

Effects of dexamethasone on the profile of cytokine secretion in human whole blood cell cultures

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Abstract

Experimental objectives: The interaction between the endocrine and immune systems is a very intriguing area. Endogenous glucocorticoids, as end-effectors of the hypothalamo–pituitary–adrenal axis, inhibit the immune and inflammatory responses and are used as immunosuppressive drugs in many inflammatory, autoimmune and allergic diseases. The aims of this study were to investigate the effects of dexamethasone on the profile of cytokine secretion in whole blood cell cultures from healthy subjects and to analyse the gender-related sensitivity to dexamethasone on each cytokine secretion. **Results:** There was a significant inhibition by dexamethasone (from 1 to 100 nM) on the secretion of monokines (IL-1 β , IL-6, IL-8 and TNF α) and lymphokines (IL-2, IL-4, IL-10 and IFN γ), either after LPS or PHA stimulation ($P < 0.01$). Interleukin 4 and IL-10 were less inhibited than IFN γ ($P < 0.05$ at 1 nM, $P < 0.01$ at 10 nM and $P < 0.001$ from 100 nM to 10 μ M). No gender difference was observed in the rate of inhibition of the secretion of each cytokine. **Conclusion:** This study shows that the inhibition of cytokine secretion by dexamethasone is more marked on Th1-type cytokines than on Th2-type cytokines. These data support the idea that glucocorticoids may induce a shift from the Th1 to Th2 profile of cytokine secretion. © 1998 Elsevier Science B.V.

Keywords: Glucocorticoids; Cytokines

1. Introduction

A very intriguing current area of research is the analysis of the multiple interactions between the endocrine and the immune systems [1]. The hypothalamic–pituitary–adrenal (HPA) axis is intimately involved in the regulation of the immune system [2]. Cytokines such as TNF α , IL-1 β and IL-6 were shown to modulate the activity of the HPA axis. Endogenous glucocorticoids, as the end-effectors of the HPA axis, suppress the immune and inflammatory responses [3,4]. The susceptibility to streptococcus cell wall-induced arthritis or to experimental allergic encephalomyelitis in susceptible Lewis and resistant Fischer rats is inversely related to the magnitude of the HPA axis response to inflammatory mediators [5].

Glucocorticoids are used as immunosuppressive drugs in many inflammatory, autoimmune and allergic diseases [6]. Differences in the sensitivity of target tissues to glucocorticoids have emerged from the different clinical responses to glucocorticoids observed among patients suffering from inflammatory and autoimmune diseases. Not only the number or the affinity of glucocorticoid receptors (GR), but each step of the GR signal transduction pathway is implicated in defining glucocorticoid sensitivity [7,8]. It has also been suggested that glucocorticoid sensitivity differences could explain some gender dimorphism in the pattern of immune responses. Glucocorticoids seem to control inflammation more efficiently in females than in males [9,10].

The pattern of cytokine secretion by Th CD4 + lymphocytes led to the definitions of two distinct subpopulations, the Th1- and Th2-type lymphocytes. Th1 cells stimulate T

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cell-mediated immunity while Th2 cells stimulate B cell-mediated immunity. Th1 cells are defined by their production of IL-2, IFN γ and TNF β , and Th2 cells are defined by their production of IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. Both types of cells produce IL-3, TNF α and GM-CSF [11,12]. The boundary between these two subsets is not sharp and there seems to exist a continuum in the cytokine profiles from Th1 to Th2 and inversely [13].

Recent studies have shown the inhibition by dexamethasone of cytokine secretion by peripheral blood mononuclear cells (PBMCs) or by cell lines. Few studies have reported the effect of glucocorticoids on cytokine secretion in human healthy subjects [14]. Whole blood cell culture is an elegant *ex vivo* method to analyse cytokine secretion, in a controlled environment, and to study the biological effects of any substance or drug on cytokine secretion [15,16]. Lipopolysaccharide (LPS) and phytohemagglutinin A (PHA) are frequently used as powerful stimulants of the secretion of cytokines, mainly but not exclusively, by monocytes and lymphocytes, respectively.

Our aim was to study the influence of dexamethasone on cytokine secretion in whole blood cell cultures from healthy subjects. We also compared the effect of dexamethasone on the profile of Th1- and Th2-type cytokines, as well as the effect of dexamethasone between men and women.

2. Materials and methods

2.1. Human samples

Fourteen healthy volunteers (age range: 25–45 years; seven women and seven men) served as blood donors. Any diseases and/or any drugs (e.g. oral contraceptives) were ruled out. Blood from female volunteers was drawn during the oestrogenous phase of their cycle. Blood samples were collected in apyrogenic heparinised tubes provided by Biosource/Medgenix (Fleurus, Belgium).

2.2. Whole blood cell cultures

The blood was treated as previously described [14]. The blood was immediately processed and diluted with 1/10 RPMI 1640 supplemented with 2 mM glutamine, 100 U/ml penicillin, 100 μ g/ml streptomycin (BioWittaker, Belgium), and distributed in 2-ml wells. RPMI 1640 medium was tested for apyrogenicity. Although the culture medium was not supplemented with exogenous serum, whole blood cell cultures was performed in 10% autologous serum. Two activators were then added in separate wells: LPS (endotoxin from *Salmonella enteritidis*, Sigma, St Louis, MO, USA) at a final concentration of 25 μ g/ml; and PHA (phytohemagglutinin A, Wellcome Diagnosis, UK) at a final concentration of 1 μ g/ml. These activators were

used at this concentration to reach an optimal effect on cytokine secretion, as was previously described [15,16]. A synthetic glucocorticoid, dexamethasone, was added at a final concentration ranging from 10^{-9} to 10^{-5} M in separate wells containing either PHA or LPS. Plates were incubated at 37°C with 5% CO $_2$. The incubation period was 24 h for LPS-stimulated whole blood and 72 h for PHA-stimulated whole blood [15,16]. The contents of the wells were then collected and centrifuged at $900 \times g$ for 10 min. Supernatants were then recovered and stored at -20°C before analysis.

2.3. Immunoassays

Human natural cytokines were measured with specific immunoassays from Biosource/Medgenix (Fleurus, Belgium). Four monokines (IL-1 β , IL-6, IL-8 and TNF α) and four lymphokines (IL-2, IL-4, IFN γ and IL-10) were specifically detected without any cross-reactivity. Each immunoassay was performed according to the information sheet. The detection limit was 2 pg/ml for IL-1 β , 2 pg/ml for IL-6, 0.7 pg/ml for IL-8, 3 pg/ml for TNF α , 0.1 UI/ml for IL-2, 1 pg/ml for IL-4, 1 pg/ml for IL-10 and 0.03 UI/ml for IFN γ . Plates were read at 460 nm by a microplate EASIA reader from Medgenix Diagnostics. Absorbancy was transformed to cytokine concentration using a standard curve computed by Medgenix Elisa software.

2.4. Statistical analyses

The inhibition and stimulation percentage of control were computed for each dexamethasone dose. Results were expressed as mean \pm SEM. Log transformation was used for the percentage of response to normalise the distribution. A generalised linear mixed model (SAS Proc Mixed) was used to analyse the dexamethasone general effect on cytokine secretion and the dexamethasone dose effect on cytokine secretion. Another generalised linear mixed model with random effects (SAS Proc Mixed) was performed for each cytokine to evaluate the hypothesis of a sex effect on the response. SAS Proc Mixed is a strict analysis; statistically mixed models are needed because of correlations between repeated measurements on the same subject. All results were considered to be significant at the 5% critical level ($P < 0.05$). Statistical analyses were carried out using SAS software package (SAS Institute Inc., Cary, NC, USA).

3. Results

There was a significant dose–response inhibition from 1 to 100 nM of dexamethasone on the secretion of the four different monokines and the four different lymphokines after LPS and PHA stimulation, respectively ($P < 0.01$).

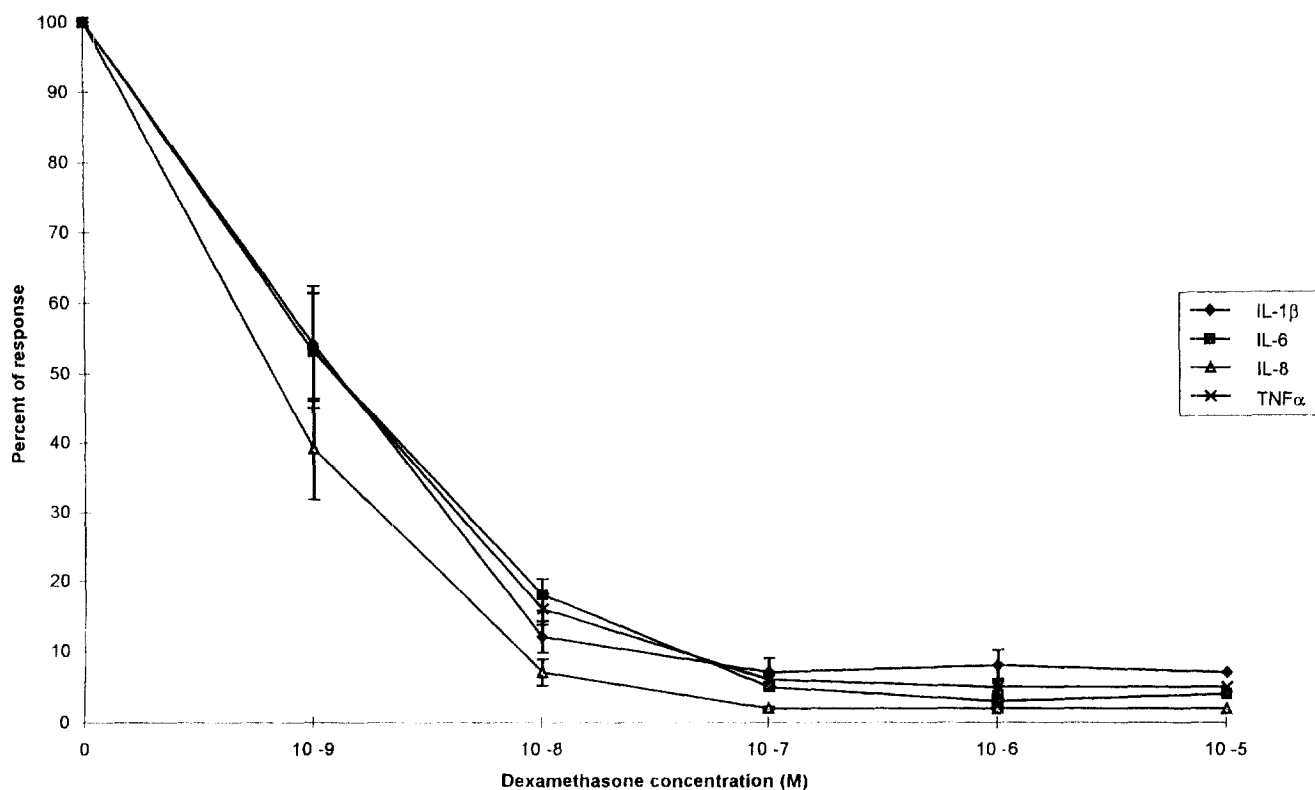


Fig. 1. Effect of dexamethasone on LPS-induced cytokine (IL-1 β , IL-6, IL-8 and TNF α) secretion in whole blood cell cultures from 14 healthy subjects (seven males and seven females). Data are expressed as the mean \pm SEM.

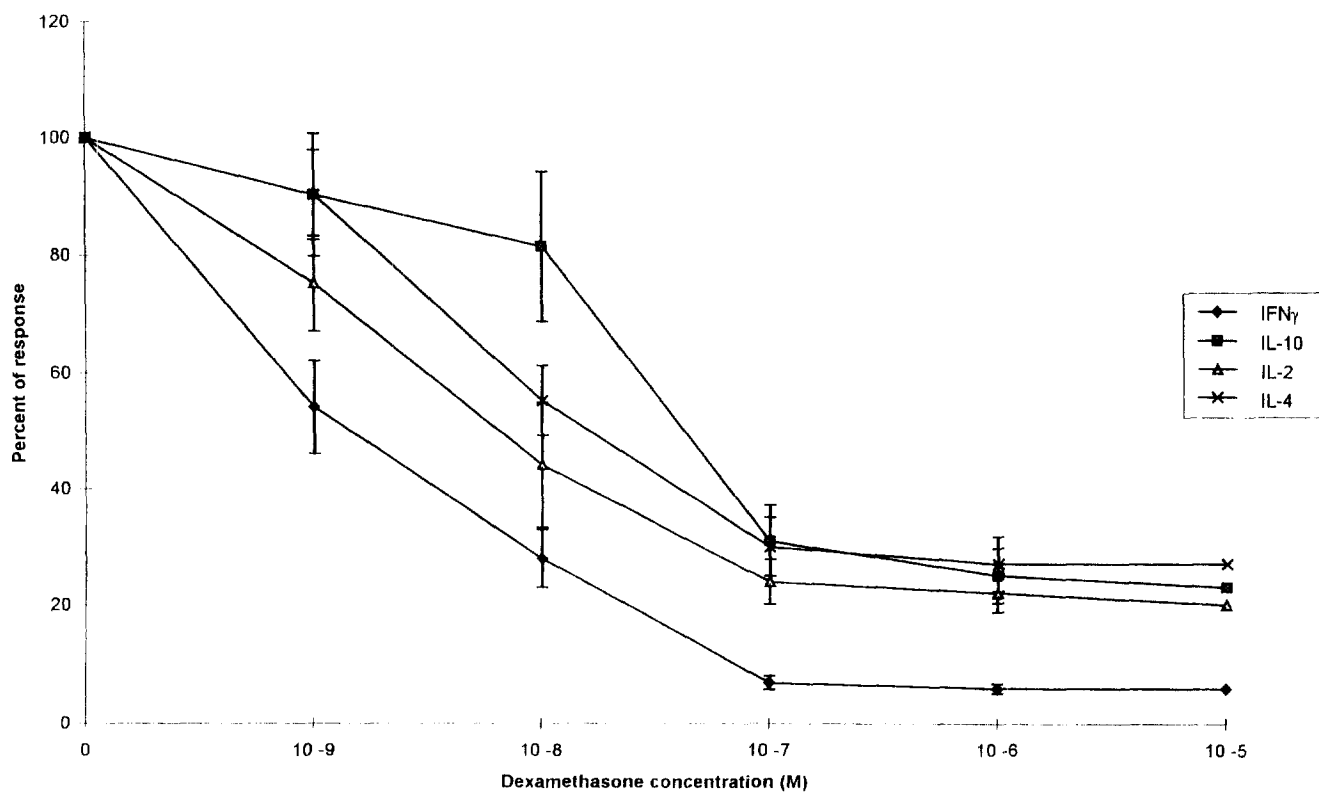


Fig. 2. Effect of dexamethasone on PHA-induced cytokine (IL-2, IL-4, IFN γ and IL-10) secretion in whole blood cell cultures from 14 healthy subjects (seven males and seven females). Data are expressed as the mean \pm SEM.

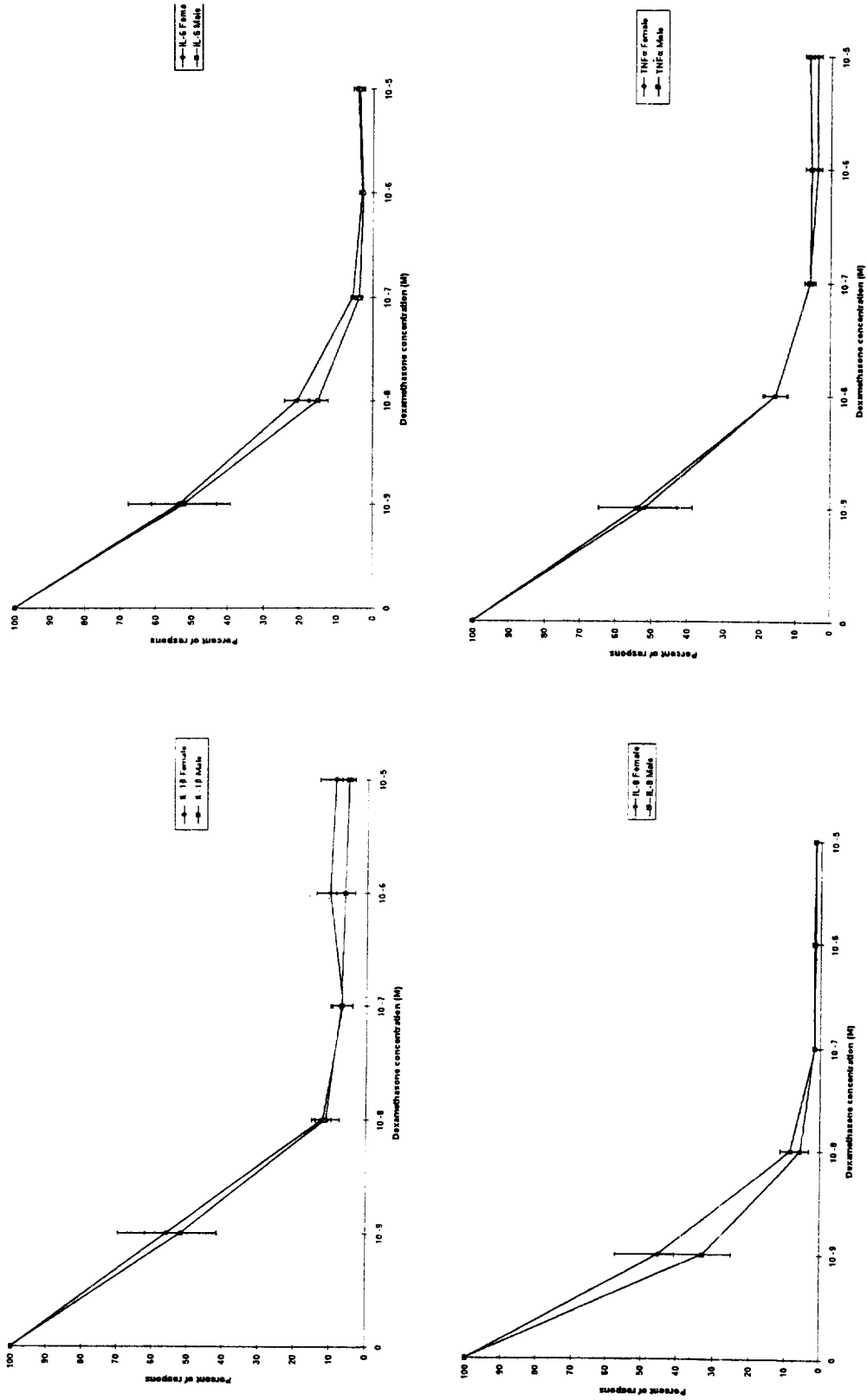


Fig. 3. Comparison between males and females of the effect of dexamethasone on LPS and PHA-induced cytokine secretion in whole blood cell cultures in 14 healthy subjects (seven males and seven females). Data are expressed as the mean \pm SEM.

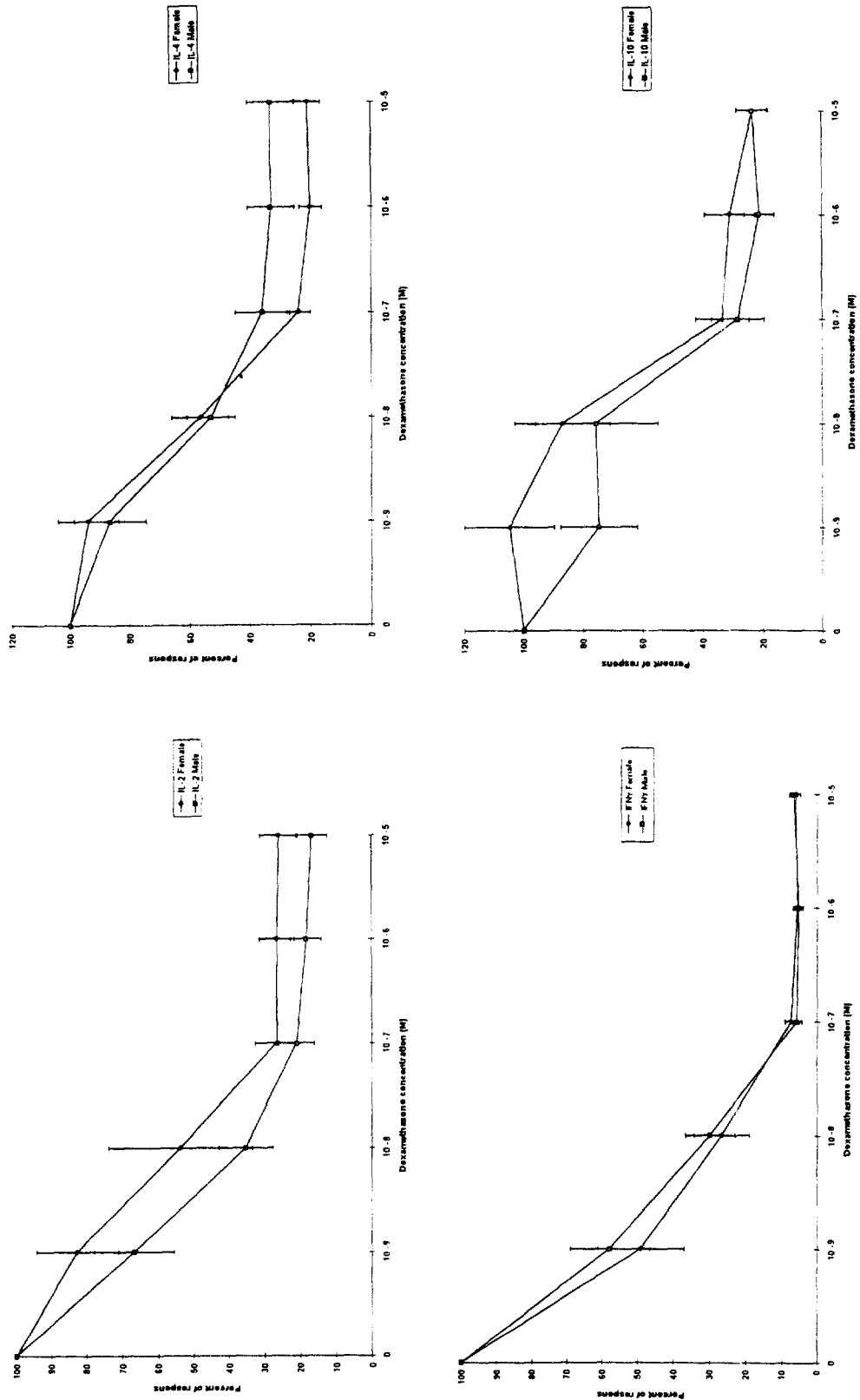


Fig. 3. (continued)

From 100 nM to 10 μ M, the inhibition was complete. The optimal secretion of cytokines was observed after 24 h with 25 pg/ml of LPS stimulation and after 72 h with 1 μ g/ml of PHA [15,16]. There was a significant difference in the rate of inhibition by dexamethasone between monokines and lymphokines following LPS and PHA stimulation, respectively ($P < 0.05$).

Considering the profile of monokine secretion, there was a significant difference in the rate of inhibition by dexamethasone between IL-8 and IL-1 β , TNF α and IL-6 ($P < 0.01$). The chemokine IL-8 was more inhibited than IL-1 β , IL-6 and TNF α by dexamethasone at 10 and 100 nM ($P < 0.05$) (Fig. 1). The dexamethasone concentration producing 50% inhibition of cytokine secretion (IC_{50}) was 0.8×10^{-9} M for IL-8 and 1.1×10^{-9} M for IL-1, IL-6 and TNF α .

Considering the profile of lymphokine secretion, there was a different rate of inhibition between IL-2, IL-4 and IL-10 on the one hand, and IFN γ on the other hand ($P < 0.01$). The level of inhibition by each dose of dexamethasone was significantly different between IL-4 and IL-10 on the one hand, and IFN γ on the other hand ($P < 0.05$ at 1 nM, $P < 0.01$ at 10 nM and $P < 0.001$ from 100 nM to 10 μ M) (Fig. 2). There was a difference in the level of inhibition by 1 nM of dexamethasone: 10% inhibition for IL-4 and IL-10, and 40% inhibition for IFN γ . At 10 nM, the level of inhibition was 15% for IL-10, 45% for IL-4 and 70% for IFN γ (Fig. 2). No significant difference was observed between IL-2 and IFN γ nor between IL-2 and IL-4 and IL-10 at each dose of dexamethasone. The dexamethasone concentration producing a 50% inhibition of cytokine secretion (IC_{50}) was 1.14×10^{-9} M for IFN γ , 7.9×10^{-9} M for IL-2, 20×10^{-9} M for IL-4 and 60×10^{-9} M for IL-10.

The analysis of the data with a generalised linear mixed model with random effects (SAS Proc Mixed) showed no difference in the rate of inhibition of monokine and lymphokine secretion between males and females (Fig. 3).

4. Discussion

The profile of eight cytokines evaluated in this study represents a large repertoire of mediators in the immune system. Interleukin 1 β , IL-6 and TNF α are the main cytokines of natural immunity and mediate nonspecific inflammation. Interleukin 8 is a chemokine involved in chemotaxis and activation of leukocytes. Interleukin 2 and IL-4, produced by antigen-specific CD4+ T cells, are cytokines that regulate lymphocyte activation, growth and differentiation. Interleukin 10 and IFN γ , derived from antigen-activated CD4+ and CD8+ T cells, play key roles in cell-mediated immune responses (monocytes, T cells, NK cells, leukocytes and others).

Cytokine levels in human blood were described as disease activity markers and as prognostic markers for

clinical relapse. Their pathophysiologic role in diseases is the subject of intense investigations [17].

Although our method does not allow the definition of the precise contribution from each cell population, dexamethasone-induced modulation of cytokine secretion was investigated in the natural environment of blood cells in accordance with the final objectives of this study. Monokines were more inhibited by dexamethasone in a dose-dependent fashion than lymphokines. Interleukin 8 was more inhibited than IL-1 β , IL-6 and TNF α . Each lymphokine was inhibited by dexamethasone in a different way. Dexamethasone inhibited IL-2 and IFN γ (Th1-type cytokines) and IL-4 and IL-10 (Th2-type cytokines) in a dose-dependent manner. The inhibition of IL-4 and IL-10, two important Th2-type cytokines, was less strong than that of IFN γ , an important Th1-type cytokine. Interleukin 2, a Th0- and Th1-type cytokine, was inhibited at a level between that of IL-4, IL-10 and IFN γ . One recent study has shown different modulation by dexamethasone between IL-10 and IL-12. Elenkov et al. showed a potent inhibition of IL-12 at 10^{-7} M, but they did not observe significant IL-10 inhibition [14]. This discrepancy with our own observations on the IL-10 modulation could be explained by different stimulatory pathways and incubation periods. In the Elenkov study, the stimulation of IL-10 in whole blood cell culture was performed with LPS over an 18-h incubation, whereas we used PHA over a 72-h incubation. In a recent study, dexamethasone was also shown to inhibit IL-10 expression and secretion by PBMCs [18]. Lipopolysaccharide mainly stimulates monocytes through CD14 and is a polyclonal mitogen for B lymphocytes, whereas PHA is a polyclonal mitogen mainly for T lymphocytes. Unlike IL-2, IL-4 and IFN γ , IL-10 is secreted equally by monocytes and T lymphocytes. This could explain the difference observed in IL-10 modulation by glucocorticoids. The most clear difference in Th1-type/Th2-type cytokine modulation by dexamethasone was observed with IFN γ , a Th1-type cytokine, and with IL-4, IL-10, Th2-type cytokines. Thus, both of these studies support a shift from Th1 to Th2 which could be induced by dexamethasone.

In a recent paper, steroid-resistant asthma patients were found to have a dysregulation in Th1-type/Th2-type cytokines. Interleukin 4 and IL-2 concentrations in the bronchoalveolar lavage fluids were higher in steroid-resistant asthma than in steroid-sensitive asthma patients, whereas IFN γ levels were similar [19]. Kam et al. demonstrated in vitro that the combination of IL-4 and IL-2 induced a decrease in GR binding affinity on PBMCs from healthy subjects, while IFN γ antagonised this effect [20]. PBMCs from steroid-resistant asthma patients have a marked decrease of glucocorticoid receptor binding affinity. It is noteworthy that it was shown to return to normal values when PBMCs were cultivated in culture medium in the absence of added cytokines. A decrease of the GR binding affinity was observed again when PBMCs were

rechallenged with IL-4 and IL-2 [21]. Thus, IL-4, IL-2 and IFN γ seem to modulate GR binding affinity. In our study, the glucocorticoid inhibition dose–response curves of IL-2, IL-4 and IFN γ were significantly different. Altogether, these data suggest that a regulatory loop could exist in the glucocorticoid sensitivity between IL-2 and IL-4 on the one side, and IFN γ on the other.

It has been suggested that a difference in glucocorticoid sensitivity could explain gender-related differences in immune response [10]. A similar pattern of inhibition of each cytokine by dexamethasone was observed in this study between men and women. In addition, it has been previously reported that phenol red, a component of RPMI 1640, exerts some estrogen-like effect [22]. It is unlikely, however, that such an effect could influence the significance of our findings on dexamethasone effects since whole blood cell cultures from both sex have been performed in identical conditions. The hypothesis of gender-related differences in the modulation of cytokine profile by endogenous and exogenous glucocorticoids needs to be further investigated.

In conclusion, our data show a dexamethasone-mediated inhibition of cytokine secretion in human whole blood cell cultures. The inhibition was more marked on Th1-type than on Th2-type cytokines, and antiinflammatory cytokines were less inhibited than proinflammatory cytokines. This difference in the inhibition rate could represent a shift from Th1- to Th2-type that would be induced by glucocorticoids. Further studies using whole blood cell cultures will help in the study of the effects of glucocorticoids on the Th1/Th2 balance and the implication of this effect in quiescent or active, corticoid-sensitive or corticoid-resistant inflammatory diseases.

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