipocortins or annexins, characterized by the presence of homologous repeats of 70–80 amino acids that form the core of the protein [2, 3]. Connected to this 4- or 8-repeat core is an N-terminus of differing length that is unique to each member of the family and, in the case of LC1, is 33 amino acids long [1]. The N-terminus region of LC1 plays an important modulatory role in the biological effects of the protein. For example, it governs the interaction of the protein with biological membranes [4, 5] and the aggregation of phospholipid vesicles [5].

We have recently investigated the biological activity of a peptide derived from human LC1 N-terminus. This 24-amino acid peptide represents the majority of the LC1 N-terminus residues, and it is hereafter referred to as human Ac2-26. Following local administration into a rat paw, human Ac2-26 mimicked the anti-inflammatory action of the parent protein [6]. It was subsequently found to inhibit potently cell recruitment into an inflammatory site in response to several stimuli: human Ac2-26 reduced polymorphonuclear leukocyte (PMN) accumulation into a 6-day-old murine air-pouch elicited by interleukin-1 [6], interleukin-8 [7], and substance P [8].

The present study was undertaken to investigate the effect of peptide human Ac2-26 on several parameters of PMN activation *in vitro* with the dual aim of (a) extending our knowledge of the biological activities of this LC1 mimetic, and (b) discovering a mechanism of action that could underlie the anti-inflammatory effects observed following administration of human Ac2-26 *in vivo*.

MATERIALS AND METHODS

Preparation of human peripheral blood PMN

PMN were prepared by single-step centrifugation of diluted blood (1:1 in sterile PBS) collected from healthy volunteers, 6 mL of which were layered over 3 mL histopaque 1077 (Sigma Chem. Co., Poole, U.K.) and 3 mL histopaque 1119 (Sigma). After centrifugation at 400 g for 30 min at room temperature, the bottom layer was harvested and washed twice in sterile PBS. An enriched population of PMN (≥95% pure) was obtained following removal of erythrocytes by hypotonic lysis (10 sec in cold water). Cells were finally washed twice in RPMI 1640 (Sigma) prior to further use. Cell number was adjusted following staining in Turk's solution and counting in a Neubauer haematocytometer.

Effect of peptide human Ac2-26 on PMN activation

fMLPL binding. FMLP binding to PMN was measured with the probe FITC-conjugated fMLPL (Penin-

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Abbreviations: LC1, lipocortin 1; DNP, di-nitrophenol; 2DG, 2-deoxy-glucose; NBD, 7-nitrobenz-2-2-oxa-1,3-diazole; PLA_2, phospholipase A_2; FACS, fluorescence-activated cell sorter; MFI, mean fluorescence intensity; fMLP, formyl-Met-Leu-Phe; fMLPL, formyl-Met-Leu-Phe-Leu; AA, arachidonic acid; PAF, platelet-activating factor; LTB_4, leukotriene B_4; PMN, polymorphonuclear leukocyte; HBSS, Hank's balanced salt solution; FCS, foetal calf serum; DMEM, Dulbecco's modified Eagle medium.

sula Laboratories, St. Helens, Merseyside, U.K.) [9]. PMN ($0.5–1 \times 10^6$) were incubated on ice for 5 min with or without peptide prior to the addition of FITC-fMLPL. After 1 hr, cells were washed and fixed in an equal volume of 2% paraformaldehyde in PBS. Flow cytometry analysis was performed using a FACScan II analyser (Becton Dickinson, Mountain View, CA) with air-cooled 100 mW argon ion laser tuned to 488 nm and Consort 32 computer running Lysis II software. Data are reported as units of fluorescence (mean fluorescence intensity, MFI) measured in the FL1 channel.

Release of elastase activity. $1–3 \times 10^6$ PMN were incubated in the presence of 5 μg/mL cytochalasin B (Sigma) with or without different concentrations on human Ac2-26 for 15 min at 37°C prior to challenge with specific activators, such as LTB_4 (30 nM), PAF (1 μM), or fMLP (30 nM) (all from Sigma). After 15 min, the reaction was stopped in ice. The elastase activity (EC 3.4.21.37) in the supernatant was quantified as recently described [10]. Aliquots (50 μL) were added to 50 μL of elastase substrate, 1 mM methoxy-Suc-Ala-Ala-Pro-Val-*p*-nitroaniline (1 mM; Sigma) in Trizma buffer at pH 8.0. Absorbance at 405 nm was then measured, and the amount of p-nitroaniline released calculated from a standard curve (0–50 nmoL *p*-nitroaniline). Results are expressed as *p*-nitroaniline formed in nmol/min/10^6 PMN. In control experiments, human Ac2-26 was added to the PMN supernatant stimulation with fMLP, and elastase reaction carried out as described above. Cell viability was preserved in these conditions (≥99% viable cells).

Adhesion to endothelial monolayers. E.A.hy926 cells, a generous gift of Dr. C.-J. Edgell [11], were grown to form monolayers in 96-well plates.

To study β_2-integrin-mediated adhesion, 50 μL of peptide solution or adhesion medium (HBSS + 0.2% BSA + 1.3 mM Ca^{2+} and Mg^{2+}) were added to each well together with 50 μL of freshly prepared PMN (10^5). After 15 min at 37°C, fMLP was added (0.1 μM) and incubation carried out for a further 30 min. At the end of the incubation period, non-adherent cells were washed off, and the extent of the cell adhesion measured using the myeloperoxidase assay. Cells were solubilised with 100 μL 1% triton X-100; then hydrogen peroxide 0.5% (25 μL) and ortho-dianisidine 4.7 mM (25 μL) were added for 30 min. The reaction was stopped with sodium azide 0.4%, and absorbance read at 450 nm. The percentage adhesion was then calculated in comparison to the absorbance measured in samples containing the total number of neutrophils added in each well. The role of β_2-integrin in these conditions was confirmed by the effect of a specific anti-human CD11b mAb (clone 44; Serotec, Oxford, U.K.), which abrogated PMN adhesion.

To study P-selectin-mediated adhesion, different experimental conditions were employed. Human Ac2-26 (100 μg/mL) was added to monolayers of EA.hy926 cells prior to stimulation with PAF (3–10 μM) or histamine (100 μM) for 15 min at 37°C. After washing in cold HBSS, plates were put on ice, and PMN (10^5 in 100 μL of adhesion medium) added for a further 30 min. At the end of the incubation, non-adherent cells were removed by gentle washing, and the extent of PMN adhesion quantified as described above.

CD11b up-regulation and L-selectin shedding. PMN (6×10^6/mL) were incubated with or without human Ac2-26 (100 μg/mL) for 15 min prior to addition of fMLP (0.1 μM) or PAF (1 μM). After 30 min at 37°C, cell aliquots (100 μL) were plated in 96-well plates before addition of 20 μL of human IgG (15 mg/mL) and 10 μL of a specific mAb anti-human CD11b (clone 44, Serotec) or 10 μL of mAb anti-human L-selectin (clone FMC46; Serotec). After 1 hr at 4°C, cells were washed and stained with a $F(ab')_2$ fragment of goat anti-mouse IgG conjugated to FITC (Sigma). Flow cytometric analysis was then performed as described above, and the number of mAb molecules bound per cell obtained by converting the MFI units with reference to microbeads labelled with standard molecules of FITC (Flow Cytometry Standards Corp., NC) [12].

Arachidonic acid release. 30×10^6 PMN were incubated in 60 ml DMEM/F-12 with 10–20 μCi of [^{3}H]-arachidonic acid (5,6,8,9,11,12,14,15-[^{3}H]-AA; specific activity of 100 Ci/mol; lot 2360-235; DuPont de Nemours, NEN Division, Dreiech, Germany) for 2 hr. After extensive washes, the labelled PMN (~90% incorporation of [^{3}H]-AA) were dispensed in 0.4 mL aliquots and incubated with or without human Ac2-26 for 30 min prior to addition of fMLP at a final concentration of 0.1 μM. After a further 30 min, cells were centrifuged, and 0.4 mL of medium was removed from each well for scintillation counting. Data are reported as net fmol of [^{3}H]-AA released, calculated from the d.p.m. of radioactivity released less basal release measured in the absence of fMLP.

F-actin polymerization. F-actin polymerization in activated neutrophils was measured by a single-step technique using 7-nitrobenz-2-oxa-1,3-diazole (NBD)-phallacidin as recently described [13]. Neutrophils (5×10^6/mL) were incubated with human Ac2-26 (100 μg/mL) prior to addition of 0.1 μM fMLP. Aliquots (100 μL) were collected before human Ac2-26 addition, after incubation with the peptide and 30 sec, 60 sec, and 5 min

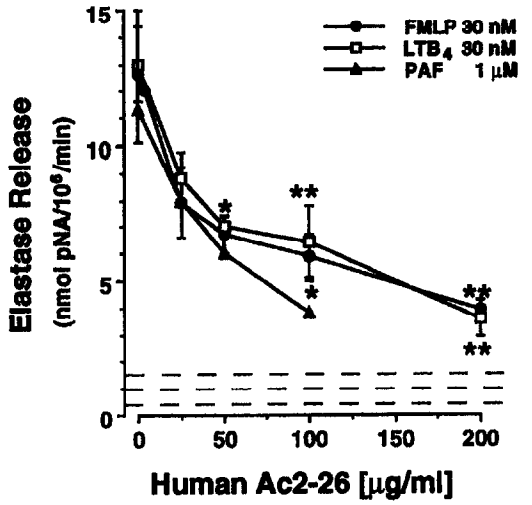

Fig. 1. Human Ac2-26 inhibited elastase release from activated PMN. Human PMN were incubated for 15 min with or without human Ac2-26 prior to stimulation with cell activators for a further 15 min. Elastase activity in the supernatants was measured as release of *p*-nitroaniline (pNA). Values are a mean ± *SEM* of 4 experiments for fMLP and LTB_4, and one experiment with PAF, performed in triplicate. Dashed lines indicate unstimulated release (mean ± *SEM*, $n = 6$). $^*P < 0.05$; $^{**}P < 0.01$ vs dose 0 values.

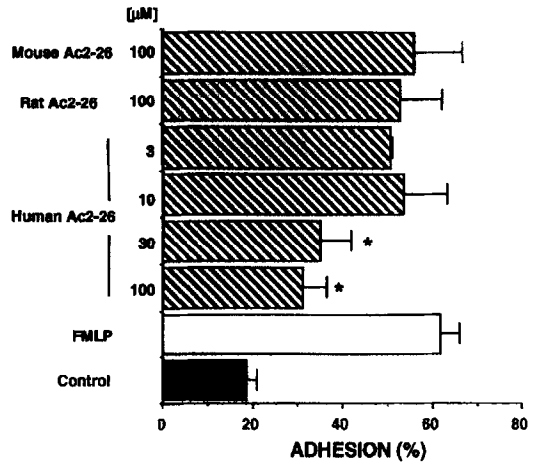

Fig. 2. Human Ac2-26 inhibited fMLP-induced PMN adhesion to endothelial monolayers. 1×10^5 PMN were added to monolayers of EA.hy926 cells in 96-well plates incubated with or without different concentrations of human Ac2-26, or mouse and rat Ac2-26, for 15 min prior to addition of fMLP 0.1 μM. After 30 min at 37°C, adhesion was quantified by measuring myeloperoxidase activity in each well and compared to the total activity measured with 1×10^5 PMN. Results are a mean ± *SEM* of 4 experiments performed in quadruplicate. $*P < 0.05$ vs fMLP control group (open bar). Unstimulated adhesion (closed bar) was measured in the absence of fMLP and peptides.

after stimulation with fMLP, and added to 100 μL of PBS containing 8% paraformaldehyde, 0.2 mg/mL lysophosphatidylcholine (Sigma), and 0.3 μM NBD-phallacidin (Molecular Probes Inc., Eugene, OR). Fluorescence was analysed by flow cytometry and measured in the FL1 channel.

Human ^{125}I-Ac2-26 internalisation

Human Ac2-26 (1 mg) was iodinated for 5 min in the presence of IODO BEADS (Pierce and Warriner, Chester, U.K.) using 1 mCi ^{125}I (Amersham Interna-

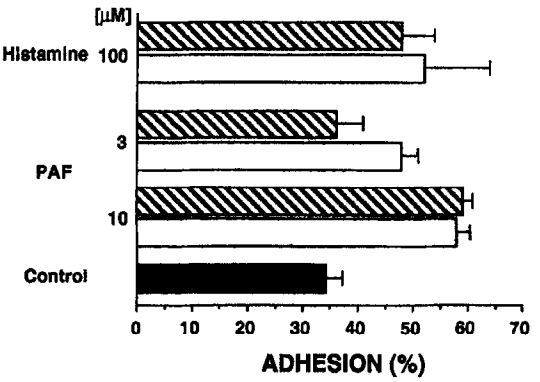

Fig. 3. Human Ac2-26 did not inhibit PMN adhesion to activated endothelial monolayers. Different concentrations of PAF or histamine, in the absence (open bars) or presence of human Ac2-26 100 μg/mL (hatched bars), were added to monolayers of EA.hy926 cells for 15 min at 37°C. After a wash, 1×10^5 PMN per well were added and incubated for a further 30 min. The % adhesion was measured as described in the legend of Fig. 2. Data are mean ± *SEM* of three experiments performed in quadruplicate. The closed bar indicates unstimulated adhesion.

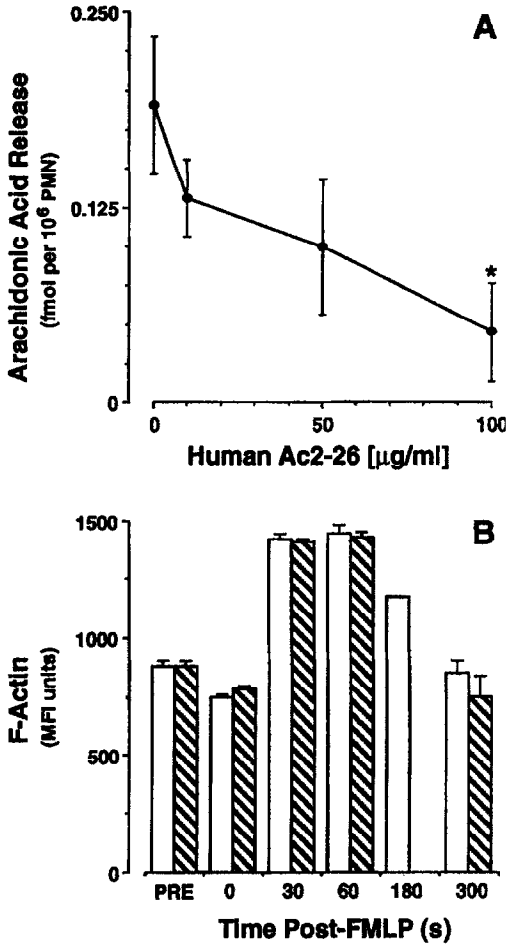

Fig. 4. (A) Human Ac2-26 inhibited [^{3}H]-arachidonic acid ([^{3}H]-AA) release from fMLP stimulated PMN. Data (mean ± *SEM* of three experiments performed in quadruplicate) are expressed as fmol of [^{3}H]-AA released in the supernatant per 10^6 PMN upon fMLP stimulation – basal release. Ac2-26 did not affect basal release (not shown). $*P < 0.05$ vs fMLP alone (dose 0 group). (B) Human Ac2-26 did not inhibit F-actin formation in stimulated PMN. Cells were incubated in the absence (open columns) or presence (hatched columns) of human Ac2-26 (100 μg/mL), then stimulated with fMLP (0.1 μM) at time 0. Aliquots (0.5×10^6) taken 30 min prior to (PRE), at time 0 before fMLP addition and at several times after fMLP, were stained with NBD-phallacidin. Data (mean ± *SEM* of three experiments performed in triplicate) are reported as Mean Fluorescence Intensity (MFI) units. fMLP caused a significant ($P < 0.05$) actin polymerization at 30 sec, 60 sec, and 180 sec time-points, with or without human Ac2-26.

tional, Little Chalfont, Buckinghamshire, U.K.) in a total reaction volume of 200 μL PBS. The labelled peptide (specific activity ~16 ± 3 mCi/μmoL, M ± *SEM* of three distinct iodinations) was then separated from free iodine on a Sephadex G-10 (Sigma) mini-column (Biorad, Hemel Hempstead, Herts, U.K.). 3×10^6 human PMN were incubated in 1 mL DMEM/F-12 (Sigma), and ^{125}I-Ac2-26 added to a total activity of 100 μCi/mL (7.0 ± 1.5 nmol per well). The cultures were incubated at either 4°C or 37°C, with or without the metabolic inhibitors di-nitrophenol (1 mM, DNP, Sigma) and 2-deoxy-glucose (45 mM, 2DG, Sigma), and the cells harvested at 30-min, 4-hr, and 24-hr intervals. PMN were then

Table 1. Lack of effect of peptide human Ac2-26 on CD11b up-regulation and L-selectin shedding in human PMN *in vitro*

Human AC2-26 (100 μg/mL)	fMLP 0.1 μM	No. of mAb molecules bound per cell	Δ (%)	No. of exps.
CD11b				
–	–	99964 ± 8319	—	10
–	+	140142 ± 9657	+41	8
+	–	96364 ± 10138	—	4
+	+	137152 ± 10917	+42	4
L-selectin				
–	–	7910 ± 571	—	3
–	+	2284 ± 490	–71	3
+	–	9094 ± 1561	—	3
+	+	1814 ± 334	–80	3

PMN were incubated with or without human Ac2-26 for 15 min at 37°C prior to stimulation with fMLP for further 30 min. The number of molecules for each antigen was calculated following staining of cells with mAb 44 (for anti-CD11b) and mAb FMC46 (for L-selectin) and flow cytometric analysis. Values are mean ± SEM of *n* experiments. The effect of fMLP both on CD11b up-regulation and L-selectin shedding was significant ($P < 0.01$) irrespective of the presence of the peptide.

washed in 0.5 ml PBS containing 1 mM EDTA to recover cell-surface bound peptide, and then lysed in 0.5 mL PBS, 10 mM EDTA, 1% Triton X-100 to recover peptide taken up by the cells. Cell extracts were then subjected to SDS-PAGE with 12% acrylamide, followed by autoradiography using standard procedures.

Peptides

Several separate batches of human Ac2-26 (acetyl-AMVSEFLKQAWFIENEEQEYVQTVK; 3 kDa) were used. They were obtained either from Bachem (Saffron Walden, Essex, U.K.) or were generously supplied by Dr. M. Toda (ONO Pharmaceutical Co., Osaka, Japan). In the latter case, two batches of human Ac2-26 were accompanied by two peptides corresponding to amino acid 2-26 of murine or rat LC1 sequence, acetyl-AMVSEFLKQARFLENQEQEYVQAVK and acetyl-AMVSEFLKQACYIEKQEQEYVQAVK, respectively. In all cases, peptide preparations were more than 95% pure as analysed by HLPC. Amino acid composition and Mw were confirmed by mass spectrometry. Peptide solutions were always made fresh in sterile media supplemented with 0.1% or 0.2% low endotoxin BSA (Sigma).

Statistics

Values are expressed as mean ± *SEM* of *n* experiments. Statistical differences were analysed by ANOVA followed by Bonferroni test for inter-group comparisons [14], or by Student's *t*-test when only two experimental groups were compared. A *P* value ≤ 0.05 was taken as significant.

RESULTS

When neutrophils were incubated with human Ac2-26 prior to subsequent stimulation, a concentration-dependent inhibition of the release of elastase activity was observed (Fig. 1). The peptide was active against fMLP and LTB_4 to a similar extent, and an approximate EC_{50} of 100 μg/mL (33 μM) was calculated, with a maximal inhibition of 59% and 65%, respectively ($n = 3$–4 experiments). In one experiment, human Ac2-26 also showed a potent inhibitory effect upon PAF-induced elastase release (Fig. 1). The potential interference of human Ac2-26 with the enzymatic reaction itself was assessed by adding the peptide to the PMN supernatant after cell stimulation with fMLP had occurred; the detection of elastase activity was marginally but not significantly affected by Ac2-26, with no inhibition at 50 μg/mL, 15% inhibition at 100 μg/mL, and 5% inhibition at 200 μg/mL ($n = 2$–3 experiments performed in triplicate).

Addition of FITC-fMLPL to PMN resulted in a concentration-dependent binding, maximal at 1 μM (125 MFI units), and still above autofluorescence at 1 nM (64 vs 32 MFI units). PMN incubation with human Ac2-26 (100 μg/mL) did not modify the binding measured either with 1 μM or 0.1 μM fMLP: 83 vs 96 MFI units and 62 vs 63 MFI units, in the absence or presence of the peptide, respectively.

When PMN were incubated with human Ac2-26, a concentration-dependent inhibition of fMLP-induced adhesion to endothelial monolayers was observed (Fig. 2), again with an approximate EC_{50} of 100 μg/mL (33 μM). Human Ac2-26 was still effective at 30 μg/mL. Cell incubation with mouse Ac2-26 (100 μg/mL) or rat Ac2-26 (100 μg/mL) did not significantly modify fMLP effect (Fig. 2). EA.hy926 cell stimulation with PAF or histamine evoked a dose-dependent adhesion of PMN. Addition of human Ac2-26 (100 μg/mL) together with PAF or histamine did not modify subsequent PMN adhesion to any extent (Fig. 3).

Incubation of PMN with human Ac2-26 (100 μg/mL) did not alter either fMLP up-regulation of the CD11b antigen of the surface of PMN or L-selectin shedding (Table 1). Human Ac2-26 alone had no effect on the basal expression of these adhesion molecules.

Incubation of PMN with different concentrations of human Ac2-26 resulted in a dose-dependent reduction of fMLP-stimulated [^{3}H]-AA release in the supernatant, with a maximal inhibition of 77% at the highest concentration tested of 100 μg/mL (33 μM) (Fig. 4A). Addition of human Ac2-26 alone did not significantly modify basal release (not shown). fMLP addition to human PMN resulted in polymerization of cellular actin transiently increasing the amount of F-actin. Human Ac2-26

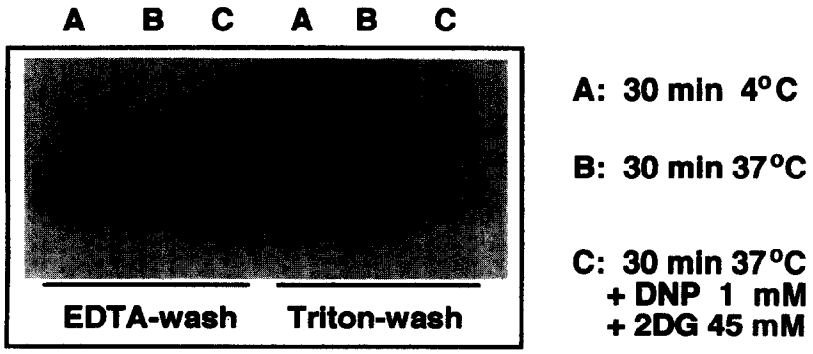

Fig. 5. Internalisation of human ^{125}I-Ac2-26 by PMN. ^{125}I-labelled Ac2-26 was added to 3×10^6 PMN and incubated for 30 min at 4°C (A lanes), at 37°C (B lanes), or at 37°C with 45 mM 2-deoxy-glucose (2DG) and 1 mM dinitrophenol (DNP) (C lanes). After washes, ^{125}I-Ac2-26 was removed from the cell surface with PBS supplemented with 1 mM EDTA (EDTA-wash); cell-associated peptide was recovered by cell lysis with PBS containing 10 mM EDTA and 1% Triton X-100 (Triton-wash). Equal amounts of protein from each fraction were subjected to SDS-PAGE and autoradiography. Representative of three experiments. Densitometric analysis (taking total as 100%): EDTA-wash, A: 41%, B: 26%, C: 78%; Triton-wash, A: 59%, B: 74%, C: 22%.

neither modified basal levels of F-actin nor the rapid increase induced by fMLP (Fig. 4B).

Finally, addition of human ^{125}I-Ac2-26 to human PMN resulted in a specific uptake of the peptide. A major portion (~80%) of ^{125}I-Ac2-26 was found inside the cells after a 30-min incubation period at 37°C (Fig. 5, B lanes): ^{125}I-Ac2-26 appeared to be unmodified, giving a single band of ~3 kDa on SDS-PAGE. Longer incubations of 4 hr or 24 hr did not further increase the uptake (not shown). ^{125}I-Ac2-26 internalisation was attenuated when PMN were incubated at 4°C (Fig. 5, lane A) or when oxidative metabolism was inhibited with 2DG and DNP (Fig. 5, C lanes).

DISCUSSION

This study investigated the effect of an LC1-derived N-terminus peptide on neutrophil functions *in vitro*. Peptide human Ac2-26 potently inhibited PMN migration *in vivo* [7], and the effect on this cell type has been now studied in detail *in vitro* using several models of PMN activation.

Incubation of human Ac2-26 with human neutrophils resulted in a selective alteration of some of the biochemical events activated in these cells by agents such as fMLP, LTB_4, and PAF. The effect exerted by human Ac2-26 on the degranulation of primary granules was assessed by measuring the release of elastase activity [15]. Human Ac2-26 was a potent inhibitor of this process irrespective of the stimulus used. This suggests that the peptide affects a specific activation step downstream of the interaction of chemoattractant with its receptor. Indeed, human Ac2-26 did not interfere with fMLP binding to its receptor as assessed by flow cytometry using the FITC-fMLPL probe. Significantly, this LC1-derived peptide was not a nonspecific inhibitor of neutrophil activation and, though inhibiting the release of elastase from primary granules, it did not modify the up-regulation of CD11b molecules, which is caused by degranulation of tertiary granules, or adhesomes [15,16].

Amongst the first intracellular events following chemoattractant application are the transient polymerization of actin and the rise in free Ca^{2+} [17]. Human Ac2-26 did not interfere with F-actin formation, and preliminary data showed that this peptide did not interfere with $[Ca^{2+}]_i$ either (Mauro Perretti, unpublished data). In separate experiments, we confirmed that fMLP addition to human PMN resulted in a release of free arachidonic acid, and reported that addition of human Ac2-26 significantly inhibited the release of this biochemical marker of PMN activation. Human Ac2-26 effect was again concentration-dependent, with an EC_{50} of 33 μM (100 μg/mL), similar to that calculated from the elastase release experiments. Recent studies have focussed on a high Mw cytosolic form of PLA_2 as the major regulator of AA release in some cells. Human LC1 may modify the activity of the enzyme following a direct interaction [18]. It is not known whether the N-terminus region of LC1 is directly involved in this interaction.

To address the question of which cell type may be the major target for human Ac2-26 during acute inflammation, PMN adhesion to endothelial monolayers *in vitro* was then investigated. Human Ac2-26 significantly affected PMN adhesion when this was achieved by PMN activation, but was ineffective when it resulted from stimulation of the endothelial cells. These *in vitro* data therefore support the observations made *in vivo*, and confirm that the neutrophil is a major cell target for the anti-inflammatory activity of human Ac2-26. The data obtained with the adhesion assay not only point to the PMN as a major target for human Ac2-26, but perhaps also highlight the existence of a species-specificity: human, but not rat or mouse, Ac2-26 inhibited fMLP-stimulated human PMN adhesion.

It is interesting to note that addition of human Ac2-26 to human PMN was followed by an energy-dependent uptake of the peptide. A good portion of the peptide (~80%) could not be removed by EDTA washing when the incubation was carried out at 37°C, whereas a higher proportion was removed by an EDTA wash following incubations at 4°C. The energy-dependence of this process was confirmed by blocking cell oxidative metabolism, in which situation all the radioactivity remained in the EDTA-wash fraction. This data suggests the existence of a mechanism that can internalise human Ac2-26. It is not clear whether such a process also exists for the full-length protein.

Strong evidence suggests a role for LC1 in the com-

plex phenomenon of exocytosis in human PMN [19, 20], where it may promote the fusion between specific granules and the plasma membrane [21, 22]. One possibility is that the LC1 N-terminus peptide interferes with the exocytotic process following internalisation by the PMN. The fact that an exogenously added peptide inhibits a process that the endogenous protein can promote is not uncommon, as already reported for a peptide derived from the consensus sequence, characteristic of the lipocortin/annexin family [23]. In another study, more relevant to this mechanism, the addition of neutrophil-derived LC1 was observed to promote fusion of the granules with plasma membrane with a bell-shaped curve, and LC1 actually inhibited this process at concentrations ≥1 μg/mL [21]. In addition, two independent studies have reported abolition of LC1-mediated granule fusion with the neutrophil plasma membrane by an antibody raised against the N-terminus region; this confirms the modulatory role for this part of the protein and points to the N-terminus as being directly involved in granule fusion [21, 22].

A mechanism of action based upon interference with the exocytotic process would explain other biological actions ascribed to LC1 and its active fragments, like the antipyretic property, which is mediated by an interference with the release of corticotrophin [24], as well as the ability to reduce hormone release from the pituitary [25].

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