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EFFECT OF DEXAMETHASONE ON α_1-PROTEINASE INHIBITOR SYNTHESIS IN HUMAN CELLS OF MONOCYTIC ORIGIN

Joanna Cichy*, Jan Potempa*, and James Travis[&,1]

*The Department of Microbiology and Immunology, Institute of Molecular Biology, Jagiellonian University, 31-120 Kraków, Poland

& The Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602

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In this study we provide evidence for downregulation of α_1-Proteinase Inhibitor (α_1-PI) synthesis in cells of monocytic origin by the glucocorticoid analog, dexamethasone. This factor significantly reduced the basal level of α_1-PI expression as well as antagonized the effect of lipopolysaccharide and interleukin-6, stimulators of α_1-PI synthesis in cells of monocyte/macrophage lineage. Since increased levels of all of these mediators are observed in both acute infections and inflammatory reactions, their combination may affect the contribution of monocytes to the synthesis of α_1-PI.

α_1-PI is believed to play an important role in inflammation due to its ability to rapidly inactivate neutrophil elastase (20, 14). This glycoprotein is a major serine proteinase inhibitor in human plasma, whose concentration is increased during the host response to inflammation or tissue injury (2). α_1-PI is predominantly synthesized by the liver, but its expression has also been demonstrated in extrahepatic cells, including human monocytes and macrophages (13). Although production of this inhibitor in these cells is about 100-times less than in cells of hepatic origin (11), its presence may be important at sites of injury or inflammation, where monocytes tend to accumulate. The synthesis of α_1-PI in human hepatic cells is regulated by the interleukin-6-type cytokines, including IL-6, interleukin-11, leukemia inhibitory factor, oncostatin M and ciliary neurotrophic factor (2). Moreover, glucocorticoids which stimulate the hepatic synthesis of several proteins in synergy with cytokines appear to enhance the effect of IL-6 on α_1-PI production in these cells (10). IL-6 has also been shown to mediate an increase in α_1-PI synthesis

[1]To Whom Correspondence Should Be Addressed. fax:(706) 542-3719.

ABBREVIATIONS:
α_1-PI, α_1-Proteinase Inhibitor; DEX, dexamethasone; PMA, Phorbol 12-myristate 13-acetate; LPS, lipopolysaccharide; IL-6, interleukin-6; U937, human histiocytic lymphoma; U937/PMA, PMA-differentiated U937 cells; FBS, fetal bovine serum; SDS-PAGE, SDS-polyacrylamide gel electrophoresis.

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in human monocytes and macrophages (13). However, in contrast to hepatic cells, monocytes can produce increased amounts of α_1-PI following LPS stimulation (1). Significantly, during macrophage activation by LPS there is an increase in the number of glucocorticoid receptors which may influence macrophage sensitivity to glucocorticoids (16). These observations suggest that the synthesis of α_1-PI in cells of mononuclear phagocyte lineage may be affected by glucocorticoids, especially since the level of these hormones is known to increase during inflammation.

In this study we examined the effect of the glucocorticoid analog, dexamethasone (DEX), on α_1-PI production in both the monocyte-like U937 cell line and human peripheral blood monocytes. Our results demonstrate that DEX significantly reduced the level of α_1-PI expression in both type of cells. Since negative regulation of α_1-PI synthesis by DEX seems to be specific for cells of monocyte/macrophage lineage, glucocorticoids may act in favor of hepatic production of this inhibitor at a late stage of inflammation, when these hormones appear to be released.

MATERIALS AND METHODS

Phorbol 12-myristate 13-acetate (PMA), dexamethasone (DEX) and lipopolysaccharide from *E. coli* 055:B5 (LPS) were purchased from Sigma (St. Louis, Mo).

Cell culture and stimulation

U937 human histiocytic lymphoma was obtained from the American Type Culture Collection (Rockville, MD). Cells were cultured in RPMI 1640 supplemented with 100 U/ml penicillin G, 100 µg/ml streptomycin (all from Gibco, Grand Island, NY) and 10% FBS (Atlanta Biologicals, Norcross, GA). U937 cell differentiation toward monocytes was induced by the addition of PMA to a final concentration of 35 ng/ml, 24 h before treatment. The culture medium containing unattached cells was then replaced by one-half the volume of fresh serum-free medium containing 35 ng/ml of PMA and stimulating factors.

Human monocytes were isolated from peripheral blood of healthy volunteers by separation on Mono-Poly Resolving Medium (ICN, Costa Mesa, CA), according to the manufacture's instruction. The cells were washed twice in PBS, resuspended in RPMI 1640 supplemented with 10% of autologous serum, and allowed to adhere to plastic tissue culture wells for 1 h. Lymphocytes were removed by several washings with PBS. Cell purity was monitored by differential cell counting with Giemsa staining and by staining for α-naphthyl acetate esterase (Sigma). Monocyte preparations were more than 90% pure. The cells were cultured in RPMI 1640 supplemented with 100 U/ml penicillin G, 100 µg/ml streptomycin and 10% FBS for 24 h and then treated in serum free medium containing indicated factors.

Northern blot analysis

Total RNA was isolated by the SDS-phenol procedure (18, 15). RNA was then subjected to formaldehyde-agarose gel electrophoresis (17), transferred to a Hybond-N membrane (Amersham, Arlington Hts., IL), according to the manufacture's instruction, and hybridized with a ^{32}P-labeled probe overnight at 65°C in a mixture containing 1M NaCl, 1% SDS and 10% dextran sulfate. The probe, a 1.4 kb EcoRI-EcoRI restriction fragment of human α_1-PI cDNA (9), was labeled using the Megaprime Labeling Kit (Amersham).

Biosynthetic labeling and immunoprecipitation

Cells were treated for indicated times with 10^{-6} M DEX and/or 5 U/ml LPS. They were then rinsed and incubated for 3 h in methionine-free medium containing various factors and 250 μCi/ml [^{35}S] methionine/cysteine (Tran ^{35}S-label), (ICN). Aliquots of medium were pretreated with preimmune serum and Pansorbin (Calbiochem, La Jolla, CA), as previously described (17) and then incubated at 4°C in 20 mM Tris-HCl pH 7.5, 140 mM NaCl, 1% Triton X-100 and 5 mM EDTA, overnight, with excess of anti-α_1-PI antibody (Dako, Carpinteria, CA). Immune complexes were precipitated with Protein A-Agarose (Sigma), washed, released by boiling in Laemmli sample buffer and subjected to 9% SDS-PAGE (8). α_1-PI was detected by fluorography, as previously described (4).

RESULTS

The effect of DEX on the expression of α_1-PI was initially studied in PMA-differentiated U937 cells. This cell line is an accepted model of monocyte effector function since, in the presence of PMA, it undergoes morphological, functional and biochemical changes toward maturation to mono-nucleated cells (6). α_1-PI is synthesized in PMA-differentiated U937 cells with its production being upregulated by LPS as observed at RNA levels at 24 h (Fig. 1A) and at protein levels at 24 h (Table 1) and more clearly at 48 h (Fig. 1B, Table 1). Addition of DEX, however, results in a decrease in α_1-PI synthesis in control cells. Furthermore, treatment of cells with DEX decreased the effect of LPS given 24 h earlier and still present at the time of DEX addition. When cells were preincubated with 10^{-6} M DEX, the addition of LPS (5 U/ml) did not increase the level of α_1-PI (Table 1, Fig. 1) but, instead, appeared to downregulate α_1-PI synthesis. Inhibition of α_1-PI production by DEX was observed even in the presence of higher levels of LPS (100 U/ml) (data not shown). The effect of DEX clearly involved a pretranslational mechanism as demonstrated by a downregulation of α_1-PI mRNA levels which generally paralleled the decrease in the level of secreted α_1-PI (Fig. 1). At least two different transcripts of α_1-PI were found to be present in cells of monocytic origin (5, 7), and could be observed in Fig. 1. Addition of DEX prevented appearance of the faster migrating band and downregulated the levels of both transcripts noted during prior LPS stimulation.

Since U937 cells were treated with DEX in the presence of PMA we next examined how the latter factor influenced the effect of DEX. The results are shown in Fig. 2. In comparison to PMA differentiated U937 cells, the untreated cells express a low levels of α_1-PI and DEX did not have a detectable effect on inhibitor synthesis (Fig. 2C). In contrast, PMA differentiated cells produce large amounts of α_1-PI (Fig. 2A and 2B), and addition of DEX profoundly impedes its synthesis either in the presence (Fig. 2A) or the absence of PMA (Fig. 2B). This clearly indicates

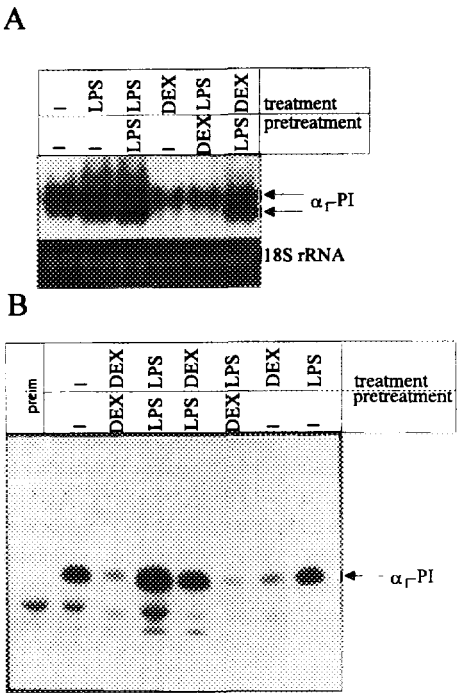

Fig. 1. Effect of DEX on α_1-PI synthesis in PMA-differentiated U937 cells.

PMA-differentiated U937 cells were pretreated in serum-free RPMI containing 35 ng/ml PMA and either 10^{-6} M DEX and/or 5 U/ml LPS for 24 h (pretreatment) and then were treated for another 24 h (A) or 21 h (B) in RPMI containing PMA, factors used for pretreatment, and indicated treatment factors (treatment). (A) Total cellular RNA was isolated and subjected to Northern blot analysis. The blot was photographed to demonstrate equal loading (bottom panel) and then hybridized with an α_1-PI probe (upper panel). (B) Cells were subjected to radiolabeling for 3 h [^{35}S] methionine/cysteine. Aliquots of culture medium were then immunoprecipitated with preimmune serum (Preim) or human anti-α_1-PI and immunoprecipitates were subjected to 9% SDS-PAGE followed by fluorography.

that DEX-exerted inhibition of α_1-PI expression does not occur through quenching of the direct stimulatory effect of PMA.

Finally, we examined whether DEX is capable of reducing α_1-PI synthesis in human peripheral blood monocytes. As illustrated in Fig. 3 and Table 1 the effect of DEX on either PMA differentiated U937 cells or monocytes was similar and was manifested by a decrease in α_1-PI production either in control or LPS-stimulated cells. However, some differences were also noted. Monocytes were generally more sensitive to LPS and if first preincubated with LPS followed by a combination of LPS and DEX, the effect of the latter factor was less apparent than in the U937/PMA system already described. This is consistent with the fact that addition of LPS to monocytes first preincubated with DEX still increased α_1-PI synthesis, although its level was

Table 1. Inhibition of α_1-PI expression in PMA-differentiated U937 cells and monocytes by DEX

Pretreatment	Treatment	% production relative to control	
		U937/PMA cells	Monocytes
Control	---	100	100
DEX	DEX	25	40
LPS	LPS	250	570
LPS	DEX	105	390
DEX	LPS	12	150
DEX	IL-6	n/d	45
---	DEX	28	n/d
---	LPS	130	550
---	IL-6	n/d	400

PMA-differentiated U937cells, or monocytes, were pretreated in serum-free RPMI supplemented with indicated factors for 24 h (Pretreatment) and then treated for another 24 h with indicated factors in the presence of factors used during pretreatment (Treatment) (in the case of U937 cells, medium additionally contained 35 ng/ml PMA). Factors were used at the following concentrations: 10^{-6} M DEX, 5 U/ml LPS, 50 ng/ml IL-6. ^{35}S-labeled α_1-PI was identified by fluorography after immunoprecipitation and SDS-PAGE. Quantification of α_1-PI was accomplished by liquid scintillation counting of bands excised from the dried gels. Mean of three determinations of two separate experiments is shown. n/d=non detected.

significantly reduced if compared to cells treated only with LPS. Finally, Table 1 also shows that in monocytes DEX also antagonized the effect of IL-6, another stimulator of α_1-PI production. The slower migrating bands shown in Fig. 3, (undetected when preimmune serum was used), probably represent α_1-PI complexed to elastase, which was previously suggested for these cells (13).

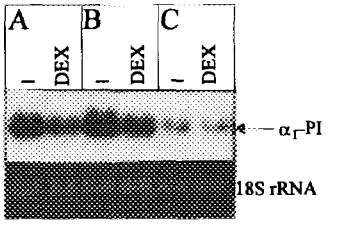

Fig. 2. Effect of PMA on DEX-exerted inhibition of α_1-PI expression in U937 cells.

Cells were incubated in RPMI supplemented with 10% FBS (C) or 10% FBS and 35 ng/ml PMA (A & B) for 48 h. They were then treated with 10^{-6} M DEX in serum-free RPMI (B & C) or serum-free RPMI containing 35 ng/ml PMA (A). At 24 h total cellular RNA was extracted and subjected to Northern blot analysis. Similar amount of ethidium-bromide-stained 18S rRNA was visualized in bottom panel. The autoradiogram obtained after hybridization of RNA with an α_1-PI specific probe is shown in upper panel.

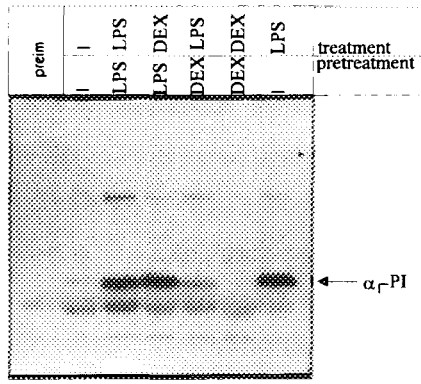

Fig. 3. Effect of DEX on α_1-PI synthesis by monocytes.

Cells were incubated for 24 h in serum-free RPMI containing 10^{-6} M DEX or 5 U/ml LPS (pretreatment) and then treated with indicated factors in the presence of factors used during pretreatment for another 21 h (treatment). Cells were then subjected to radiolabeling for 3 h. Aliquots of culture medium were immunoprecipitated with preimmune serum (Preim) or human anti-α_1-PI. Immunoprecipitates were then subjected to 9% SDS-PAGE followed by fluorography.

DISCUSSION

The inflammatory mediators, IL-6 and LPS, have been previously shown to regulate the expression of α_1-PI in human cells of mononuclear phagocyte lineage (1, 12). Although these factors are potent stimulators of inhibitor production in cells in culture, it is likely that *in vivo* they act in cooperation with other mediators. Several observations have suggested that glucocorticoids could potentially modulate α_1-PI expression in monocytes or macrophages. These include the finding that; *(i)*, these hormones increase in concentration during inflammation (2); *(ii)*, glucocorticoids appear to act in synergy with IL-6 on stimulation of α_1-PI synthesis in hepatic cells (10); and *(iii)*, LPS induces an increase in either α_1-PI synthesis or glucocorticoid receptor number in macrophages (16). Indeed, as illustrated in both figures 1 and 3, and Table 1, α_1-PI expression was regulated by DEX, a glucocorticoid analog. Moreover, these results indicate that in cells of monocytic origin the level of α_1-PI synthesis seems to be dependent on an equilibrium between positive (LPS, IL-6) and negative (DEX) regulators. Although a previous report has suggested that DEX did not antagonize the effect of IL-6 on α_1-PI synthesis in monocytes (12), a 10,000 lower concentration of DEX used in their study might explain this discrepancy. It is important to note that the DEX concentration used in our *in vitro* assay was comparable to the physiological concentration reached by glucocorticoids in the circulation following inflammation (3, 19).

The results which have been obtained raise the question as to why inhibition of α_1-PI synthesis by DEX is monocyte-specific. Since the upregulation of glucocorticoid levels appear to be associated with the late stage of inflammation, the data shown here suggest that α_1-PI synthesis by monocytes are likely contribute to the local tissue levels of this protein at an early stage of the inflammatory process. Thus, the stimulation of α_1-PI expression in monocytes which is triggered by LPS or IL-6 could be important in controlling proteolytic enzymes released in large quantities at the site of injury where such cells accumulate. However, once the systemic defense mechanisms are recruited, release of glucocorticoids curtails production of the inhibitor by monocytes, and tissue levels of α_1-PI become dependent on the inhibitor diffusion from plasma where its concentration is increased during the late stage of the acute phase response to inflammation or injury.

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