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Expression of Endothelin-1 in Human Broncho-Epithelial and Monocytic Cell Lines: Influence of Tumor Necrosis Factor-α and Dexamethasone

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ABSTRACT. Abnormal endothelin-1 (ET-1) expression has been observed in bronchial asthma and systemic sclerosis with lung involvement. The purpose of this study was to analyze the synthesis of ET-1 in human airway epithelial cells and macrophages under basal conditions and after challenge with tumor necrosis factor-α (TNFα) or with the glucocorticoid dexamethasone. The ET-1 mRNA level and peptide release were measured in the broncho-epithelial cells BEAS-2B and the monocytic cell line U937. At baseline, U937 cells released low amounts of ET-1 peptide, whereas ET-1 was not detectable in BEAS-2B cells. After TNFα treatment, BEAS-2B cells, but not U937 cells, showed a significant increase in ET-1 expression, both at the mRNA and peptide levels. In contrast, dexamethasone elicited an increased amount of ET-1 peptide in U937 medium, but not in BEAS-2B cells. In this latter cell line, dexamethasone pretreatment was unable to inhibit the TNFα-induced expression. We conclude that response to TNFα and glucocorticoids is cell-type specific with respect to ET-1 production. The response of lung tissue to these agents *in vivo* is likely to be the overall balance of induction and inhibition in local microenvironments. BIOCHEM PHARMACOL 53;4:547–552, 1997. © 1997 Elsevier Science Inc.

KEY WORDS. endothelin; bronchi; epithelium; corticosteroids; monocyte; asthma

The lung is a major source of endothelin in the organism and is also involved in its clearance [1]. In the airways, ET-1§ and ET-3 can be produced by bronchial epithelial cells [2] and macrophages [3], together with endothelial cells from the bronchial vasculature. After the initial report by Nomura *et al.*, describing an increased content of ET-1 in the BALF from a patient with *status asthmaticus* [4], several reports have confirmed that asthmatics produce a higher amount of ET-1 than controls, as measured in the BALF [5], or as evaluated by immunocytochemistry [6] and *in situ* hybridization [7]. Treatment with corticosteroids, the most widely used anti-inflammatory agents in asthma, may decrease this overexpression *in vivo* [5], although this latter point was not confirmed by another group [8].

The potentially deleterious activities of ET-1 in asthma are: 1. a constriction of the airway smooth muscle [9]; 2. a mitogenic effect on smooth muscle cells and fibroblasts [10]; that may lead to airway remodeling and narrowing; 3. an activation of the pathway of the arachidonic acid metabolism, resulting in the generation of proinflammatory mediators [11]; and 4. the induction of histamine release from mast cells [12].

The regulation of ET-1 and ET-3 expression in the airways, and especially in bronchial epithelial cells and lung macrophages, is still poorly understood. In the adenocarcinoma cell line A549, Calderón and colleagues have recently demonstrated that glucocorticoids were able to downregulate ET-1 release, both under basal conditions and after stimulation with interleukin-2 [13]. The results concerning the effects of glucocorticoids on ET-1 production in human airway epithelial cells *in vivo* are conflicting [5, 8], whereas the influence of corticosteroids on ET-1 synthesis by macrophages has not yet been reported.

The purpose of the present work was, therefore, to analyze two cell lines as a model to study the expression of ET-1 in human airway epithelial cells and macrophages. For the former, we used BEAS-2B cells, a nontumorigenic cell line with a phenotype close to that of basal bronchial cells [14]. U937 cells, derived from a monocytic leukemia [15], were used to study the monocyte/macrophage lineage. We then tested if these cells were able to express ET-1 at the mRNA and protein levels under basal conditions. In addition, we treated these cells with TNFα in the presence or absence of dexamethasone to determine the respective in-

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§ *Abbreviations:* ET, endothelin; ET-1, endothelin-1; TNFα, tumour necrosis factor-α; BALF, broncho-alveolar lavage fluid; PMA, phorbol myristate acetate; SSPE, standard saline phosphate EDTA; GAPDH, glyceraldehyde phosphate dehydrogenase; PBMC, peripheral blood mononucleated cells.

fluence of a physiological mediator and a pharmacological substance on ET-1 expression *in vitro*.

MATERIALS AND METHODS

Cell Lines and Reagents

BEAS-2B cells were obtained from the National Cancer Institute, Bethesda, MD (Dr. C. C. Harris). U937 cells and prepro-ET-1 and -ET-3 cDNA were from American type culture collection (ATCC, Rockville, MD). Peripheral blood mononucleated cells (PBMC) were obtained from the buffy coat of 6 healthy blood donors at our hospital by centrifugation on Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). The LHC-9 cell culture medium was purchased from Biofluids (Rockville, MD). All other cell culture reagents were from Gibco (Basel, Switzerland). ET-1 radioimmunoassay was from Nichol's Institute (Geneva, Switzerland); Sep-Pack C_{18} columns from Millipore (Volketswil, Switzerland); recombinant hTNFα (8.33×10^8 U/mg) from Genzyme (Cambridge, MA); ^{32}[P]dATP from Amersham (Zürich, Switzerland); and the cDNA random prime labeling kit was from Pharmacia. The other chemicals were from Sigma (Buchs, Switzerland).

Cell Culture and Treatment

BEAS-2B cells were cultured on collagen, fibronectin, and albumin-coated flasks in LHC-9 serum-free medium as previously described [14]. U937 cells and PBMC were grown in RPMI 1640 medium supplemented with 10% fetal calf serum [15]. When the cells reached approximately 80% confluence (BEAS-2B) or were at 5×10^5 cells/mL (U937), they were both incubated under serum-free conditions for 24 hr in the presence or absence of 10^{-6} M dexamethasone. They were then washed and reincubated for another 24-hr period in serum-free medium, with or without dexamethasone, rhTNFα (50 ng/mL), or PMA 10^{-6} M. The supernatants were harvested and kept at −20°C until analysis. The cells were detached with trypsin and EDTA, counted in a hematocytometer and, in some experiments, lysed for RNA analysis (see below).

ET-1 Immunoassay

The cell supernatants were acidified with an equal volume of 4% (v/v) acetic acid, centrifuged, and extracted by reverse phase on C_{18} columns, using 4 mL (BEAS-2B) or 5 mL medium (U937). The columns were washed with 25% ethanol, eluted with 85% methanol and 4% acetic acid, and the samples dried overnight in a vacuum evaporator. Radioimmunoassay using ^{125}I-ET-1 was then performed in duplicate, following the recommendation of the supplier, and the radioactivity measured in a γ counter (LKB Wallac 1470, Uppsala, Sweden). According to the supplier, cross-reactivity of the assay was 52% with ET-2, 92% with ET-3, and 7% with Big-ET-1. Recovery after reverse phase extraction was evaluated by adding a trace amount (5 nCi) of ^{125}[I]ET-1 through the C_{18} columns and counting the eluate with the γ counter. The recovery was 95 ± 2% on 4 experiments. The intra-assay and inter-assay coefficients of variation were 5.4 and 7.1%, respectively.

Northern Analysis

RNA analysis was performed as previously described [16]. Briefly, total cellular RNA was extracted in a guanidinium/phenol/chloroform mixture according to the technique of Chomczynski *et al.* [17]. RNA was separated on a 0.6 M formaldehyde, 1% agarose gel, and transferred onto nylon membranes according to standard blotting procedures. cDNA probes for human prepro-ET-1 and rat GAPDH were labeled using the random priming procedure. Filters were hybridized overnight with 0.5 μCi/mL of labeled cDNA at 42°C in 5 × SSPE (1 × SSPE; 150 mM NaCl, 10 mM NaH_2PO_4, 1 mM EDTA), 2% Denhardt, 0.5% SDS, 50% deionized formamide, and 100 μg/mL sonicated salmon DNA. After washing under increasingly stringent conditions, the filters were exposed to Kodak XAR film with intensifying screen at −80°C. Signals were measured with a laser densitometer (Molecular Dynamics and ImageQuant v.3.3) and normalized to the GAPDH mRNA signal. Data from treated cells were expressed as a percentage, with basal conditions arbitrarily set at 100%.

Data Analysis

Results, given as mean ± SEM, were analyzed using the nonparametric Kruskal-Wallis test. Significance was set at $p = 0.05$.

RESULTS

ET-1 Content in Cell Culture Medium

Under basal, serum-free conditions, ET-1 peptide was secreted at low levels in the U937 cells and in PBMC, and was not detectable in supernatants from BEAS-2B cells (Figs. 1 and 2). Challenge with rhTNFα induced ET-1 release in the broncho-epithelial cells at 21.2 ± 4.7 pg/10^7 cells/24 hr (mean ± SEM, n = 4), but was without effect on the U937 cells or on PBMC. Treatment with the protein kinase C activator phorbol myristate acetate (PMA, 10^{-6} M for 24 hr) induced a modest ET-1 release in BEAS-2B cells (6.6 ± 1.9 pg/10^7 cells/24 hr), but no change in U937 cells. PMA-induced monocytic differentiation into a macrophage phenotype (10^{-8} M for 48 hr), as described elsewhere [18], did not modify the response of the U937 cells after TNFα (data not shown).

We then tested the influence of the glucocorticoid dexamethasone at a pharmacological concentration of 10^{-6} M for 48 hr. This treatment was without effect on BEAS-2B cells, but was able to increase basal ET-1 release in U937 cells from 19.7 ± 3.3 to 37.4 ± 3.9 pg/10^7 cells/24 hr ($n = 6$, $p < 0.001$). Unlike U937 cells, dexamethasone did not modify ET-1 release from PBMC (baseline: 4.50 ± 2.61 pg/

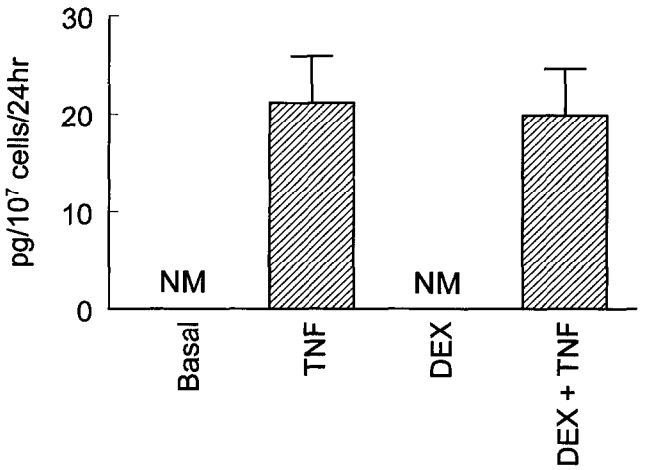

FIG. 1. ET-1 peptide content measured in the conditioned medium from BEAS-2B cells after 24-hr incubation. Data are the mean ± SEM from ⩾4 independent experiments. NM, not measurable (detection threshold: 10 pg/10^7 cells); TNF, 50 ng/mL hTNFα; DEX, 10^{-6} M dexamethasone for 48 hr; TNF+DEX, both previous treatments together.

10^7 cells/24 hr, dexamethasone treated: 4.12 ± 1.41 pg/10^7 cells/24 hr). As previously shown, PMA-pretreated U937 cells reacted similarily to PMA-naive cells. To determine if glucocorticoids were able to inhibit TNFα-induced ET-1 expression in the broncho-epithelial cells, the BEAS-2B cells were first incubated with dexamethasone 10^{-6} M alone for 24 hr. The medium was then changed and the cells incubated for another 24-hr period with TNFα (50 ng/mL) and dexamethasone. ET-1 peptide release was comparable with this treatment and with TNFα alone (Fig. 1). The same experiments performed with U937 cells and PBMC, either with dexamethasone alone or with dexamethasone followed by TNFα, showed that ET-1 production was similar under both conditions (Fig. 2).

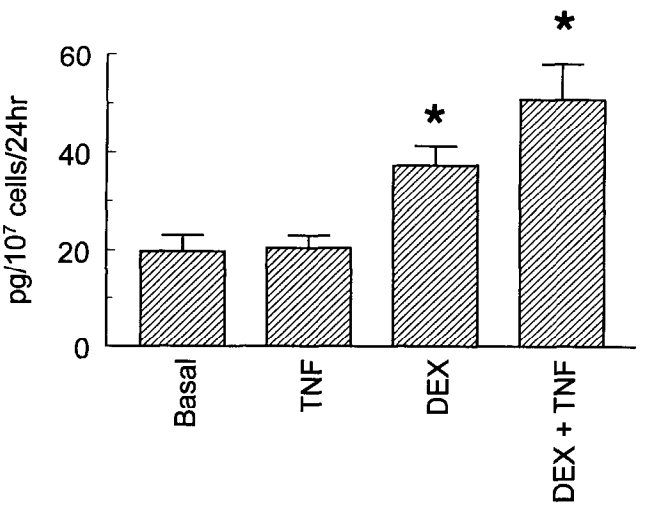

FIG. 2. ET-1 peptide content measured in the conditioned medium from U937 cells after 24 hr incubation. Data are the mean ± SEM from >4 independent experiments. Same abbreviations as in Fig. 1. p = 0.0018 (Kruskal-Wallis).

Northern Analysis

Under basal conditions, prepro-ET-1 mRNA was weakly detectable in Northern blots obtained from U937 and BEAS-2B (Fig. 3A,B). Treatment of both cell lines with PMA 10^{-6} M induced a strong mRNA band with a stimulation from 2 to 24 hr in BEAS-2B cells, but no significant induction in U937 cells. Challenge with dexamethasone and TNFα were performed according to the same experimental design as for the cell supernatant studies. In BEAS-2B cells, TNFα, but not dexamethasone, was able to increase the prepro-ET-1 mRNA signal (Fig. 4A,B). Pretreatment of these cells with dexamethasone did not decrease TNFα-induced expression of prepro-ET-1 mRNA. In contrast, in dexamethasone-treated U937 cells, there was a tendency toward higher prepro-ET-1 mRNA expression, although these data did not reach statistical significance (Fig. 5A,B). After TNFα treatment, no significant induction was observed. Under neither condition was prepro-ET-3 mRNA detectable (data not shown).

We then tested whether or not the induction by TNFα of prepro-ET-1 mRNA in BEAS-2B cells was dependent on new protein synthesis. Incubation of cells with the protein synthesis inhibitor cycloheximide 10 μg/mL for 4 hr led to an increased signal for prepro-ET-1 mRNA (Fig. 6A,B). After coincubation with TNFα and cycloheximide, the mRNA was more abundant than with cycloheximide alone, demonstrating that TNFα was able to increase expression of this mRNA without the need for new protein synthesis.

DISCUSSION

This *in vitro* study demonstrated that the modulation by biological mediators or pharmacological agents of ET-1 expression differed according to the cell line analyzed. In the BEAS-2B broncho-epithelial cell line, TNFα, but not dexamethasone, was able to induce ET-1 synthesis. How-

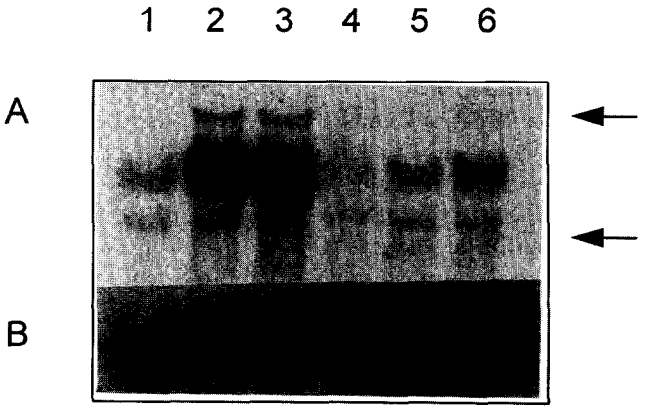

FIG. 3. Northern blot from BEAS-2B and U937 RNA. The total cellular RNA (20 μg/lane) was hybridized with the prepro-ET-1 probe (panel A) and the rGAPDH probe (panel B). Lanes 1–3, BEAS-2B cells lanes 4–6, U937 cells; lanes 1 & 4, basal conditions; lanes 2 & 5, 10^{-6} M PMA 2 hr; lanes 3 & 6, 10^{-6} M PMA 4 hr, Arrows, 28S and 18S RNA.

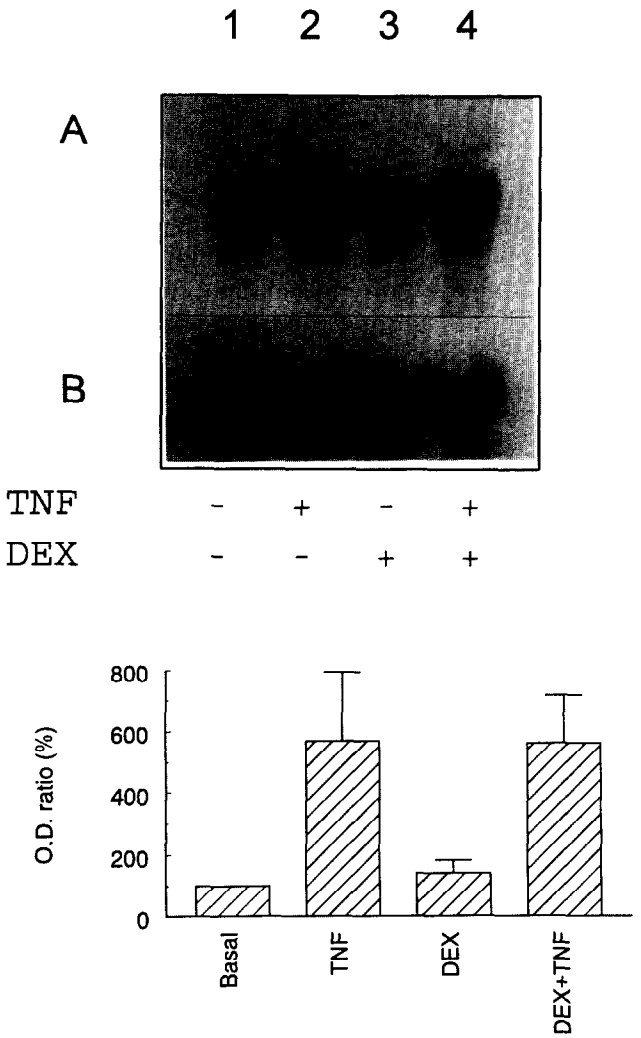

FIG. 4. Northern blot from BEAS-2B cells hybridized for ET-1 (panel A) and GAPDH (panel B). Treatment conditions were similar to those in Fig. 1. The lower graph represents quantification of the prepro-ET-1 mRNA signal, normalized to GAPDH signal to correct for unequal loading. Data are the mean ± SEM from ⩾4 independent experiments, expressed as a percentage of the baseline arbitrarily set at 100%. $p = 0.002$.

ever, in the monocytic U937 cells, the reverse was observed. In BEAS-2B cells, Northern blot analyses were overall concordant with data obtained with secretion of the peptide, showing that this modulation takes place essentially through enhanced transcription or/and increased mRNA half-life. After PMA treatment, however, the mRNA signal was much higher than expected considering the modest amount of peptide released in the medium, suggesting that this nonphysiological stimulus does not generate an efficient peptide synthesis. In U937 cells, there was no significant correlation between Northern blot analyses and peptide release, indicating that posttranscriptional regulation might prevail in this cell line.

The ET-1 immunoassay used in this study cross-reacts with both ET-2 and ET-3. Because ET-3 has been detected in macrophages [3] and bronchial epithelial cells [2], some of the endothelin measured here could be the type 3 isopeptide. However, Northern blotting with human specific ET-3 cDNA did not show any signal, either under basal or stimulated conditions, suggesting that ET-3 synthesis is comparatively low in these two cell lines.

Immunoassay in conditioned medium from cell culture reflects peptide release by these cells, which could significantly differ from *de novo* synthesis if the peptide is stored in secretory granules within the cytoplasm. For ET-1, several lines of evidence suggest, however, that the release of the peptide does require *de novo* synthesis, and not liberation of intracellularly stored precursors [19]. In addition, the parallel increase of prepro-ET-1 mRNA strongly suggests an increased gene expression. TNFα is known to induce ET-1 production in endothelial cells through transcriptional regulation [20]. Airway epithelial cells from the guinea pig [21] are also known to respond to this cytokine, whereas the human alveolar carcinoma cell line A549 has been shown to respond to IL-2 and to fetal bovine serum [13]. We demonstrate here that the human BEAS-2B cells,

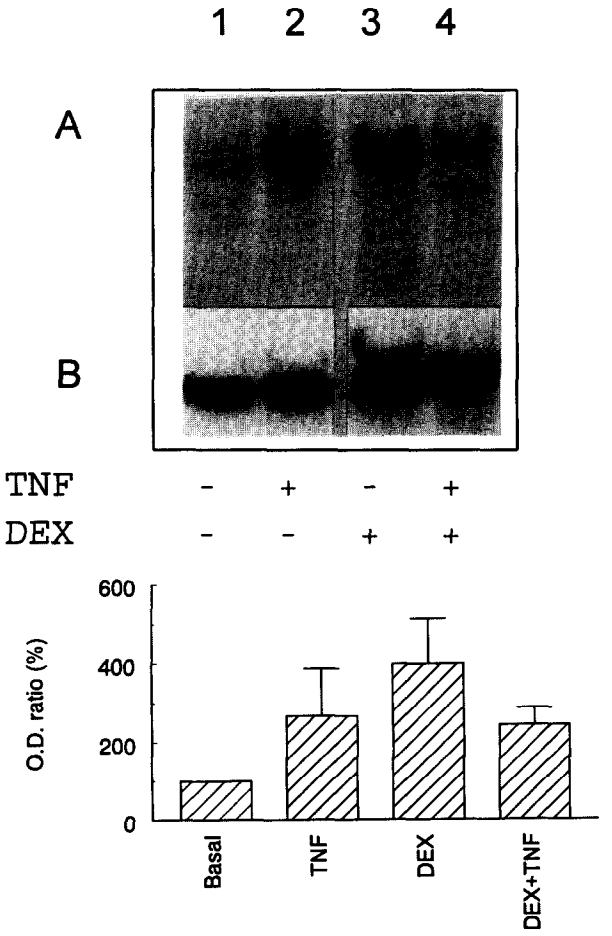

FIG. 5. Northern blot from U937 cells hybridized for ET-1 (panel A) and GAPDH (panel B). Treatment conditions are similar to those in Fig. 2. The graph represents quantification of the prepro-ET-1 mRNA signal, normalized to GAPDH signal to correct for unequal loading. Data are the mean ± SEM from ⩾4 independent experiments, expressed as a percentage of the baseline arbitrarily set at 100%.

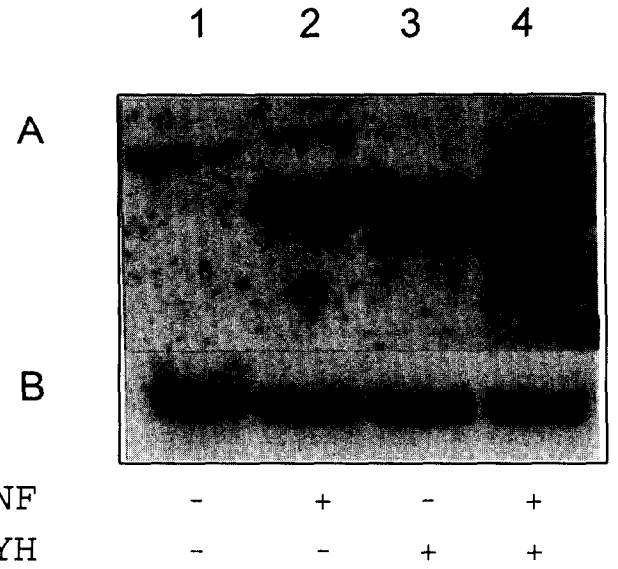

FIG 6. Northern blot from BEAS-2B cells hybridized for ET-1 (panel A) and GAPDH (panel B). TNF, 50 ng/mL hTNFα 4 hr; CYH, 10 μg/mL cycloheximide 4 hr; TNF+CYH, both treatments together.

derived from bronchial epithelium, behave similarly after TNFα exposure, although the amount of ET-1 produced is much lower than what has been measured in other epithelial cells [13, 21]. Whether this lower expression is due to differences in the culture conditions (in particular, the serum-free medium) or is specific to this cell line will need further study. In addition, we have shown that the mechanisms involved in the regulation of TNFα-induced prepro-ET-1 mRNA expression in BEAS-2B cells did not require new protein synthesis, in agreement with observations made in bovine endothelial cells [20]. Surprisingly, U937 cells were not responsive to TNFα, either at the mRNA or at the peptide level, although this cell line is known to express TNFα receptors. Even after inducing a macrophage-like phenotype through incubation with the phorbol ester PMA [18], we did not notice any response to TNFα treatment. The failure to respond to TNFα was confirmed in mononucleated cells from healthy donors.

Glucocorticoids have been shown to have the potential to induce ET-1 expression in vascular smooth muscle cells [22], and in breast cancer cell lines [23]. Although not yet clarified, the mechanism is likely to be indirect, because the ET-1 gene does not possess any identifiable glucocorticoid response element in its promoter region [24]. Our study demonstrates that U937 cells behave similarly and that the induction persists at least for 72 hr. On the contrary, PBMC and BEAS-2B cells did not respond to dexamethasone; moreover, dexamethasone was unable to modify the TNFα-induced ET-1 expression in the epithelial cells, although they do possess functional glucocorticoid receptors [25]. The LHC-9 medium used to grow the BEAS-2B cells contains 2×10^{-7} M hydrocortisone [14]. As such, it can be argued that this basal level could have desensitized these cells to pharmacological doses of glucocorticoids. However, it has been demonstrated in published experiments with a similar design that 10^{-6} M dexamethasone was still able to downregulate the TNFα-mediated IL-6 expression in BEAS-2B cells, *irrespective* of the presence or absence of hydrocortisone in the medium [25]. Therefore, these experimental conditions most likely reflect the situation of a pharmacological treatment *in vivo* in human patients, with a mean plasma cortisol concentration of 4×10^{-7} M.

The discrepancy between the behavior of U937 cells and PBMC in response to corticosteroids is intriguing. Because U937 are a leukemia cell line, they may phenotypically differ from normal blood monocytes, even after differentiation with PMA. We are presently evaluating the response of other nonneoplastic monocytic cells, such as lung tissue and broncho-alveolar macrophages.

It has already been observed that modulation of ET-1 release after glucocorticoid treatment is cell type- or tissue-specific: for example, dexamethasone elicits ET-1 release from vascular smooth muscle, but not from endothelial cells [22]. Our data from bronchial epithelial cells contrast with those reported in asthmatic patients *in vivo* [5, 6], in animal models [26], or with the A549 cell line [13]. We speculate that *in vivo* glucocorticoids are able to achieve a downregulation of ET-1 in the bronchi indirectly, through inhibition of the release of proinflammatory mediators, such as TNFα and/or IL-2, that can increase ET-1 release. The discrepancy with the results reported by Vittori *et al.* [7], where hydrocortisone downregulated ET-1 secretion in primary epithelial cells cultured from asthmatics, might originate from the difference in the cell types analyzed, in the ET-1-inducing factors, or dissimilar culture conditions, although these latter points would require specific experiments.

In conclusion, our study demonstrates that the modulation of ET-1 expression and secretion in human bronchial epithelial and monocytic cells is phenotype-specific. The complexity of ET-1 regulation lies, in part, in the structure of the ET-1 gene, which can be transcribed by alternative promoters [24, 27]. Therefore, in conjunction with the numerous ways of posttranslational regulation, the human body has set up a very sophisticated multilevel control system that can prevent noxious effects from such a potent peptide. To modulate pharmacological ET-1 activity in human lung diseases, results obtained from both *in vitro* and *in vivo* studies will need to be taken into account.

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References

1. Barnes PJ, Endothelins and pulmonary diseases. *J Appl Physiol* 77: 1051–1059, 1994.
2. Black PN, Ghatei MA, Takahashi K, Bretherton-Watt D, Krausz T, Dollery CT, Bloom SR, Formation of endothelin by

cultured airway epithelial cells. *FEBS Lett* **255:** 129–132, 1989.
3. Ehrenreich H, Anderson RW, Fox CH, Rieckmann P, Hoffman GS, Travis WD, Coligan JE, Kehrl JH, Fauci AS, Endothelins, peptides with potent vasoactive properties, are produced by human macrophages. *J Exp Med* **172:** 1741–1748, 1990.
4. Nomura A, Uchida Y, Kameyama M, Saotome M, Oki K, Hasegawa S, Endothelin and bronchial asthma. *Lancet* **2:** 746–747, 1989.
5. Mattoli S, Soloperto M, Marini M, Fasoli A, Levels of endothelin in the bronchoalveolar lavage fluid of patients with symptomatic asthma and reversible airflow obstruction. *J Allergy Clin Immunol* **88:** 376–384, 1991.
6. Springall DR, Howarth PH, Counihan H, Djukanovic R, Holgate ST, Polak JM, Endothelin immunoreactivity of airway epithelium in asthmatic patients. *Lancet* **337:** 697–701, 1991.
7. Vittori E, Marini M, Fasoli A, De Franchis R, Mattoli S, Increased expression of endothelin in bronchial epithelial cells of asthmatic patients and effect of corticosteroids. *Am Rev Respir Dis* **146:** 1320–1325, 1992.
8. Reddington AE, Springall DR, Ghatei MA, Lau LCK, Bloom SR, Holgate ST, Polack JM, Howarth PH, Endothelin in bronchoalveolar lavage fluid and its relation to airflow obstruction in asthma. *Am J Respir Crit Care Med* **151:** 1034–1039, 1995.
9. Rae GA, Calixto JB, D'Orléans-Juste P, Effects and mechanisms of action of endothelins on non-vascular smooth muscle of the respiratory, gastrointestinal and urogenital tracts. *Reg Peptides* **55:** 1–46, 1995.
10. Noveral JP, Rosenberg RA, Anbar NA, Grunstein MM, Role of endothelin-1 in regulating proliferation of cultured rabbit airway smooth muscle cells. *Am J Physiol (Lung Cell Mol Physiol)* **263:** L317–L324, 1992.
11. Wu T, Rieves RD, Larivée P, Logun C, Lawrence MG, Shelhamer JH, Production of eicosanoids in response to endothelin-1 and identification of specific endothelin-1 binding sites in airway epithelial cells. *Am J Respir Cell Mol Biol* **8:** 282–290, 1993.
12. Egger D, Geuenich S, Denzlinger C, Schmitt E, Mailhammer R, Ehrenreich H, Dormer P, Hultner L, Il-4 renders mast cells functionally responsive to endothelin-1. *J Immunol* **154:** 1830–1837, 1995.
13. Calderón E, Gómez-Sánchez CE, Cozza EN, Zhou M, Coffey RG, Lockey RF, Prockop LD, Szentivanyi A, Modulation of endothelin-1 production by a pulmonary epithelial cell line. *Biochem Pharmacol* **48:** 2065–2071, 1994.
14. Reddel RR, Ke Y, Gerwin BI, McMenamin MG, Lechner JF, Su RT, Brash DE, Park JB, Rhim JS, Harris CC, Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40 hybrid virus, or transformation via strontium phosphate coprecipitation with a plasmid containing SV40 early region gene. *Cancer Res* **48:** 1904–1909, 1988.
15. Sundström C, Nilsson K, Establishment and characterization of a human histiocytic lymphoma cell line (U937). *Int J Cancer* **17:** 565–571, 1976.
16. Aubert JD, Hayashi S, Hards J, Bai R, Paré PD, Hogg JC, Platelet-derived growth factor and its receptor in lungs with asthma and chronic airflow obstruction. *Am J Physiol (Lung Cell Mol Physiol)* **266:** L655–L663, 1994.
17. Chomczynski P, Sacchi N, Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162:** 156–162, 1987.
18. Hoff T, Spencker T, Emmendoerffer A, Goppelt-Struebe M, Effects of glucocorticoids on the TPA-induced monocytic differentiation. *J Leukoc Biol* **52:** 173–182, 1992.
19. Fabbrini MS, Vitale A, Patrono C, Zamai M, Vaghi F, Caiolfa V, Monaco L, Benatti L, Heterologous in vivo processing of human preproendothelin 1 into bioactive peptides. *Proc Natl Acad Sci USA* **88:** 8939–8943, 1991.
20. Marsden PA, Brenner BM, Transcriptional regulation of the endothelin-1 gene by TNF-α. *Am J Physiol (Cell Physiol 31)* **262:** C854–C861, 1992.
21. Endo T, Uchida Y, Matsumoto H, Suzuki N, Nomura A, Hirata F, Hasegawa S, Regulation of endothelin-1 synthesis in cultured guinea-pig airway epithelial cells by various cytokines. *Biochem Biophys Res Com* **186:** 1594–1599, 1992.
22. Kanse SM, Takahashi K, Warren JB, Ghatei M, Bloom SR, Glucocorticoids induce endothelin release from vascular smooth muscle cells but not endothelial cells. *Eur J Pharmacol* **199:** 99–101, 1991.
23. Schrey MP, Patel KV, Tezapsidis N, Bombesin and glucocorticoids stimulate human breast cancer cells to produce endothelin, a paracrine mitogen for breast stromal cells. *Cancer Res* **55:** 1786–1790, 1992.
24. Inoue A, Yanagisawa M, Takuwa Y, Mitsui Y, Kobayashi M, Masaki T, The human preproendothelin-1 gene. Complete nucleotide sequence and regulation of expression. *J Biol Chem* **264:** 14954–14959, 1989.
25. Levine SJ, Larivée P, Logun C, Angus CW, Shelhamer JH, Corticosteroids differentially regulate secretion of IL-6, IL-8, and G-CSF by a human bronchial epithelial cell line. *Am J Physiol (Lung Cell Mol Physiol)* **265:** L360–L368, 1993.
26. Andersson SE, Eirefelt S, Zackrisson C, Hemsén A, Dahlbäck M, Lundberg JM, Regulatory effects of aerosolized budesonide and adrenalectomy on the lung content of endothelin-1 in the rat. *Respiration* **62:** 34–39, 1995.
27. Benatti L, Bonecchi L, Cozzi L, Sarmientos P, Two preproendothelin 1 mRNAs transcribed by alternative promoters. *J Clin Invest* **91:** 1149–1156, 1993.