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# Signal transduction pathways leading to the production of IL-8 by human monocytes are differentially regulated by dexamethasone

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## SUMMARY

Previous studies have shown that IL-8 gene expression is enhanced by various stimuli, which induce different signal transduction pathways. A lipopolysaccharide (LPS)-induced pathway has been reported to be inhibited by glucocorticoids in monocytes. We have now examined the effect of dexamethasone on the LPS-induced and other signal transduction pathways leading to the production of IL-8 by human monocytes. Dexamethasone inhibited the production of IL-8 stimulated with a cyclic adenosine monophosphate analog or LPS. In contrast, dexamethasone had no significant effect on a phorbol ester (PMA)-stimulated IL-8 production. These results suggest that the signal transduction pathways leading to the production of IL-8 by human monocytes are differentially regulated by dexamethasone.

**Keywords** glucocorticoids IL-8 monocytes signal transduction

## INTRODUCTION

IL-8 is a chemotactic factor for neutrophils [1] and T cells [2]. It is produced by monocytes, neutrophilic granulocytes [3], epithelial cells of various origins, T cells, fibroblasts and endothelial cells after appropriate stimulation [4]. The IL-8 gene contains potential binding sites for several nuclear factors (NF) including activation factor-1 (AP-1), AP-2, glucocorticoid receptor [5], NF-κB-like factor and NF-IL-6-like factor [6]. The expression of IL-8 gene is enhanced by IL-1, tumour necrosis factor (TNF), phorbol esters, lipopolysaccharide (LPS), or agents which increase the level of intracellular cyclic adenosine monophosphate (cAMP) [5]. The binding sites for NF-κB and NF-IL-6-like factors are essential and sufficient for the IL-8 induction by IL-1, TNF or phorbol myristate acetate (PMA). LPS-induced IL-8 production is inhibited by glucocorticoids [5].

Glucocorticoid hormones regulate gene expression by the ligand-induced activation of the specific steroid receptor. The receptor acts as a transcription factor that binds to the glucocorticoid response element [7] or acts on another transcription factor, AP-1 [8–10]. Positive effects of glucocorticoids are mediated by the glucocorticoid responsive element (GRE) while the negative regulation is exerted through the AP-1 binding site. The pathways induced by LPS are not completely known. Protein kinase C (PKC) is involved in the signal transduction induced by LPS [11]. The cAMP signal is mediated by PKA [12]. PMA is a PKC activator and thus may affect either AP-1 or AP-2. However, both of these are of minor importance in the induction of IL-8 gene expression by PMA [6].

We have investigated the effects of dexamethasone on the signal transduction pathways induced by LPS, dbcAMP and PMA leading to the production of IL-8 by human monocytes.

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## MATERIALS AND METHODS

*Separation of monocytes*

Peripheral blood mononuclear cells (PBMC) were isolated from leucocyte-rich buffy coats (Finnish Red Cross Blood Transfusion Service, Helsinki, Finland) by centrifugation on Ficoll-Isopaque (Pharmacia, Uppsala, Sweden). Monocytes were further purified by adherence on plastic for 1 h at 37°C in culture medium: RPMI 1640 (Flow Laboratories, Irvine, UK) substituted with 10% heat inactivated human AB serum (Finnish Red Cross), 10 mM HEPES buffer (Sigma Chemical Co., St Louis, MO), 2 mM L-glutamine (Flow) and antibiotics. The adherent cells were harvested with a rubber policeman at 4°C and resuspended in culture medium at the concentration of $10^6$ cells/ml of which 80–90% were monocytes.

*Stimulation of monocytes*

Monocytes were stimulated by addition of one of the following reagents or their combinations in culture medium for 24 h: LPS 1–1000 ng/ml (LPS B, *E. coli* 026:B6, Difco Laboratories, Detroit, MI), 0·1–1·0 mM dbcAMP, PMA 1–10 ng/ml, 1 μM prostaglandin E2 ($PGE_2$) or 0·5 mM isobutylmethylxanthine (IBMX) purchased from Sigma Chemical Co.

**Table 1.** Effects of the different stimuli on IL-8 protein production by monocytes

| Stimulus | IL-8 (ng/ml) | | | |
|---|---|---|---|---|
| | Donor 1 | Donor 2 | Donor 3 | Mean (s.d.) |
| None | 12 | 22 | 3 | 12 (10) |
| PMA 1 ng/ml | 16 | 52 | 4 | 24 (25) |
| PMA 10 ng/ml | 317 | 268 | 212 | 266 (53) |
| dbcAMP 0·1 mM | 134 | 17 | 7 | 53 (70) |
| dbcAMP 1 mM | 248 | 527 | 218 | 331 (170) |
| LPS 1 ng/ml | 411 | 526 | 289 | 409 (119) |
| LPS 10 ng/ml | 657 | 379 | 188 | 408 (236) |
| LPS 100 ng/ml | 508 | 422 | 263 | 398 (124) |
| LPS 1000 ng/ml | 741 | 481 | 431 | 551 (166) |
| $PGE_2$ 1 μM | 156 | 11 | 19 | 62 (82) |
| IBMX 0·5 mM | 482 | 357 | 159 | 333 (163) |
| $PGE_2$+IBMX | 779 | 487 | 235 | 500 (272) |

PMA, Phorbol myristate acetate; LPS, lipopolysaccharide; $PGE_2$, prostaglandin E2; IBMX, isobutylmethylxanthine; dbcAMP, dibutyryl cyclic AMP.

In some experiments $10^{-5}$–$10^{-6}$ M dexamethasone (Decadron, Merck Sharp et Dohme B.V., Haarlem, The Netherlands) was added 1 h before stimulation. To inhibit the effects of dexamethasone, $10^{-5}$–$10^{-6}$ M promegestone was used together with dexamethasone. Promegestone (RU486, Roussel-Uclaf, Romainville, France) was kindly donated by Dr Oskari Heikinheimo (University of Helsinki, Finland).

*Assay for IL-8*

Monocyte cultures were lysed by two cycles of freezing and thawing. IL-8 in the lysates was measured using a double ligand immunoassay (Sandoz Research Institute, Vienna, Austria) based on specific [13] mouse anti-IL-8 and goat polyclonal anti-IL-8 MoAbs linked to alkaline phosphatase. The detection limit of the assay was 20 pg/ml of recombinant IL-8.

*Statistical analysis*

The summary of concentrations of IL-8 is expressed as mean and s.d. Student's two-sided *t*-test for paired data was used to assess differences between the IL-8 concentrations of untreated cells and cells treated with dexamethasone. In Fig. 2, the effect of dexamethasone is expressed as mean percentage of inhibition ±s.e.m. Each cell group that had received either dexamethasone or dexamethasone plus RU 486 was compared with the group of cells that did not receive dexamethasone.

## RESULTS

*Production of IL-8 by monocytes*

LPS, PMA and dbcAMP, a cell-permeable structural analog of cAMP, enhanced the IL-8 protein production of monocytes (Table 1). These results are in accordance with previous studies on IL-8 mRNA levels [5]. $PGE_2$, a cAMP inducer, alone did not induce significant IL-8 production in all donors. However, the IL-8 production induced by a combination of $PGE_2$ and a phosphodiesterase inhibitor, IBMX, was similar to the IL-8

**Table 2.** Effect of dibutyryl cyclic AMP (dbcAMP) on IL-8 production by human monocytes stimulated with phorbol myristate acetate (PMA) or lipopolysaccharide (LPS)

| Stimulus | IL-8 (ng/ml) | |
|---|---|---|
| | Donor 4 | Donor 5 |
| None | 5 | 4 |
| dbcAMP 1 mM | 128 | 377 |
| PMA 10 ng/ml | 200 | 189 |
| PMA 10 ng/ml+dbcAMP 1 mM | 386 | 666 |
| LPS 10 ng/ml | 264 | 565 |
| LPS 10 ng/ml+dbcAMP 1 mM | 176 | 233 |

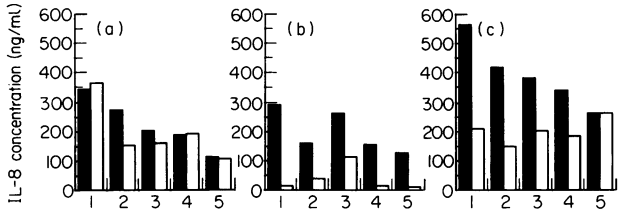


**Fig. 1.** The effect of dexamethasone on IL-8 production by stimulated human monocytes derived from five independent blood donors. Monocytes were stimulated with 10 ng/ml phorbol myristate acetate (PMA) (a); 1 mM dibutyryl cyclic AMP (dbcAMP) (b); or 10 ng/ml lipopolysaccharide (LPS) (c) in the presence (□) or absence (■) of $10^{-5}$ M dexamethasone. The IL-8 content was measured after 24 h culture.

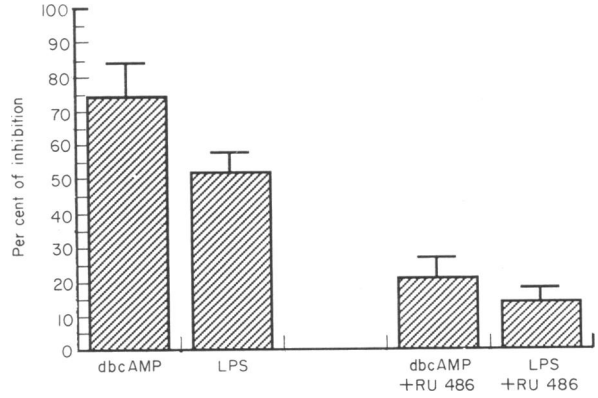


**Fig. 2.** The influence of RU 486 on the inhibitory effect of dexamethasone on IL-8 production. Monocytes were stimulated with either 1 mM dibutyryl cyclic AMP (dbcAMP) or 10 ng/ml lipopolysaccharide (LPS) in the presence or absence of either dexamethasone ($10^{-5}$ M) or dexamethasone plus RU 486 ($10^{-6}$ M). After 24 h the cultures were harvested and their IL-8 content was determined by an IL-8 specific ELISA. The per cent of inhibition (either in the presence of dexamethasone alone or dexamethasone plus RU 486) was calculated as described in Materials and Methods. The values represent mean of three independent experiments. Bars indicate inhibition percentages ±s.e.m.

production induced by 1 mM dbcAMP. IBMX alone induced lower IL-8 protein levels than in combination with $PGE_2$. The stimulatory effects of PMA and dbcAMP on IL-8 production were additive. However, dbcAMP reduced the LPS-induced IL-8 production by human monocytes (Table 2). The reducing effect of dbcAMP on LPS-induced IL-8 production is comparable to its effects on IL-1$\beta$ production [14]. The stimulatory effect of PMA and dbcAMP on IL-8 production was only additive. Their effect on IL-1 production was synergistic [14].

A high concentration of dexamethasone partially inhibited IL-8 production induced by LPS ($P<0{\cdot}05$, Fig. 1). The inhibitory effect of dexamethasone on cAMP-induced IL-8 production was statistically significant ($P<0{\cdot}02$). In contrast, the effect of dexamethasone on IL-8 production induced by PMA was not significant. Optimal concentrations of RU 486 (a glucocorticoid antagonist on receptor level) reduced the inhibitory effect of dexamethasone on IL-8 production by human monocytes (Fig. 2). This confirms that the results obtained with dexamethasone were specific steroid effects mediated by the glucocorticoid receptor.

## DISCUSSION

This study shows that the signal transduction pathways leading to the production of IL-8 by human monocytes are differentially regulated by dexamethasone. The stimulus dependence of the effects of glucocorticoids on both the protein production and mRNA expression of other cytokines has been reported previously. The LPS-induced production of IL-1$\beta$ and TNF are significantly inhibited by glucocorticoids, whereas the PMA-induced production of TNF was only minimally reduced [15] and the production of IL-1$\beta$ was in fact enhanced [16] by glucocorticoids in human monocytes. In accordance with the regulation of IL-1$\beta$ and TNF, the present data could not show a significant decrease in the PMA-induced production of IL-8. However, unlike the findings on IL-1$\beta$, any significant enhancing effect of glucocorticoids could not be demonstrated with IL-8.

The signal transduction systems activated by phorbol esters and by cAMP have been reported to synergize with each other when leading to IL-1$\beta$ production [14] but to be only additive when leading to IL-6 production [17]. This study suggests that these two signal transduction systems do not interact with each other when leading to IL-8 production as their stimulating effects were additive. In contrast, dbcAMP reduced the production of IL-8 induced by LPS, which indicates that the pathways used by these two signals are not independent of each other. Similar results have been observed with the regulation of the production of IL-1$\beta$ by human monocytes [14,18].

In a recent study, Mukaida *et al.* [6] showed that the binding sites for NF-KB- and NF-IL-6-like factors are responsible for the PMA-induced expression of IL-8. As we could not show any significant effect of dexamethasone on PMA-induced IL-8 expression, we suggest that the effect of dexamethasone on IL-8 expression is not likely to be directly mediated by NF-KB- or NF-IL-6-like factors. The signal transduction pathways regulated by LPS needed higher concentrations of dexamethasone than the pathways regulated by dbcAMP. However, both concentrations were of the level known to be sufficient for gene activation [8]. The signal induced by cAMP could be mediated by the cAMP responsive element (CRE) located within the IL-8 gene [5]. Akerblom *et al.* [19] have suggested that glucocorticoids are effective inhibitors of the cAMP response of a human glycoprotein gene. The overlapping of a GRE and the CRE was suggested to be involved in the mechanism of inhibition. However, the CRE and the GRE found in the IL-8 gene are not overlapping [5]. The final mechanism of the action of dexamethasone on the production of IL-8 will remain open until intracellular levels of the different transcription factors and their functions are studied.

As monocytes are among the first cells to invade the early lesions of psoriatic skin [20], their production of IL-8 could be responsible for the later neutrophil accumulation. The chemotactic effect of IL-8 on neutrophils has also been demonstrated *in vivo* by injecting IL-8 in human skin [21]. Higher IL-8 mRNA levels in psoriatic epidermis than in normal epidermis further suggests the involvement of IL-8 in the pathology of psoriasis [22]. Glucocorticoids are widely used for treatment of psoriasis and it could be assumed that their effect is partly mediated by the inhibition of IL-8 production of monocytes.

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