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Modulation of ICAM-1 Levels on U-937 Cells and Mouse Macrophages by Interleukin-1β and Dexamethasone

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Differentiation of U-937 cells with phorbol ester (10 nM) induced a time-dependent (24 h or 48 h) increase of adhesion molecules and lipocortin 1 expression on the cell surface. Stimulation with interleukin-1β for a further 16 h increased the levels of intercellular adhesion molecule-1, and this effect was inhibited by co-incubation with 0.1-1 μM dexamethasone. The effect of the glucocorticoid was not modified by addition of a specific anti-lipocortin 1 monoclonal antibody (mAb 1A, 5 μg/ml). This opposite modulatory role of interleukin-1 and dexamethasone on intercellular adhesion molecule-1 expression was also, for the first time, observed *in vivo* using mouse peritoneal macrophages: a four-fold increase in intercellular adhesion molecule-1 expression was measured after local administration of the cytokine (5 μg/kg) and this effect was greatly inhibited (>70%) by co-injection with 1 μg dexamethasone. In conclusion, modulation of intercellular adhesion molecule-1 expression by glucocorticoids is an effect independent endogenous lipocortin 1, and it is an *in vivo* feature of these potent anti-inflammatory drugs.

Adhesion molecules represent important effectors in the leukocyte migration process, mediating not only the interaction with the endothelium but also the cell movement in the sub-endothelial matrix. In spite of the pleiotropic potent anti-inflammatory activity of glucocorticoid hormones, not many studies have investigated their effect upon adhesion molecule expression, especially *in vivo.* We have recently shown that a part of dexamethasone (DEX) anti-migratory action is mediated by endogenous lipocortin 1 (LC1) [1]. The actual step affected by DEX in a LC1-dependent way was one subsequent to adhesion to the endothelial wall [2]. There are no data available on the effect of LC1 on adhesion molecule expression; in a recent study we found a lack of involvement for LC1 on CD62L shedding and CD11b up-regulation on human neutrophil plasma membrane [3].

Differentiation of U-937 cells with phorbol 12-myristate 13-acetate (PMA) is associated with induction of LC1 expression. In addition, treatment of differentiated U-937 cells with DEX increases LC1 expression and translocation to the cell surface [4,5]. Differentiated U-937 cells up-regulate the cell surface expression of many adhesion molecules, including intercellular adhesion molecule-1 (ICAM-1) [6], platelet-endothelial cell adhesion molecule-1 (PECAM-1) [7] and several α subunits of the β_2-integrin class of proteins (CD11a, CD11b, CD11c) [8].

DEX has been reported to inhibit ICAM-1 induction consequent to PMA stimulation without interfering with the actual cell differentiation process [6]. Based on all these experimental observations, it seemed conceivable to use this system for investigating DEX effect upon adhesion molecule expression with the aim to relate potential inhibitory effects to endogenous LC1. The expression of ICAM-1, PECAM-1, CD11a, CD11b, CD11c, and LC1 on the surface of U-937 cells was quantified in relation to cell differentiation; changes in adhesion molecule expression after cell stimulation with interleukin-1β (IL-1β), the inhibitory action of DEX and the potential involve-

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Abbreviations used: DEX, dexamethasone; LC1, lipocortin 1; ICAM-1, intercellular cell adhesion molecule-1; PECAM-1, platelet-endothelial cell adhesion molecule-1; IL-1β, interleukin-1β; Mø, macrophage; mAb, monoclonal antibody; FACS, fluorescence-activated cell sorter; PMA, phorbol 12-myristate 13-acetate; FITC, fluoresceine isothiocyanate.

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ment of LC1 were investigated; and, finally, we gave *in vivo* relevance to our observation by measuring IL-1β and DEX effect on ICAM-1 expression by murine peritoneal macrophages.

MATERIALS AND METHODS

Preparation of U-937 cell line. U-937 cells were cultured in suspension at 0.2-1 × 10^6 cells/ml in RPMI 1640 medium supplemented with 1% pen-strept, 0.5% gentamycin, 2 mM L-glutamine and 10% heat-inactivated foetal calf serum (all materials purchased from Sigma Chemical Co., Poole, UK). Cells (seeded at a cell density of 0.5 × 10^6/ml) were differentiated by addition of 6 ng/ml PMA (10 nM; Sigma) and incubations carried out for 24 h or 48 h [4]. U-937 cells were then stimulated for a further 16 h by addition of human recombinant IL-1β (generously provided by Dr RC Newton, Du-Pont Merck, Glenolden, DE). Different concentrations of DEX were added to cultures 1 h prior to IL-1β. In other cases, the specific mouse anti-human LC1 neutralising mAb (mAb 1A; generously given by Dr JL Browning, Biogen Corp., Cambridge, MA) was used [9], and it was added to cells at a final concentration of 5 μg/ml 2 h before DEX, a protocol previously shown to neutralize the action of endogenous LC1 [10]. In all cases, U-937 cells were washed twice in RPMI 1640 after harvesting with 5 ml of non-enzymatic dissociation medium (Sigma) and prior to use for further analysis (see below).

Fluorescence-activated cell sorter (*FACS*) *analysis.* 0.1–0.2×10^6 U-937 cells were seeded in 96-well flat-bottom plates in 20 μl of PBS supplemented with 0.2% bovine serum albumin; non-specific sites were blocked by adding 20 μl of human IgG (15 mg/ml) prior to the addition of 20 μl of several anti-adhesion molecule mAbs. After 1 h at 4°C, cells were washed twice with PBS/BSA and incubated with 40 μl of diluted (1:40) $F(ab')_2$ fragment of goat anti-mouse IgG conjugated to FITC (Sigma). After further 45 min at 4°C, cells were washed, resuspended in 200 μl PBS-bovine serum albumin and fixed with an equal volume of 2% paraformaldehyde. FACS analysis was performed within 5 days 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 (Becton Dickinson). At least 5,000 events were analysed for each labelling. Data were analysed as units of fluorescence measured in the FL1 channel and converted to the number of mAb molecules bound per cell with reference to microbeads labelled with standard molecules of FITC (Flow Cytometry Standards Corp., Research Triangle Park, NC) [11].

The following mAbs were purchased from Serotec (Oxford, UK) and used at 1 μg per well (17 μg/ml): mouse anti-human ICAM-1 (clone B-C14); mouse anti-human PECAM-1 (clone HC1/6); mouse anti-human CD11a (LFA-1; clone B-B15); mouse anti-human CD11b (CR3; clone 44) and mouse anti-human CD11c (clone 3.9). The mouse anti-human LC1 mAb 1B was provided by Dr JL Browning (Biogen) and used at a final concentration of 20 μg/ml [9].

ICAM-1 levels on mouse peritoneal macrophages (*Mø*). Male Swiss Albino mice (28–32 g body weight; Interfauna, Huntingdon, Cambridgeshire) were maintained on a standard chow pellet diet and tap water *ad libitum.* Animals were injected i.p. either with sterile saline (10 ml/kg) or with 5 μg/kg murine recombinant IL-1β (provided by Dr RC Newton) alone, or in association with 1 μg DEX (corresponding approximately to 30 μg/kg). After 4 h peritoneal cavities were washed with 4 ml of PBS containing 3 mM EDTA, and cells pelleted by centrifugation at 400 g for 15 min at 4°C. Doses and time-point were selected on the basis of preliminary experiments. After two washes, 10^6 peritoneal cells (>80% Mø) were incubated in 100 μl PBS/BSA with 100 μl of homologous plasma (1:10 final dilution; freshly prepared by centrifuging 1 ml of blood at 6,500 r.p.m. in a minifuge) and 50 μl of rat IgG (9 μg/ml final concentration; Sigma) or rat anti-mouse ICAM-1 (9 μg/ml; clone KAT-1; Serotec) for 1 h at 4°C. After two washes, cells were stained with FITC-conjugated anti-rat IgG antibody (Serotec) for 5 min at room temperature. For storage purposes, cells were fixed with an equal volume of 2% paraformaldehyde. Flow cytometry was then performed as described above, and the Mø population was discriminated from

TABLE 1
Expression of Membrane Antigens upon U937 Cell Differentiation

Membrane antigen	Hours after PMA differentiation (No. of molecules per cell)		
	0	24	48
ICAM-1	63,105 ± 3,829	138,414 ± 23,235	200,265 ± 24,346
PECAM-1	117,560 ± 21,098	226,573 ± 40,632	172,483 ± 28,949
CD11a	14,954 ± 4,003	49,394 ± 17,120	62,429 ± 13,129
CD11b	1,843 ± 765	2,382 ± 1,009	7,857 ± 2,900
CD11c	1,246 ± 499	10,261 ± 2,089	39,642 ± 16,190
LC1[a]	7,302 ± 500	11,347 ± 1,250	14,155 ± 2,000

Note. Adhesion molecule and LC1 levels on U937 cell plasma membranes was determined by FACS analysis using specific mAbs as described under Materials and Methods. Data are mean ± SEM of four experiments performed in triplicate.

[a] 20–30% of cells were positive.

lymphocytes (and neutrophils in the case of animals treated with the cytokine) on the basis of the forward and side scatter characteristics. At least 10,000 events were analysed for each sample. As expected, Mø and lymphocytes were both positive for ICAM-1, however treatment with IL-1β and DEX modulated the expression of the adhesion molecule only on the first cell type.

Statistics. In vitro data are reported as mean ± SEM of n experiments performed in triplicate, whereas *in vivo* data are shown as mean ± SE of n animals per group. Statistical differences were analysed by ANOVA with the Bonferroni test for multiple comparisons, taking a P value less than 0.05 as significant.

RESULTS AND DISCUSSION

Constitutive expression of PECAM-1, and to a lesser extent of ICAM-1, was found on U-937 cell plasma membrane. Also CD11a, and to a lesser extent LC1, were positively detected on the cell surface (Table I). Treatment of U-937 cells with PMA provoked a time-dependent differentiation with adhesion to plastic, and up-regulation of membrane expression of several adhesion molecules

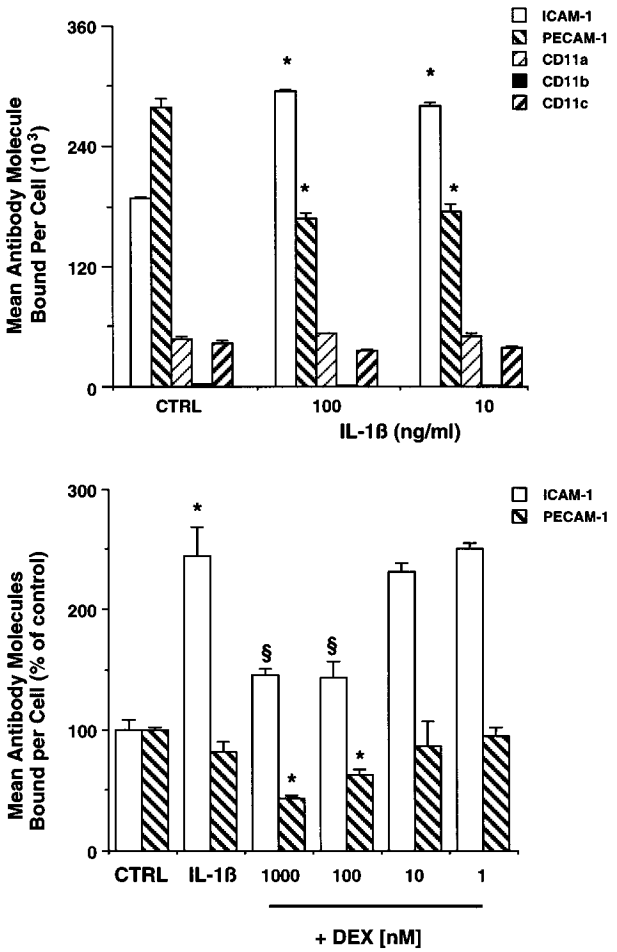

FIG. 1. Modulation of ICAM-1 expression on U-937 cell plasma membrane by IL-1β and DEX. **(Top)** U-937 cells were differentiated for 48 h with 6 ng/ml PMA, and incubated for a further 16 h with the reported concentrations of IL-1β prior to quantification of adhesion molecule expression on the plasma membrane by FACS analysis. Data are mean ± SE of triplicate observations. *P < 0.05 vs. control values (CTRL). **(Bottom).** DEX was added at the reported concentrations 1 h prior to IL-1β and adhesion molecule expression assessed 16 h later. Data are mean ± SEM of 4 experiments performed in triplicate. Control values for ICAM-1 and PECAM-1 were 117,516 and 147,717 molecules per cell, respectively. *P < 0.05 vs. control values (CTRL), and §P < 0.05 vs. IL-1β alone, as calculated on original values.

and of LC1. Whereas ICAM-1 and β_2-integrin expression increased linearly with time, PECAM-1 levels were lower ar 48 h than 24 h (Table I), and this is in agreement with the findings of a previous study [7].

IL-1 has originally been described as a good inducer of ICAM-1 expression [12]. Incubation of differentiated U-937 cells with IL-1β selectively up-regulated ICAM-1 expression (Figure 1A). Co-incubation with DEX abolished IL-1β effect on ICAM-1 levels with a significant effect from 100 nM (Figure 1B). This data extends IL-1β to the list of inducers which effect on ICAM-1 expression in U-937 cells is suppressed by the steroid [6,13], and adds U-937 cells to fibroblasts and an adenocarcinoma cell line as experimental systems suitable to study the opposite modulatory action of IL-1 and DEX on ICAM-1 expression [14]. As a negative control, we monitored PECAM-1 levels finding, with surprise, that cell stimulation with IL-1 stimulation was often (6 positive experiments out of 9 performed in total) associated with a reduced expression of PECAM-1 (Figure 1A). Since processing of PECAM-1 on the cell surface following cell activation has been described [7,15], it is likely that a similar mechanism could have occurred here. DEX interfered with the mechanisms leading to IL-1β-dependent ICAM-1 induction but not with those causing PECAM-1 shedding. Cumulative data (n = 6 experiments) showed that PECAM-1 levels were reduced by 20 ± 5% following cell incubation with 100 ng/ml IL-1β, and this was increased to 38 ± 7% reduction when 100 nM DEX was added to the cultures. It should also be said that we have observed a similar dual behaviour between ICAM-1 and PECAM-1 expression also in endothelial cell lines (MP and SKW, unpublished data). Further studies will be required for a full investigation on this matter, and the role played by DEX.

Since U-937 cells respond to PMA and DEX treatment with induction and translocation of LC1 (see Table I, and references [4,5]), the potential role played by endogenous LC1 was investigated using the neutralizing mAb 1A [9,10]. However, tested against either 1 μM or 100 nM DEX, pre-incubation of cells with 5 μg/ml mAb 1A did not alter the inhibitory action of the steroid on ICAM-1 induction by IL-1β (Figure 2). Therefore, whereas membrane-bound LC1 plays a role in

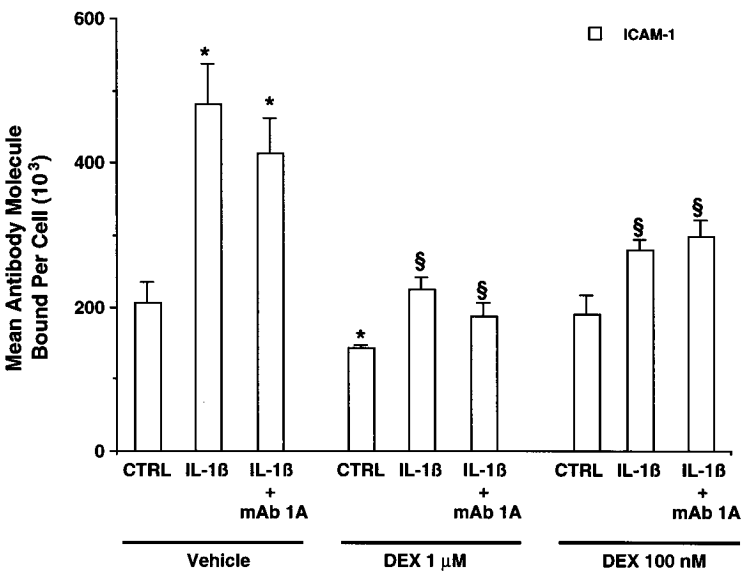

FIG. 2. Lack of involvement for LC1 in DEX mediated suppression of ICAM-1 up-regulation by IL-1β. U-937 cells were differentiated for 48 h with 6 ng/ml PMA, and incubated with a specific anti-LC1 mAb (5 μg/ml, mAb 1A) for 2 h prior to addition of the reported concentrations of DEX or vehicle. IL-1β was added 1 h after the steroid and ICAM-1 expression monitored 16 h later by FACS analysis. Data are mean ± SEM of 3 experiments performed in triplicate. *P < 0.05 vs. control values (CTRL), and §P < 0.05 vs. IL-1β in the vehicle group.

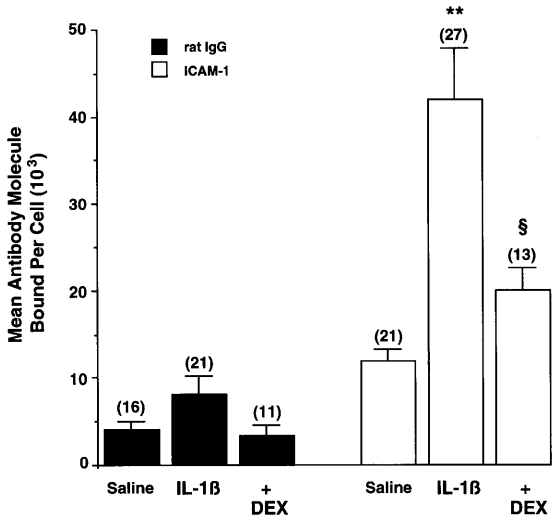

FIG. 3. DEX inhibits IL-1β-induced ICAM-1 up-regulation on mouse peritoneal Mø. Mice were treated with sterile saline (10 ml/kg i.p.), mouse IL-1β alone (5 μg/kg i.p.) or supplemented with 1 μg DEX (≈30 μg/kg) 4 h prior to washing the peritoneal cavities. Cells (>80% Mø) were stained with an anti-ICAM-1 mAb or with control rat IgG (9 μg/ml in both cases) prior to FACS analysis. Data are mean ± SE of (n) mice per group. $^{**}P < 0.01$ vs. rat IgG values and §$P < 0.05$ vs. IL-1β group.

the inhibitory action exerted by DEX on eocosanoid generation [4], this mediator does not appear to be involved in the steroid effect on ICAM-1 induction.

Finally, the occurrence of IL-1β and DEX effects upon ICAM-1 expression after *in vivo* administration was also investigated. Consistent ICAM-1 expression was detected on mouse peritoneal Mø (≈12×10^3 ICAM-1 molecules per cell) which responded strongly to IL-1β, with almost a four-fold increase 4 h after i.p. administration of the cytokine (and a net increase of 30×10^3 ICAM-1 molecules per cell) (Figure 3). Co-injection of 1 μg DEX into the mouse peritoneal cavity together with the cytokine potently inhibited ICAM-1 up-regulation on the Mø plasma membrane, with a net increase of only 8×10^3 ICAM-1 molecules per cell ($P < 0.05$ vs. IL-1β alone; Figure 3). These data indicate that ICAM-1 expression on Mø, and likely on other leukocytes, is under the dual control of pro-inflammatory cytokines and anti-inflammatory glucocorticoid hormones.

In conclusion, we report that DEX inhibited ICAM-1 induction consequent to cell activation with IL-1β both *in vitro* and *in vivo,* and that the effect of the steoroid appeared to be LC1-independent. The observed inhibitory effect of DEX on ICAM-1 induction by a pro-inflammatory agent such as IL-1β may be relevant not only to the process of leukocyte adhesion and extravasation, but also to other cell-cell interactions mediated by this adhesion molecule, including antigen presentation, T-cell mediated cytotoxicity, antibody-dependent cytotoxicity mediated by monocytes and neutrophils, and the interaction chondrocyte-mononuclear leukocytes [16].

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