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Dexamethasone regulation of the expression of cytokine mRNAs induced by interleukin-1 in the astrocytoma cell line U373MG

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BSF-2/IL-6, GM-CSF and IL-1β mRNAs were induced by recombinant IL-1 in human astrocytoma cell line U373MG. The induction of BSF-2/IL-6 and IL-1β mRNAs did not require de novo protein synthesis while that of GM-CSF mRNA required a newly synthesized protein. Dexamethasone inhibited the induction of these cytokine mRNAs by IL-1. This process seems to require continued protein synthesis. These results suggest that the production of these cytokines are positively and negatively controlled by IL-1 and glucocorticoids, respectively, in astrocytes.

Interleukin-1; B-cell stimulatory factor-2; Interleukin-6; Granulocyte-macrophage colony-stimulating factor; Dexamethasone; Astrocytoma

1. INTRODUCTION

Interleukin-1 is a cytokine which has multiple biological activities involved in the regulation of the immune, inflammatory, endocrine and central nervous system [1–4]. Its biological activities include induction of various cytokines. IL-1 has been shown to induce the production of granulocyte-macrophage colony-stimulating factor [5–13], granulocyte CSF (G-CSF) [9–12] and B-cell stimulatory factor-2/interleukin-6/interferon-β_2 (IFN-β_2)/26-kDa protein [14–17]. Recently it has also been reported that IL-1 can induce its own synthesis [18–20]. However it has not been clear whether IL-1 can induce all of these cytokines in a given cell type or different cells respond differently to IL-1 action. The role of IL-1 in regulation of the expression of various cytokines at its molecular level remains to be elucidated.

Glucocorticoids, the immunosuppressive and anti-inflammatory agents, have been shown to decrease mitogen induced production of IL-2 [21], IL-3 [22] or GM-CSF [22,23], and also production of IL-1 by lipopolysaccharide (LPS) [24–28] or phorbol ester [29].

During the course of our studies on the effect of IL-1 on CSF production, we found that CSF activities were induced by IL-1 in human astrocytoma cell line U373MG. Therefore we examined what kind of cytokine mRNAs were induced by IL-1 as well as the regulatory mechanism of their induction by IL-1. We also studied glucocorticoids regulation of the induction of cytokine mRNAs by IL-1. In this paper, we describe that IL-1 induced BSF-2, GM-CSF and IL-1β mRNAs in U373MG cells and dexamethasone decreased the induced level of these mRNAs.

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Abbreviations: IL-1, interleukin-1; BSF-2, B-cell stimulatory factor-2; IL-6, interleukin-6; GM-CSF, granulocyte-macrophage colony-stimulating factor; rIL-1, recombinant IL-1; LPS, lipopolysaccharide

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2. MATERIALS AND METHODS

2.1. *Cell culture*

Human astrocytoma cell line U373MG was obtained from the American Type Culture Collection (ATCC). U373MG cells were grown at 37°C in 90 mm-diameter dish in Eagle's minimal essential medium supplemented with 10% fetal calf serum. Upon reaching confluence, the culture medium was then replaced with fresh medium. The cells were treated with recombinant human IL-1β (rIL-1β, final concentration of 10 ng/ml) [30] alone, or rIL-1β with cycloheximide (final concentration of 10 μg/ml) and/or dexamethasone (final concentration of 10^{-6} M) for 8 h.

2.2. *RNA isolation and analysis*

At the end of the incubation period, cells were lysed in guanidine isothiocyanate and total cellular RNA was isolated by centrifugation through cesium chloride cushions [31]. RNA samples (10 μg) were denatured with glyoxal-DMSO, electrophoresed in 1.2% agarose gel and blotted onto nitrocellulose membrane [32] or serial dilutions of the RNA samples were spotted onto nitrocellulose membrane [33]. Prehybridization and hybridization were carried out as described [32] with nick-translated cDNA probe.

2.3. *DNA probes*

BSF-2/IL-6 was detected using BSF-2 cDNA fragment (1.1 kb) isolated from plasmid pBSF-2.38.1 [34]. GM-CSF was detected using a *Pst*I-*Nco*I fragment (0.5 kb) of GM-CSF cDNA derived from pGM-CSF 22-25 isolated from PHA and PMA stimulated human tonsillar cDNA library (unpublished). IL-1β was detected using a *Pst*I-*Pvu*II fragment (0.7 kb) of IL-1β cDNA from pcD-GIF-16 [35]. β-Actin DNA probe was purchased from Wako Pure Chemical Industries Ltd.

3. RESULTS AND DISCUSSION

In initial experiments, we observed that BSF-2, GM-CSF and IL-1β mRNAs were induced by rIL-1α or rIL-1β in U373MG cells. After the addition of rIL-1β, BSF-2 mRNA was detectable after 1 h which reached its maximum level at 4 h and then remained constant for at least additional 12 h. On the other hand, GM-CSF and IL-1β mRNAs were detectable only 8 h after rIL-1β treatment. Moreover the level of these mRNAs was lower than that of BSF-2 (fig.1). We have observed that G-CSF mRNA was also induced although its level was very low (not shown), and therefore we did not study G-CSF mRNA induction further. Seelentag et al. [10] have reported that IL-1 induced the expression of GM-CSF, G-CSF and M-CSF mRNAs in human endothelial cells and Yasukawa et al. [17] have shown BSF-2 mRNA induction by rIL-1β in U373MG cells. Our results showed that IL-1β induced not only GM-CSF and

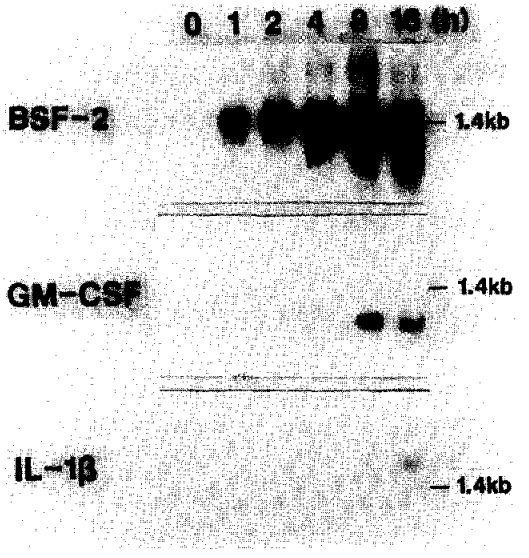

Fig.1. Time course of BSF-2, GM-CSF and IL-1β mRNAs induction by rIL-1β. U373MG cells were incubated for the indicated time periods with rIL-1β. Total cellular RNA (10 μg) was subjected to Northern blot analysis. The blot was hybridized with the indicated ^{32}P-labeled cDNA probe on the left of each panel.

G-CSF mRNAs but also those of BSF-2 and IL-1β in astrocytoma cell line. This suggests that IL-1 can induce the production of several cytokines in the same cell.

The requirement for newly synthesized proteins in the induction of these cytokine mRNAs was explored by the use of cycloheximide, an inhibitor of protein synthesis. The level of BSF-2 and IL-1β mRNAs induced by rIL-1β increased in the presence of cycloheximide. On the other hand, the level of GM-CSF mRNA induced by rIL-1β decreased markedly in the presence of cycloheximide. The level of β-actin mRNA remained unchanged (figs 2 and 4). These data indicate that the induction of BSF-2 and IL-1β mRNAs by rIL-1β does not require de novo protein synthesis, while GM-CSF mRNA induction by rIL-1β is dependent upon de novo protein synthesis. Therefore it appears that BSF-2 and IL-1β mRNAs induction by rIL-1β might be regulated by a common mechanism. It is not clear whether GM-CSF mRNA induction by IL-1 is a consequence of direct action of IL-1 or is brought about by a protein (intracellular or secreted) that is synthetically regulated by IL-1. Therefore it is possible that

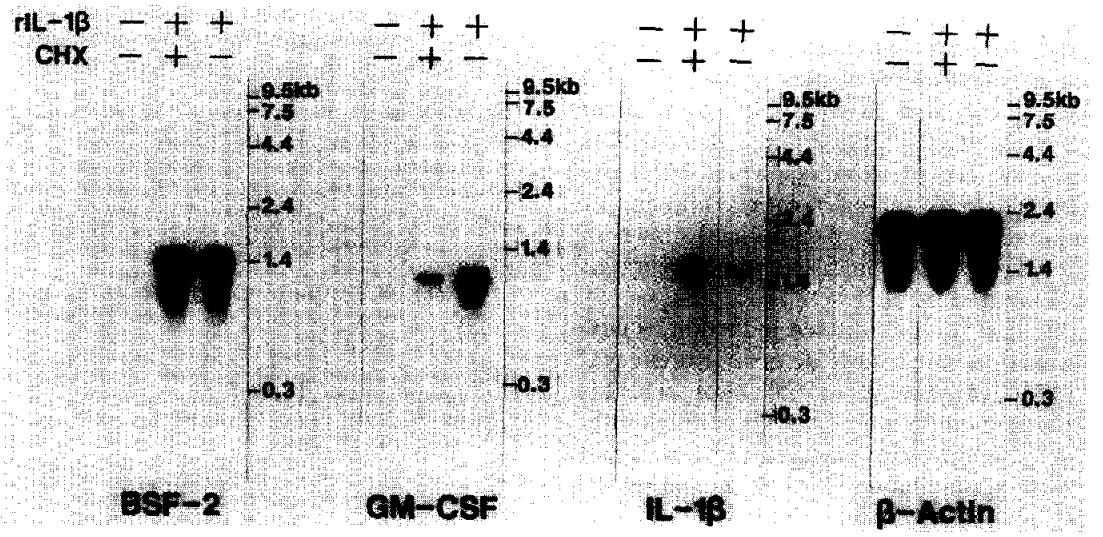

Fig.2. Effect of cycloheximide on the induction of BSF-2, GM-CSF and IL-1β mRNAs by rIL-1β. U373MG cells were incubated for 8 h in the presence (+) of rIL-1β with (+) or without (−) cycloheximide (CHX). RNA was analyzed as described in the legend to fig.1. cDNA probes used are shown at the bottom of each panel. Molecular size markers of RNA (BRL) are shown on the right of each panel.

mRNAs for various cytokines may be regulated entirely by different mechanisms by IL-1 in the same cell line.

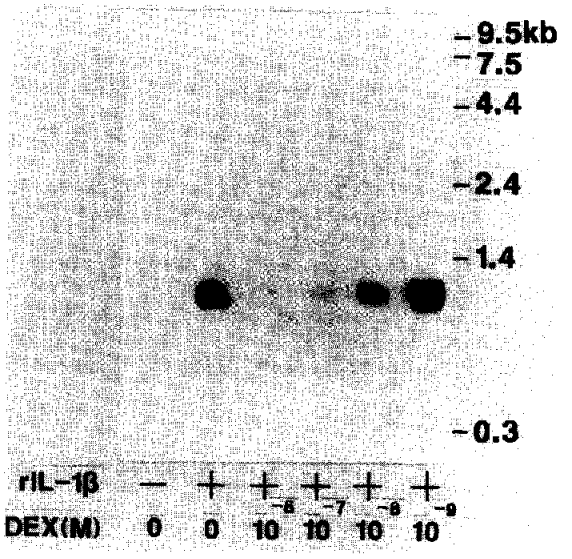

Fig.3. Concentration dependence of dexamethasone inhibition on GM-CSF mRNA induction. U373MG cells were incubated for 8 h in the presence (+) of rIL-1β with the indicated concentration of dexamethasone (DEX). RNA was analyzed as described in the legend to fig.1. Molecular size markers are described in the legend to fig.2.

Seelentag et al. [10] have described that the induction of GM-CSF mRNA by IL-1 in human endothelial cells was not affected by dexamethasone, while Thorens et al. [23] have reported that dexamethasone completely prevented GM-CSF mRNA induction by LPS. We therefore studied the effect of dexamethasone on the induction of BSF-2, GM-CSF and IL-1β mRNAs by rIL-1β. Our results showed that dexamethasone blocked the induction of these cytokine mRNAs by rIL-1β (fig.4). As shown in fig.3, dexamethasone inhibited rIL-1β induced GM-CSF mRNA level in a

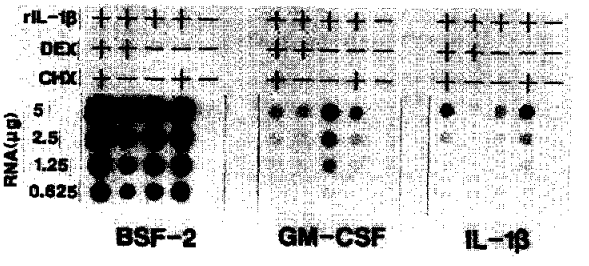

Fig.4. Effect of dexamethasone and cycloheximide on the induction of BSF-2, GM-CSF and IL-1β mRNAs by rIL-1β. U373MG cells were incubated for 8 h with (+) or without (−) the indicated reagents. The indicated amount of total cellular RNA was applied onto nitrocellulose filter. The filter was hybridized with the cDNA probe indicated at the bottom of each panel.

dose-dependent fashion. The suppressive dose range (10^{-6}–10^{-8} M) of dexamethasone was in the pharmacological to physiological range. We also observed a dexamethasone dose-dependent decrease in the level of BSF-2 and IL-1β mRNAs induction by rIL-1β (not shown). Therefore glucocorticoids may inhibit not only mitogen or LPS induced production of cytokines shown in several reports [21–29], but also the production of cytokines induced by IL-1.

It has been shown that mRNA induction by dexamethasone does not require de novo protein synthesis [36]. However it is not clear whether dexamethasone inhibition of mRNA induction requires de novo protein synthesis or not. Fig.4 shows that accumulation of BSF-2 and IL-1β mRNAs by rIL-1β is not inhibited by dexamethasone in the presence of cycloheximide. Our results indicate that dexamethasone inhibition of BSF-2 and IL-1β mRNAs induction require de novo protein synthesis. The accumulation of GM-CSF mRNA might be also inhibited by dexamethasone in the similar mechanism. Therefore as yet undiscovered gene product(s) that may be induced by dexamethasone might be involved in the decrease of the level of BSF-2, GM-CSF and IL-1β mRNAs.

It has been reported that astrocytes produce IL-1 [37] and IL-1 stimulates the proliferation of astrocytes [38]. Our results suggest that IL-1 induces the production of several cytokines and glucocorticoids regulate their production negatively in astrocytes. These cytokines that are differentially regulated by IL-1 and glucocorticoids may play important roles in central nervous system. The regulatory mechanism of the expression of these cytokines might be also common to the target cells of IL-1.

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