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THE TRANSCRIPTION OF THE INTERLEUKIN 1β GENE IS INDUCED WITH PMA AND INHIBITED WITH DEXAMETHASONE IN U937 CELLS

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SUMMARY: Interleukin 1β mRNA was induced with phorbol ester, not LPS, in the human histiocytic lymphoma cell line U937, but interleukin 1α mRNA was not induced. Nuclear run-on analysis showed that phorbol ester induced the transcription of interleukin 1β but did not induce it in the presence of cycloheximide. This indicates that the induction of the transcription with PMA requires de novo protein synthesis. Dexamethasone, an immunosuppressive and anti-inflammatory agent, decreased the level of interleukin 1β mRNA induced with phorbol ester. The transcriptional analysis showed that dexamethasone inhibits the transcription of the interleukin 1β gene.

Interleukin 1(IL-1) is a mediator of immunologic and inflammatory responses (1). Two distinct forms of IL-1 are produced by monocytes and macrophages (2 - 5). These two forms of IL-1, termed IL-1α and IL-1β, have been cloned in humans (6 - 9). Both forms of IL-1 have identical biological activities (1,10) and bind to the same receptor(11). The production of IL-1 can be induced in culture cells by various stimuli and in most cases, IL-1β mRNA is induced predominantly(7,9,12,13). It has been reported that IL-1β mRNA is rapidly induced and falls to a low, but constant level within 6 hours in THP-1 human monocytic leukemia cells stimulated with LPS (14). However the regulatory mechanism of IL-1β gene expression as well as IL-1α gene expression remains to be elucidated.

Glucocorticoids are used clinically as immunosuppressive and anti-inflammatory agents. Snyder and Unanue have reported that glucocorticoids inhibit IL-1 production from murine peritoneal macrophages (15).

In order to examine the regulatory mechanism of IL-1 gene expression including effects of glucocorticoids, we studied the expression of IL-1

Abbreviations: IL-1, interleukin 1; LPS, lipopolysaccharide; PMA, phorbol-12-myristate-13-acetate; Dex, dexamethasone; CHX, cycloheximide.

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mRNA using human histiocytic lymphoma cell line U937 (16) which is known to differentiate to macrophages (17). In this paper, we describe that PMA induced the transcription of IL-1β which required de novo protein synthesis, and dexamethasone(DEX) inhibited the transcription induced with PMA.

MATERIALS AND METHODS

Cell culture : The human histiocytic lymphoma cell line U937 was cultured to 4×10^5 cells/ml in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum (FCS) (Gibco). Cells were induced and treated as follows: cells were washed and resuspended in fresh RPMI 1640 medium supplemented with 10% FCS and 25 ng/ml of phorbol-12-myristate-13-acetate (PMA) (Pharmacia) in the presence or absence of 10^{-6}M dexamethasone (Dex) (Sigma) or 10 μg/ml of cycloheximide, and incubated at 37°C in 5% CO_2 and 95% air for the indicated period.

RNA isolation and hybridization : Total cellular RNA was isolated by the guanidine isothiocyanate - cesium chloride method (18). RNA samples (10 μg) were denatured with glyoxal-DMSO, electrophoresed in 1.2% agarose and blotted onto nitrocellulose membrane (Schleicher & Schuell) (19). Cytoplasmic RNA was isolated from the post-nuclear supernatant (20) and transferred to nitrocellulose membranes as described (21). Prehybridization and hybridization were carried out as described (19) with nick-translated IL-1β cDNA probe (PstI - PvuII fragment of pcD-GIF-16 (9)).

Nuclear run-on transcription : Nuclei were isolated from U937 cells treated as indicated by lysing the cells in NP40 lysis buffer as described (20). Nuclear transcription and RNA extraction were performed essentially as decribed (22) except that [α-^{32}P]GTP (400 Ci/mmol, Amersham) was used to label elongating RNA chains. RNA samples of equal radioactivity were hybridized with 2 μg of linearized plasmid DNA immobilized on nitrocellulose filters. Prehybridization, hybridization and washing of the filters were performed as described (23).

RESULTS AND DISCUSSION

Expression of IL-1β gene in U937 cells induced with PMA

We have previously reported that both IL-1α and IL-1β cDNAs were isolated from mRNA derived from U937 cells, which were differentiated to adhered cells with PMA and ConA, and then stimulated with LPS, MDP, and PMA (9). In the process of our investigation of the kinetics of IL-1 mRNAs, we found that IL-1β mRNA accumulates in U937 cells treated with only PMA, but IL-1α mRNA does not. IL-1β mRNA can be observed within 4 hours after PMA induction while no IL-1β mRNA can be detected in uninduced U937 cells (Fig.1, left). The levels of IL-1β mRNA remain essentially unchanged from 8 hours to 3 days after PMA induction (Fig.1). This is in contrast to the transient expression of IL-1β mRNA in THP-1 human monocytic leukemia cells reported by Fenton et al., (14). It may be due to a difference in mRNA stability since PMA treatment induces the stable expression of c-fos mRNA in U937 cells (24) and GM-CSF mRNA in T

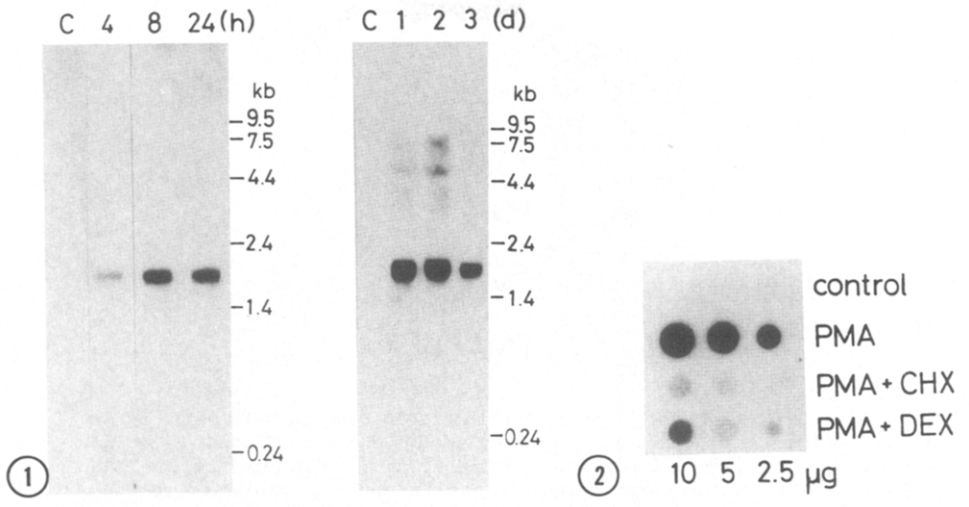

Figure 1. Time course of IL-1β mRNA expression in U937 cells. Cells were treated with PMA at 25 ng/ml for the indicated periods. Total cellular RNA was blotted on each lane (10 μg). Control (C) represents the total cellular RNA from uninduced U937 cells. Molecular size markers of RNA (BRL) are shown on the right. h,hours; d,days.

Figure 2. Accumulation of IL-1β mRNA in U937 cells after various treatments. Cells were treated with indicated reagents for 8 hours. Cytoplasmic RNA was extracted and the indicated amounts of cytoplasmic RNA was applied onto nitrocellulose filter. Control shows cytoplasmic RNA from non-treated U937 cells.

lymphocytes (25). Next we examined whether IL-1β mRNA expression induced with PMA requires the newly synthesized proteins. Dot blot analysis using cytoplasmic RNA 8 hours after PMA treatment showed that IL-1β mRNA is induced with PMA only, but is not induced with PMA in the presence of 10 μg/ml of cycloheximide(CHX), the inhibitor of de novo protein synthesis (Fig. 2). The run-on analysis using nuclei showed that CHX inhibits the transcription of IL-1β (Fig. 3). Our data indicate that PMA induces the transcription of IL-1β, which requires the newly synthesized proteins in U937 cells. Recently S.W.Lee et al., have reported that IL-1β mRNA induced with PMA and LPS is not inhibited with CHX in U937 cells (26). Therefore, it may be suggested that the additional signal by LPS replaces the newly synthesized proteins which are required in the transcription of IL-1β induced with PMA. Since IL-1β mRNA was not induced with LPS only in U937 cells (data not shown), the signal with PMA is essential for the induction of IL-1β mRNA.

Inhibition of the transcription of the IL-1β gene by dexamethasone

In order to examine the effect of Dex on the expression of IL-1β mRNA induced with PMA, we isolated cytoplasmic RNA from PMA-induced and

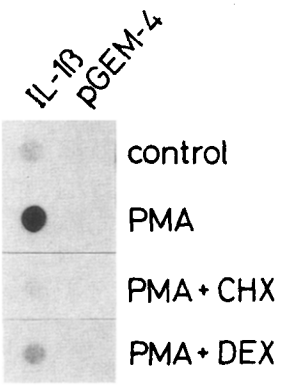

Figure 3. Transcriptional analysis of the IL-1β gene in U937 cells after various treatments. Cells were treated as indicated in Figure 2 legend and their nuclei were isolated. Equal counts of radiolabeled RNAs synthesized in run-on transcription assays were hybridized to the linearized and denatured IL-1β (pcD-GIF-16) and pGEM-4 plasmid DNA immobilized on nitrocellulose filters.

PMA/Dex-treated cells. Dot blot anlysis showed that the expression of IL-1β mRNA induced with PMA was inhibited with Dex (Fig. 2). Nuclear run-on analysis showed that the transcription of IL-1β was inhibited in the presence of PMA and Dex (Fig. 3). This indicates that the transcription of IL-1β induced with PMA is inhibited by Dex in U937 cells. Recently P.J.Knudsen et al., have reported that the expression of IL-1β mRNA in bacterial toxin-stimulated U937 cells is inhibited with Dex (27), and S.W.Lee et al., have shown that the transcription of IL-1β induced with PMA and LPS is inhibited with Dex (26). Our data and their reports show that Dex inhibits the expression of IL-1β mRNA in U937 cells although IL-1β mRNA was induced with the different stimuli, respectively.

Glucocorticoids have been shown to stimulate the transcription of various genes by mechanisms involving the interaction of the glucocorticoid hormone receptor with glucocorticoid response elements (GRE) (28). Recent evidence demonstrates that glucocorticoids can also negatively regulate specific gene expression (29 - 31). The IL-1β gene has been reported to have a sequence homologous to GRE in intron 5 (32). However two more homologous sequences to the GRE consensus sequence (33,34) can be seen in 5'-flanking region (position -588 to -574) and in exon 6 (position 5344 to 5358). Whether these GRE sequences are involved in the negative regulation of transcription by Dex is presently unknown.

It has been reported that IL-1 induces glucocorticoids and that they inhibit the production of IL-1 in vivo (35). Our data suggest that the block of IL-1 production by glucocorticoids occur at the transcriptional level in vivo.

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