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Dexamethasone inhibits cytokine-induced intercellular adhesion molecule-1 up-regulation on endothelial cell lines

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Abstract

Intercellular adhesion molecule-1 (ICAM-1) expression on three endothelial cell lines was differently modulated by pro-inflammatory cytokines, such as interleukin-1β and tumour necrosis factor-α (TNF-α) and the glucocorticoid hormone dexamethasone. Incubation of EA.hy926 cells with 1 μM dexamethasone prior to addition of TNF-α consistently reduced ICAM-1 induction by approximately 40%. EA.hy926 cell responsiveness to the steroid was validated by detecting specific dexamethasone binding, with a calculated affinity constant of 1.3 nM and a maximal number of sites of 35×10^3 per cell. To establish the generality of dexamethasone inhibition upon ICAM-1 up-regulation, two other endothelial cell lines were assessed. Incubation of LT4 and ECV304 cells with interleukin-1β or TNF-α produced a significant increase in ICAM-1 expression on their cell surface (ranging from a 2-fold increase for interleukin-1β to a 5-fold increase for TNF-α). Addition of dexamethasone was again able to significantly reduced this induction. Finally, the effect of the steroid on cytokine-induced ICAM-1 up-regulation was functionally related to its ability to suppress in vitro neutrophil trans-endothelial passage. Overall these data indicate that ICAM-1 is a likely molecular target for the anti-inflammatory action exerted by dexamethasone. Inhibition of ICAM-1 up-regulation may, at least in part, mediate the potent anti-migratory action displayed by this class of anti-inflammatory drugs. © 1997 Elsevier Science B.V.

Keywords: Adhesion molecule; Glucocorticoid; Inflammation; PECAM-1; Interleukin-1; TNF-α

1. Introduction

Intercellular adhesion molecule-1 (ICAM-1), a member of the immunoglobulin superfamily of adhesion molecules, is basally expressed on the cell surface of endothelial cells, where its levels can be increased during inflammatory conditions (Duperray et al., 1995; Malik and Lo, 1996). In the sequela of events regulating the interaction between leukocytes and the endothelium of post-capillary venules, endothelial ICAM-1 promotes leucocyte firm adhesion and the subsequent emigration through the endothelial gaps into the sub-endothelial space (Luscinskas et al., 1991; Sligh et al., 1993).

The importance of ICAM-1 and other adhesion molecules in mediating the cellular response characteristic of inflammatory and infectious pathologies makes them ideal candidates to be targeted for anti-inflammatory therapy. However, the efficacy of the most potent anti-inflammatory drugs available, glucocorticoid hormones, in inhibiting ICAM-1 induction in human umbilical vein endothelial cells, as well as on endothelial cells from other sources, has been conflicting (Cronstein et al., 1992; Forsyth and Talbot, 1992). Similar conflicting results have been found using cells from different lineages. The synthetic glucocorticoid, dexamethasone, reduced interleukin-1β-dependent ICAM-1 induction on a bronchial epithelial cell line (van de Stolpe et al., 1993) and on primary murine macrophages (Perretti et al., 1996), whereas the steroid was ineffective when a renal tubular epithelial cell line was used (Hogan and Foster, 1996). The reported discrepancies may also be related to the stimulus used to achieve cell activation, as indicated in a recent study in which dexamethasone inhibited ICAM-1 induction following cell activation by lipolysaccharide but not by a combination of tumour necrosis factor-α (TNF-α) and γ-interferon (Burke-Gaffney and Hellewell, 1996).

In recent years the availability of endothelial cell lines to many laboratories has been particularly useful to investigate the responsiveness of the endothelium to pro- and

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anti-inflammatory agents. EA.hy926 cells were the first human umbilical vein endothelial cell-derived cell line to be available and maintained most of the characteristics of primary human umbilical vein endothelial cells (Edgell et al., 1983), including increased adhesion molecule expression on stimulation with cytokines (Thornill et al., 1993).

Using three distinct human umbilical vein endothelial cell-derived cell lines, the present study was undertaken to test the effect of dexamethasone on ICAM-1 levels on the endothelial cell surface following activation with pro-inflammatory cytokines. We found that all three cell lines displayed specific glucocorticoid receptors, and consequently responded to the steroid in terms of inhibition of ICAM-1 up-regulation. Finally, the effect of dexamethasone was evident at longer (24 h) but not shorter (4 h) time-points of cell stimulation with TNF-α.

2. Materials and methods

2.1. Cell cultures

EA.hy926 cells were kindly provided by Dr. C.-J. Edgell (Department of Pathology, School of Medicine, University of North Carolina, Chapel Hill, NC, USA). These cells are an hybridoma between human umbilical vein endothelial cells and the epithelioma A549, and retain most of the features of human umbilical vein endothelial cells, including the human factor VIII-related antigen (Edgell et al., 1983). EA.hy926 cells were cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal calf serum, 1% pen-strep, 0.5% gentamicin, 1% L-glutamine and HAT supplement (all reagents from Sigma, Poole, UK), and sub-cultured 1:3 twice a week. Cells were used between passages 25 and 45.

LT4 cells were obtained from Dr. C. Pitzalis (Department of Rheumatology, Guy's Hospital, London, UK) and are SV40-transformed human umbilical vein endothelial cells. They were used between passages 3 and 30. ECV304 cells are spontaneously transformed and immortalized human umbilical vein endothelial cells and were obtained from Dr. S. Alexander (Department of Physiology, LSUMC, Shreveport, LA, USA) (Takahashi et al., 1990). ECV304 cells do not require special growth factors, can be maintained in culture indefinitely and display most of the markers of endothelial cells (Takahashi et al., 1990; Bowie et al., 1995). Both cell types were cultured in RPMI-1640 medium containing 20% fetal calf serum and the antibiotics as above. LT4 cells were passaged 1:2 once a week whereas ECV304 were handled as the EA.hy926 cells.

The following observations were made in preliminary experiments: (i) all cell types were seeded in 6-well plates at a concentration of 0.5×10^6 cells per well (in 2 ml of the respective medium) to be confluent at the time of cytokine addition; (ii) concentrations of 100 and 10 ng/ml were found to be optimal for human recombinant interleukin-1β (generous gift of Dr. R.C. Newton, Du Pont-Merck, Wilmington, DE, USA) and human recombinant TNF-α (NIBSC, Hertsfordshire, UK), respectively; finally, (iii) with the exception of EA.hy926 cells, all cell lines up-regulated ICAM-1 expression after incubation with either interleukin-1β or TNF-α.

2.2. Cell stimulation and flow cytometry analysis

After reaching confluence ($\sim$48 h) cells were washed and incubated with or without interleukin-1β or TNF-α for 4 or 24 h. Dexamethasone (sodium phosphate salt, David Ball Laboratories, Warwick, UK) was added to the cells 1 h prior to the respective cytokine. At the end of the incubation period, cells were washed with warm medium, harvested following 10 min incubation with non-enzymatic dissociation medium (Sigma), washed again in sterile phosphate-buffered saline (Sigma) and incubated on ice in 96-well flat-bottom plates.

Adhesion molecule expression was assessed by addition of specific monoclonal antibodies (final concentration 16 μg/ml): a mouse anti-ICAM-1 (CD54, clone BC-14 from Serotec, Oxford, UK), a mouse anti-platelet-endothelial cell adhesion molecule-1 (PECAM-1, CD31, clone HC1-6, Serotec) were added, or non-immune mouse immunoglobulin G were used in control wells. Non-specific binding was minimised by addition of 6 mg/ml human immunoglobulin G (Sigma). After 1 h at 4°C, cells were washed and incubated with $F(ab')_2$ fragments of goat anti-mouse IgG conjugated to fluorescein isothiocyanate for 30 min prior to further washing and fixation in 1% paraformaldehyde. Flow cytometry analysis was performed using a FACScan II analyser (Becton Dickinson) with air-cooled 100 mW argon ion laser tuned to 488 nm and a Consort 32 computer running Lysis II software. At least 5000 events were recorded for each sample, and fluorescence measured in the FL1 channel was quantified as mean fluorescence intensity units.

2.3. In vitro assay for neutrophil migration

Human neutrophils were freshly prepared from healthy volunteers by hystopaque gradient separation as reported previously (Perretti et al., 1995). Neutrophil transmigration was performed using the transwell system™ (Costar, Cambridge, MA, USA). EA.hy926 cells (5×10^4 cells in 700 μl of DMEM-F12 supplemented with 10% fetal calf serum and antibiotics) were seeded into collagen-coated transwells (6.5 mm diameter, 3 μm pore size) and grown to confluence (2–3 days) at 37°C in an atmosphere of 95% air/5% CO_2. Cells were incubated with or without 1 μM dexamethasone 1 h prior to addition of 10 ng/ml TNF-α (10 ng/ml) and incubated further for 24 or 4 h. A 24-well culture tray was precoated with gelatin for 10 min at room temperature to prevent neutrophil adhesion. The gelatin was aspirated and 600 μl of medium supplemented with

2.5% fetal calf serum added to the compartment (as an internal control, in some cases 14 nM interleukin-8 was added to the bottom compartment). EA.hy926 cells were then washed to remove TNF-α and dexamethasone, and the transwell added to this 24-well tray and 1.5×10^6 neutrophils added in 50 μl of assay medium were placed into the transwells. After 2 h at 37°C in 95% air/5% CO_2, cells which had migrated through the filters were retrieved from the lower compartment and counted using a Neubauer hemacytometer following staining in Turk's solution.

2.4. Glucocorticoid receptor quantification

EA.hy926, and ECV304, cell responsiveness to dexamethasone prompted the assessment of glucocorticoid receptor number and affinity, using a published protocol (Schlaghecke et al., 1994). Briefly, cells (1×10^6 in DMEM supplemented with 0.2% bovine serum albumin) were incubated in a total volume of 1 ml with different concentrations (2.5–50 pmol) of [1,2,4,6,7-^{3}H]-dexamethasone (specific activity 82 Ci/mmol; code TRK.645, Amersham International, Amersham, UK) in the presence or absence of 100 nmol unlabelled dexamethasone for 1 h at 37°C in a shaking water bath. Reaction was stopped by addition of 2 ml ice cold phosphate-buffered saline and centrifugation at 2000 rpm for 10 min (four washes in total were performed). Radioactivity associated with the pellets was then measured with a β-counter, and specific binding determined: the dissociation constant (K_d) and the density (number of binding sites per cell) were then calculated according to the method of Scatchard using computer assisted linear regression (Excell software running on a Macintosh Performa 6200).

2.5. Statistics

Data are reported as mean ± S.E.M. of n experiments performed in triplicate. Differences among means were analysed by analysis of variance followed by the Bonferroni test for intergroup comparisons, taking a P value < 0.05 as significant.

3. Results

3.1. Dexamethasone effect on EA.hy926 cell activation

In basal conditions, EA.hy926 cells expressed negligible levels of ICAM-1 on their cell surface whereas PECAM-1 (monitored as an internal control, since not inducible by cytokines) was highly present (Fig. 1). Cell incubation with TNF-α produced a time-dependent increase in ICAM-1 levels (up to 100-fold increase by 24 h post-stimulation), associated with a modest (between 10 and 20%) but consistent reduction in PECAM-1 expression. Addition of dexamethasone prevented both phenomena, with the significant exception of ICAM-1 induction at 4 h post-TNF-α (Fig. 1).

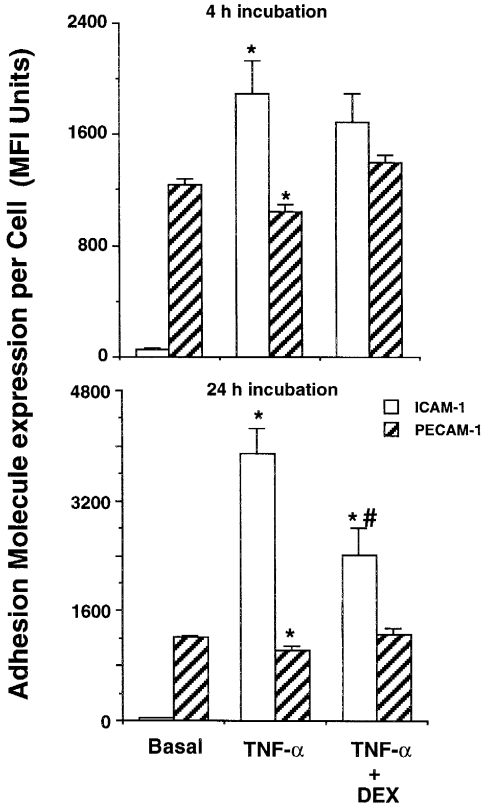

Fig. 1. Dexamethasone inhibits TNF-α-induced ICAM-1 expression on EA.hy926 cells. Cells were cultured to confluence in 6-well plates, incubated with or without 1 μM dexamethasone (DEX) 1 h prior to addition of 10 ng/ml TNF-α. ICAM-1 and PECAM-1 levels on their plasma membrane were measured by flow cytometry analysis 4 h (top panel) or 24 h (bottom panel) after addition of the cytokine. Data (mean ± S.E.M. of 3–4 experiments performed in triplicate) are shown as mean fluorescence intensity (MFI) units. * $P < 0.05$ vs. basal values; # $P < 0.05$ vs. TNF-α alone.

Dexamethasone inability to affect TNF-α-induced ICAM-1 up-regulation at the 4 h time-point was not related to the time length of cell incubation with the steroid. In a separate set of experiments, EA.hy926 cells were incubated with dexamethasone for 20 h or 1 h prior to TNF-α (10 ng/ml) addition. ICAM-1 levels were quantified 4 h later, and there was no difference amongst the groups (mean fluorescence intensity units, mean ± S.E.M. of $n = 3$): 1894 ± 67 for TNF-α alone, 1865 ± 45 in TNF-α + dexamethasone (−24 h) and 1731 ± 110 in TNF-α + dexamethasone (−1 h).

Dexamethasone effect upon TNF-α-stimulated 24 h ICAM-1 expression reached significance only at the highest concentration tested of 1 μM (39.8%) (Fig. 2). Cumulative data of 12 separate experiments showed that dexamethasone (1 μM) reduced TNF-α-induced ICAM-1 expression by $34.5 \pm 3.8\%$ ($P < 0.05$ as calculated on original values).

EA.hy926 cells sensitivity to dexamethasone was con-

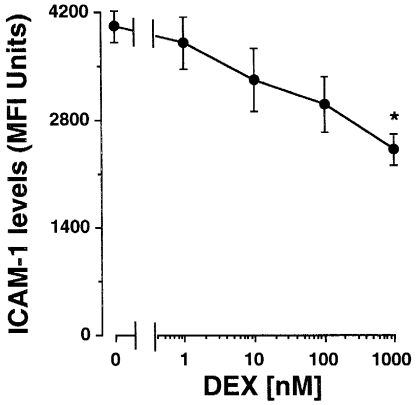

Fig. 2. Concentration-related dexamethasone inhibition of TNF-α-induced ICAM-1 expression on EA.hy926 cells. Cells were incubated with the reported concentrations of dexamethasone (DEX) 1 h prior to addition of 10 ng/ml TNF-α. ICAM-1 levels were measured by flow cytometry analysis 24 h later. Data (mean $\pm$ S.E.M. of 3 experiments performed in triplicate) are shown as mean fluorescence intensity (MFI) units. * $P <$ 0.05 vs. TNF-α alone (dose 0 group).

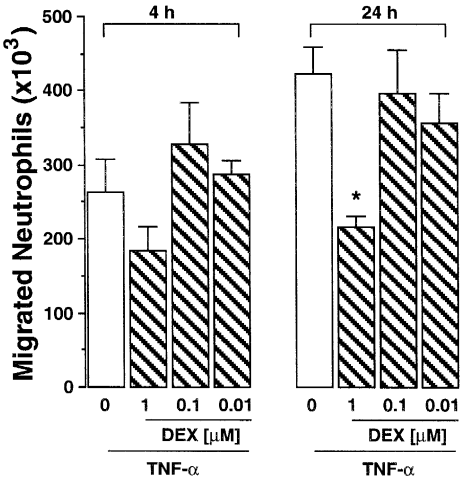

Fig. 3. Dexamethasone inhibits neutrophil passage through EA.hy926 cell monolayers activated with TNF-α. Cells were incubated in 24-well transwell™ with or without the reported doses of dexamethasone (DEX) 1 h prior to addition of 10 ng/ml TNF-α. Neutrophils (1.5×10^6) were added 4 h or 24 h later. The number of leukocytes migrated to the bottom well was assessed within 2 h. In absence of EA.hy926 cell activation, $10.5\pm2\times10^3$ neutrophils had migrated (negative control, $n=4$), whereas addition of 14 nM interleukin-8 to the bottom wells produced a migratory response of $312\pm20\times10^3$ cells (positive control, $n=6$). Data are mean $\pm$ S.E.M. of 3 experiments performed in triplicate. * $P < 0.05$ vs. TNF-α alone (dose 0 group).

firmed by assessing the presence of glucocorticoid receptors in this cell type. Scatchard analysis on the specific binding curve confirmed the existence of a single site of binding for the steroid. Table 1 reports the K_d and the binding capacity per cell measured in several distinct experiments using EA.hy926 and ECV304 cells. Similar K_d could be measured in the two cell types for dexamethasone binding, whereas ECV304 cells showed a higher number of binding sites.

The functional role of dexamethasone-induced inhibition of ICAM-1 up-regulation following TNF-α stimulation was investigated by measuring in vitro neutrophil trans-endothelial migration. Consistent with the profile of ICAM-1 induction shown in Fig. 1, TNF-α activated the endothelial monolayers to produce a significant neutrophil passage at 4 h, but a higher extent of cell transmigration was seen at the 24 time-point (Fig. 3). Addition of dexamethasone to EA.hy926 cells prior to the cytokine inhibited leucocyte passage only at the 24 h time-point.

3.2. Dexamethasone effect on LT4 and ECV304 cell activation

The possibility that dexamethasone inhibition on ICAM-1 induction could be restricted to EA.hy926 cells was challenged using two other human umbilical vein endothelial cell-derived cell lines.

In basal conditions, LT4 cells presented remarkable amounts of ICAM-1 on their plasma membrane (Fig. 4), much more than those found on EA.hy926 cells (see Fig. 1). These cells responded better to TNF-α than interleukin-1β stimulation with significant increases in their ICAM-1 levels (5-fold vs. two-fold increase for TNF-α

Table 1
Glucocorticoid (GC) receptor characteristics in EA.hy926 and ECV304 cells

Experiment	Cell type	K_d		GC receptor density	
		nM	Mean $\pm$ S.E.M.	$\times10^3$/cell	Mean $\pm$ S.E.M.
Exp #1	EA.hy926	1.21		30.0	
Exp #2	EA.hy926	1.35	1.30 $\pm$ 0.04	38.9	35.0 $\pm$ 2.6
Exp #3	EA.hy926	1.34		36.0	
Exp #1	ECV304	0.70		78.8	
Exp #2	ECV304	1.55	1.20 $\pm$ 0.25	45.6	63.8 $\pm$ 9.7
Exp #3	ECV304	1.34		66.9	

Illustrated are the data of separate binding experiments performed in duplicate as described in Section 2. No difference between the two cell lines was measured in terms of dissociation constant (K_d), whereas higher binding capacity was displayed by the ECV304 cells ($P < 0.05$).

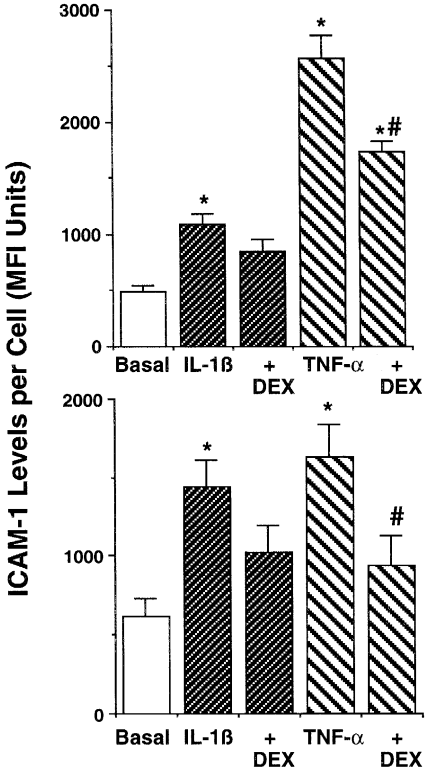

Fig. 4. Dexamethasone inhibits cytokine-induced ICAM-1 expression on LT4 (top panel) and ECV304 cells (bottom panel). Cells were cultured to confluence in 6-well plates, incubated with or without 1 μM dexamethasone (DEX) 1 h prior to addition of 10 ng/ml TNF-α or 100 ng/ml interleukin-1β (IL-1β). ICAM-1 levels on their plasma membrane were measured by flow cytometry analysis 24 h after addition of the cytokine. Data (mean ± S.E.M. of 4 experiments performed in triplicate) are shown as mean fluorescence intensity (MFI) units. * $P < 0.05$ vs. basal values; # $P < 0.05$ vs. TNF-α alone.

and interleukin-1β, respectively). Pre-incubation with 1 μM dexamethasone reduced both interleukin-1β- and TNF-α-induced expression of this adhesion molecule, with an inhibitory effect reaching more than 30% for either cytokine. LT4 cells were also PECAM-1 positive (794 ± 115 mean fluorescence intensity units, $n = 7$ experiments). However, in contrast to EA.hy926 cells no reproducible changes in PECAM-1 levels were seen after LT4 cell incubation with cytokines in the absence or presence of dexamethasone (not shown).

Fig. 4 (bottom panel) illustrates that essentially similar data were obtained with ECV304 cells. These cells were PECAM-1 negative (no signal above control IgG in $n = 6$ experiments). Incubation of ECV304 cells with interleukin-1β or TNF-α produced a similar degree of ICAM-1 up-regulation, and this was again significantly attenuated by addition of 1 μM dexamethasone. The steroid produced a calculated 30% and 61% of inhibition on interleukin-1β and TNF-α response, respectively (Fig. 4).

4. Discussion

The data obtained in this study contribute to clarify the apparent disparity in published reports which have investigated the ability of dexamethasone to inhibit ICAM-1 induction on the plasma membrane of endothelial cells. Using three human umbilical vein endothelial cell-derived cell lines we show that dexamethasone significantly reduced TNF-α- or interleukin-1β-induced endothelial cell activation (measured as ICAM-1 up-regulation and, for EA.hy926 cells, by the modest PECAM-1 shedding). A positive relationship between the effect of dexamethasone on ICAM-1 expression and the inhibition of neutrophil trans-endothelial passage is found.

Cronstein et al. (1992) firstly reported that dexamethasone is able to inhibit ICAM-1 and CD62E (E-selectin) up-regulation on human umbilical vein endothelial cells activated with lipopolysaccharide. This property of the steroid was functionally related to its inhibitory capacity on neutrophil adhesion to endothelial monolayers. A subsequent study failed to confirm these observations (Forsyth and Talbot, 1992). More recently, using EA.hy926 cells, Burke-Gaffney and Hellewell (1996) found dexamethasone to inhibit ICAM-1 up-regulation after cell activation with lipopolysaccharide but not after activation with a mixture of TNF-α and γ-interferon. In the present study we report that activation of EA.hy926 cells with TNF-α alone allows the detection of dexamethasone inhibition on ICAM-1 up-regulation. It is likely that addition of γ-interferon complicates the in vitro experimental system by activating pathways which are dexamethasone insensitive. Indeed, a reduced ability of dexamethasone to inhibit ICAM-1 up-regulation when EA.hy926 cells were stimulated with lipopolysaccharide + γ-interferon was also observed (Burke-Gaffney and Hellewell, 1996).

In our experimental conditions, dexamethasone produced a significant inhibition of ICAM-1 up-regulation only at the 24 h time-point, whereas the steroid did not modify the early (4 h) effect of TNF-α. The mechanism(s) behind this delay in dexamethasone efficacy is difficult to identify on the basis of these data. TNF-α activation of ICAM-1 gene and subsequent up-regulation of ICAM-1 protein is a process requiring not only translocation of specific nuclear factors (such as nuclear factor-kB) into the nucleus (Caldenhoven et al., 1995) but also full functioning of the proteasome multicatalytic complex (Read et al., 1995). The fact that dexamethasone was overall able to produce only a partial inhibition (approximately 40%) suggests that multiple factors are operating to achieve ICAM-1 up-regulation on endothelial cells (discussed in Burke-Gaffney and Hellewell, 1996), and that only a portion of these (or only one) are affected by the steroid. Dexamethasone sensitive mechanisms, at least in EA.hy926 cells, become clearly involved in ICAM-1 induction at time-points > 4 h. This was not related to the length of cell incubation with the steroid, because dexamethasone

failed to inhibit TNF-α-induced 4 h ICAM-1 induction even when preincubated with the EA.hy926 cells for 20 h prior to addition of the cytokine. Further studies are required to clarify this aspect of steroid biology.

Whatever the mechanism, we detected dexamethasone inhibition on ICAM-1 expression on all three human umbilical vein endothelial cell-derived cell lines. Differently from EA.hy926 cells, LT4 and ECV304 cells responded to both TNF-α and interleukin-1β with increased ICAM-1 expression. In all cases, addition of 1 μM dexamethasone was able to reduce this phenomenon. Therefore the ability of dexamethasone in modulating ICAM-1 expression is not restricted to EA.h926 cells and appears to be a relatively general phenomenon.

We could characterise further EA.hy926 cell responsiveness to dexamethasone by quantifying glucocorticoid receptor expression. Intracellular receptors for these hormones are widely distributed in mammalian tissues (Goldstein et al., 1992), and we show here that they are present in high amounts in human umbilical vein endothelial cell-derived cells lines. EA.hy926 and ECV304 cells bound labelled-dexamethasone with similar affinity, however they displayed different binding capacity. EA.hy926 cells showed a total number of glucocorticoid receptors similar to that measured in A549 cells (Ballard et al., 1978), whereas higher values could be found in immortalised human umbilical vein endothelial cell-derived ECV304 cells. These data, together with the observation that LT4 cells display similarly high glucocorticoid receptor numbers (71.5×10^3 per cell, with a K_d of 1.49 nM as determined in 1 experiment), suggests that human umbilical vein endothelial cells express higher glucocorticoid receptors than epithelial type II cells. Data generated with primary cells are necessary to confirm this proposition.

ICAM-1 up-regulation on EA.hy926 cells was associated with a modest but consistent dow-regulation of PECAM-1, and both processes were attenuated by dexamethasone. Processing of PECAM-1 on the cell surface of activated cells has been previously reported for human umbilical vein endothelial cells and U-937 cells (Goldberger et al., 1994). We have recently observed this dual behaviour for ICAM-1 and PECAM-1 expression also in interleukin-1β-activated U-937 cells (Perretti et al., 1996). Since both these adhesion molecules have been strongly implicated in the process of leucocyte migration (Luscinskas et al., 1991; Muller, 1995), the present finding may have in vivo application in the microenvironment of the leucocyte migrating through the endothelium in inflammatory conditions, and requires further investigations.

Interestingly, leucocyte trans-endothelial passage is the step preferentially affected by the steroid, as assessed by intravital microscopy (Mancuso et al., 1995). It was therefore consequent to search for a functional back-up to the data reported in this study by measuring neutrophil migration through TNF-α-activated EA.hy926 monolayers. A striking relationship between dexamethasone ability to inhibit ICAM-1 up-regulation and neutrophil trans-endothelial passage was found. In particular, dexamethasone displayed a similar capacity to inhibit 24 h, but not 4 h, TNF-α-induced ICAM-1 up-regulation and neutrophil migration. Overall, this data supports the concept that ICAM-1 may represent an important molecular target in the anti-inflammatory profile of glucocorticoid hormones. Nonetheless, the possibility that in the present experimental conditions the steroid is preventing the formation and/or release of other mediators from the activated endothelial monolayers, such as interleukin-8 or platelet-activating factor (Kuijpers et al., 1991; Smart and Casale, 1994; Zimmerman et al., 1996), should also be considered.

In conclusion, this study investigates further dexamethasone effect on ICAM-1 expression on three commonly used endothelial cell lines, and gives functional back-up to the observed inhibitory action of the steroid.

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References

Ballard, P.L., Mason, R.J., Douglas, W.H.J., 1978. Glucocorticoid binding by isolated lung cells. Endocrinology 102, 1570–1575.

Bowie, A., Moynagh, P.N., O'Neill, L.A.J., 1995. The human endothelial cell line ECV304 as a model of endothelial cell activation by interleukin-1. Biochem. Soc. Trans. 23, 109S.

Burke-Gaffney, A., Hellewell, P.G., 1996. Regulation of ICAM-1 by dexamethasone in a human vascular endothelial cell line EA.hy926. Am. J. Physiol. 270, C552–C561.

Caldenhoven, E., Liden, J., Wissink, S., van de Stolpe, A., Raaijmakers, J., Koenderman, L., Okret, S., Gustafsson, J.-A., van der Saag, P.T., 1995. Negative cross-talk between RelA and the glucocorticoid receptor: A possible mechanism for the antiinflammatory action of glucocorticoids. Mol. Endocrinol. 9, 401–412.

Cronstein, B.N., Kimmel, S.C., Levin, R.I., Martiniuk, F., Weissmann, G., 1992. A mechanism for the antiinflammatory effects of corticosteroids: The glucocorticoid receptor regulates leukocyte adhesion to endothelial cells and expression of endothelial-leukocyte adhesion molecule 1 and intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA 89, 9991–9995.

Duperray, A., Mantovani, A., Introna, M., Dejana, E., 1995. Endothelial cell regulation of leukocyte infiltration in inflammatory tissues. Med. Inflamm. 4, 322–330.

Edgell, C.-J., McDonald, C.C., Graham, J.B., 1983. Permanent cell line expressing human factor VIII-related antigen established by hybridization. Proc. Natl. Acad. Sci. USA 80, 3734–3737.

Forsyth, K.D., Talbot, V., 1992. Role of glucocorticoids in neutrophil and endothelial adhesion molecules expression and function. Med. Inflamm. 1, 101–106.

Goldberger, A., Middleton, K.A., Oliver, J.A., Paddock, C., Yan, H.-C., DeLisser, H.M., Albelda, S.M., Newman, P.J., 1994. Biosynthesis and processing of the cell adhesion molecule PECAM-1 includes production of a soluble form. J. Biol. Chem. 269, 17183–17191.

Goldstein, R.A., Bowen, D.L., Fauci, A.S., 1992. Adrenal corticosteroids. In: Gallin, J.I., Goldstein, I.M., Snyderman, R. (Eds.), Inflammation: Basic Principles and Clinical Correlates. Raven Press, New York, NY, pp. 1061–1081.

Hogan, S.P., Foster, P.S., 1996. Cellular and molecular mechanisms involved in the regulation of eosinophil trafficking in vivo. Med. Res. Rev. 16, 407–432.

Kuijpers, T.W., Hakkert, B.C., Hoogerwerf, M., Leeuwenberg, J.F.M., Roos, D., 1991. Role of endothelial leukocyte adhesion molecule-1 and platelet-activating factor in neutrophil adherence to IL-1-prestimulated endothelial cells. Endothelial leukocyte adhesion molecule-1-mediated CD18 activation. J. Immunol. 147, 1369–1376.

Luscinskas, F.W., Cybulsky, M.I., Kiely, J.-M., Peckins, C.S., Davis, V.M., Gimbrone, M.A. Jr., 1991. Cytokine-activated human endothelial monolayers support enhanced neutrophil transmigration via a mechanism involving both endothelial-leukocyte adhesion molecule-1 and intercellular adhesion molecule-1. J. Immunol. 146, 1617–1625.

Malik, A.B., Lo, S.K., 1996. Vascular endothelial adhesion molecules and tissue inflammation. Pharmacol. Rev. 48, 213–229.

Mancuso, F., Flower, R.J., Perretti, M., 1995. Leukocyte transmigration, but not rolling or adhesion, is selectively inhibited by dexamethasone in the hamster post-capillary venule. Involvement of endogenous lipocortin 1. J. Immunol. 155, 377–386.

Muller, W.A., 1995. Migration of leukocytes across vascular intima. Molecules and mechanisms. Trends Cardiovasc. Med. 5, 15–20.

Perretti, M., Wheller, S.K., Choudhury, Q., Croxtall, J.D., Flower, R.J., 1995. Selective inhibition of neutrophil function by a peptide derived from lipocortin 1 N-terminus. Biochem. Pharmacol. 50, 1037–1042.

Perretti, M., Wheller, S.K., Harris, J.G., Flower, R.J., 1996. Modulation of ICAM-1 levels on U-937 cells and mouse macrophages by interleukin-1β and dexamethasone. Biochem. Biophys. Res. Commun. 223, 112–117.

Read, M.A., Neish, A.S., Luscinskas, F.W., Palombella, V.J., Maniatis, T., Collins, T., 1995. The proteasome pathway is required for cytokine-induced endothelial-leukocyte adhesion molecule expression. Immunity 2, 493–506.

Schlaghecke, R., Beuscher, D., Kornely, E., Specker, C., 1994. Effects of glucocorticoids in rheumatoid arthritis. Diminished glucocorticoid receptors do not result in glucocorticoid resistance. Arthritis Rheum. 37, 1127–1131.

Sligh, J.E. Jr., Ballantyne, C.M., Rich, S.S., Hawkins, H.K., Smith, C.W., Bradley, A., Beaudet, A.L., 1993. Inflammatory and immune responses are impaired in mice deficient in intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA 90, 8529–8533.

Smart, S.J., Casale, T.B., 1994. TNF-α-induced transendothelial neutrophil migration is IL-8 dependent. Am. J. Physiol. 266, L238–L245.

Takahashi, K., Sawasaki, Y., Hata, J.-I., Mukai, K., Goto, T., 1990. Spontaneous transformation and immortalization of human endothelial cells. In Vitro Cell. Dev. Biol. 25, 265–274.

Thornill, M.H., Li, J., Haskard, D.O., 1993. Leucocyte endothelial cell adhesion: A study comparing human umbilical vein endothelial cells and the endothelial cell line EA.hy926. Scand. J. Immunol. 38, 279–286.

van de Stolpe, A., Caldenhoven, E., Raaijmakers, J.A.M., van der Saag, P.T., Koenderman, L., 1993. Glucocorticoid-mediated repression of intercellular adhesion molecule-1 expression in human monocytic and bronchial epithelial cell lines. Am. J. Respir. Cell Mol. Biol. 8, 340–347.

Zimmerman, G.A., McIntyre, T.M., Prescott, S.M., 1996. Adhesion and signaling in vascular cell-cell interactions. J. Clin. Invest. 98, 1699–1702.