Tumor Necrosis Factor α Decreases, and Interleukin-10 Increases, the Sensitivity of Human Monocytes to Dexamethasone: Potential Regulation of the Glucocorticoid Receptor

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ABSTRACT

Resistance to glucocorticoid therapy has been observed in patients with autoimmune/inflammatory diseases and may be related to the inflammatory process itself. The aim of this study was to examine the ability of tumor necrosis factor α (TNFα, a proinflammatory cytokine) and interleukin (IL)-10 (an anti-inflammatory cytokine) to differentially regulate the sensitivity of human monocytes/macrophages to glucocorticoids. To accomplish this, we first analyzed the pattern of TNFα and IL-10 inhibition by dexamethasone in LPS-stimulated whole-blood cell cultures. Second, we studied the modulation of the sensitivity of these cells to dexamethasone by preincubation with TNFα or IL-10 and measurement of LPS-stimulated IL-6 secretion. In addition, we evaluated the effect of dexamethasone on phorbol-myristate-acetate-stimulated IL-1 receptor antagonist secretion by the human monocytic cell line U937. Finally, we investigated whether the modulation of corticosensitivity in TNFα- and IL-10-pretreated U937 cells was related to a change of the glucocorticoid receptor concentration and affinity. Dexamethasone had different effects on LPS-induced TNFα and IL-10 secretion; whereas it suppressed TNFα in a dose-dependent fashion, its effect on IL-10 secretion was biphasic, producing stimulation at lower, and inhibition at higher doses. The concentration of LPS employed influenced the effect of dexamethasone on IL-10 secretion ($P < 0.001$). Pretreatment with TNFα diminished, and with IL-10 improved, the ability of dexamethasone to suppress IL-6 secretion in whole-blood cell cultures ($P < 0.01$ for both) and to enhance IL-1 receptor antagonist secretion by U937 cells ($P < 0.05$ for both). TNFα decreased ($P < 0.001$), while IL-10 increased ($P < 0.001$), the concentration of dexamethasone binding sites in these cells, with no discernible effect on their binding affinity. We conclude that glucocorticoids differentially modulate TNFα and IL-10 secretion by human monocytes in a LPS dose-dependent fashion and that the sensitivity of these cells to glucocorticoids is altered by TNFα or IL-10 pretreatment; TNFα blocks their effects, whereas IL-10 acts synergistically with glucocorticoids. This is accompanied by opposite glucocorticoid receptor changes, respectively opposing and favoring glucocorticoid actions. This study suggests that the pattern of pro-/antiinflammatory cytokine secretion may alter the response of patients to glucocorticoid therapy. (*J Clin Endocrinol Metab* **84:** 2834–2839, 1999)

GLUCOCORTICOIDS are widely employed in the treatment of autoimmune and inflammatory diseases (1). Varying sensitivity to glucocorticoid therapy has emerged from the different clinical responses to glucocorticoids among the multitude of patients with autoimmune/inflammatory diseases, on the basis of which they are classified as corticosteroid-sensitive or corticosteroid-resistant. The response of the disease to glucocorticoid therapy depends not only on the glucocorticoid receptor (GR) concentration and binding affinity but also on postreceptor mechanisms and interactions of the GR with DNA hormone-responsive elements and other nuclear factors, such as AP-1 and NF-κB (2, 3). The glucocorticoid resistance observed in inflammatory diseases may be induced by the inflammatory process and/or be genetically or constitutionally determined (4, 5). Cytokines, the main endocrine, paracrine, and autocrine factors of the immune/inflammatory response, have been reported to modulate the sensitivity of immune cells to glucocorticoids (6–10).

Tumor necrosis factor α (TNFα, a proinflammatory cytokine) and interleukin (IL)-10 (a T helper 2-type and antiinflammatory cytokine) are secreted by activated macrophages and play opposite roles in both innate and specific immune responses (11–15). Monocytes/macrophages, as antigen-presenting cells, are of major importance in the decision for differentiation of naive Th0 CD4+ cells toward Th1-directed cellular immunity or Th2-directed humoral immunity. Glucocorticoids promote a shift from Th1-type and proinflammatory to Th2-type and antiinflammatory cytokine secretory pattern. This is because the former is strongly inhibited by glucocorticoids, whereas the latter is not or positively affected (16–19). In this study, we hypothesized that cytokines could differentially regulate the sensitivity of normal human

monocytes/macrophages from healthy subjects to glucocorticoids in a fashion that could be influenced by the balance of proinflammatory/antiinflammatory cytokine secretion. To test this hypothesis, we first analyzed the pattern of dexamethasone-mediated inhibition of TNFα and IL-10 secretion by LPS-stimulated whole-blood cell cultures; and, second, we studied the modulation of the dexamethasone responses of human LPS-stimulated whole-blood cell cultures or of a phorbol-ester-stimulated human monocytic cell line by preincubation with TNFα or IL-10.

Materials and Methods

Whole-blood cell cultures

Ten healthy male volunteers (age range, 25–45 yr old) served as blood donors. The blood was treated as previously described (20). The whole-blood cell cultures were treated as follows: 1) LPS (endotoxin from Salmonella enteritidis, Sigma Chemical Co., St. Louis, MO) was added at a final concentration of either 100 pg/mL, 1 ng/mL, or 10 ng/mL. In whole-blood cell cultures, the mononuclear cells activated by LPS are mainly monocytes/macrophages. Dexamethasone was added at a final concentration ranging from 10^{-9}–10^{-6} mol/L in separate wells containing LPS. Plates were incubated at 37 C in 5% CO_2. The incubation periods for LPS-stimulated whole blood were 24 h and 48 h. 2) LPS was added at a final concentration of 1 ng/mL. Twenty-four hours and 48 h before LPS stimulation, the whole-blood cell culture was incubated alone or either in the presence of TNFα (1 ng/mL) or IL-10 (50 pg/mL). Dexamethasone was added at a final concentration of 10^{-8} mol/L in separate wells containing LPS. Plates were incubated at 37 C in 5% CO_2. The incubation period for LPS-stimulated whole blood was 24 h. 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.

Cell line cultures

Two types of clones (plus and minus) of the myeloid monocytic cell line, U937 cells, were a generous gift from Priscilla Biswas and Guido Poli (Ospedale San Raffaele, Immunopathogenesis Unit, Milano, Italy). Cells were maintained in RPMI 1640 (BioWhittaker, Inc. Belgium) with 10% steroid-free heat-inactivated FCS, 100 U/mL penicillin, 100 μg/mL streptomycin sulfate, and 2 mmol/L glutamine (BioWhittaker, Inc.) at 37 C in 5% CO_2. The FCS was treated with 1% dextran-coated charcoal to remove endogenous sex steroids and glucocorticoids. Cells were counted and cultured in medium alone or in the presence of TNFα (10 ng/mL) or IL-10 (250 pg/mL) (R&D Systems, UK) for 24 h and 48 h. The cells were then treated as follows: 1) The cells were removed, counted, and prepared for whole-cell dexamethasone binding assay; 2) The cells were stimulated with phorbol-myristate-acetate (PMA), at a final concentration of 10 ng/mL, for 24 h, in medium alone or in the presence of dexamethasone (10^{-8} and 10^{-6} mol/L). The supernatants were then recovered and stored at −20 C before further analyses.

Whole-cell dexamethasone binding assay

A whole-cell dexamethasone binding assay was used to quantify GR number and to determine GR affinity in untreated and TNFα- or IL-10-treated cells. Cells were incubated, in a waterbath at 37 C, for 2 h, at a final concentration of 10^7 cells/mL in RPMI 1640 buffer solution containing 0.5% BSA (Sigma, Belgium) supplemented with seven different concentrations of [^{3}H]-dexamethasone (Amersham International, Arlington Heights, IL) ranging from 2.5–50 nmol/L (SA, 82 Ci/mmol), in the presence or in the absence of 1000-fold excess of unlabeled dexamethasone (Sigma). After incubation, cells were cooled, centrifuged, and washed before the supernatant was carefully aspirated. Cells were resuspended in an equal volume of scintillation cocktail for counting. Dissociation constant (K_d) values and receptor binding capacity were determined on the basis of saturation curves using nonlinear regression computed with the Prism 2.0 program (GraphPad Software, Inc.). Sta-

tistical analyses were performed on the Scatchard analysis of the same curves.

Immunoassays

Specific enzyme-linked immunosorbent assays were used to measure human TNFα, IL-10, and IL-6 (Biosource Technologies, Inc./Medgenix, Belgium) and IL-1 Ra (receptor antagonist) (R&D Systems) and were performed according to the manufacturer's instructions. Absorbency was transformed to cytokine concentration using a standard curve computed with Medgenix enzyme-linked immunosorbent assay software.

Statistical analyses

The relative changes of cytokine production were computed for each dexamethasone dose. A logarithmic transformation was used for the percentage of response, to normalize the distribution. A generalized linear mixed model (SAS PROC MIXED) was used to analyze the dexamethasone general effect and the specific-dose effect on cytokine secretion. All results were considered to be significant at the 5% critical level ($P < 0.05$). Statistical analyses were carried out using the SAS software package (SAS Institute, Inc., Cary, NC). Nonparametric (Mann Withney and Wilcoxon tests) and parametric tests (Student's t and ANOVA tests) were also used for comparisons, as appropriate.

Results

Dexamethasone modulates TNFα and IL-10 secretion in LPS-stimulated whole-blood cell cultures (Fig. 1)

TNFα and IL-10 secretion were significantly increased in LPS-stimulated whole-blood cell cultures ($P < 0.001$). Although both absolute and percent baseline values are shown, only the statistical comparisons between cytokine levels expressed as mean percent baseline are reported (Fig. 1, *right* panels). TNFα secretion was uniformly suppressed in a dose-dependent fashion by dexamethasone (10^{-9}–10^{-6} mol/L) after three different concentrations of LPS (Fig. 1, *middle* and *right* panels). Nevertheless, the dexamethasone-induced inhibition of TNFα at 10^{-8} and 10^{-7} mol/L was less marked after 1 and 10 ng than after 0.1 ng LPS stimulation ($P < 0.001$) (Fig. 1, *right* panels). In contrast, the effect of dexamethasone on IL-10 secretion was biphasic, characterized by stimulation at the lower doses employed and inhibition at the higher doses (Fig. 1, *left* and *right* panels). LPS dose effects on dexamethasone-induced changes in IL-10 secretion were observed; IL-10 was strongly suppressed by dexamethasone (10^{-8}–10^{-6} mol/L) after 0.1 ng LPS stimulation, whereas its secretion was biphasic after 1 and 10 ng of LPS stimulation ($P < 0.001$). After exposure to 1 and 10 ng LPS, there was no difference in IL-10 modulation by dexamethasone at 10^{-9} and 10^{-8} mol/L; however, a significant difference appeared at 10^{-7} and 10^{-6} mol/L glucocorticoid ($P < 0.01$ and $P < 0.05$, respectively) (Fig. 1, *right* panels). At each of the three concentrations of LPS employed, the dexamethasone-induced modulation (10^{-9}–10^{-6} mol/L) was significantly different between IL-10 and TNFα ($P < 0.05$–0.001). After 0.1 ng LPS, however, no difference was observed in the dexamethasone-induced modulation of IL-10 and TNFα secretion at 10^{-7} and 10^{-6} mol/L glucocorticoid (Fig. 1, *right* panels). The results were similar at both 24- and 48-h incubations (Fig. 1, *right* panels).

TNFα and IL-10 modulate dexamethasone-mediated IL-6 suppression in whole-blood cell cultures (Fig. 2)

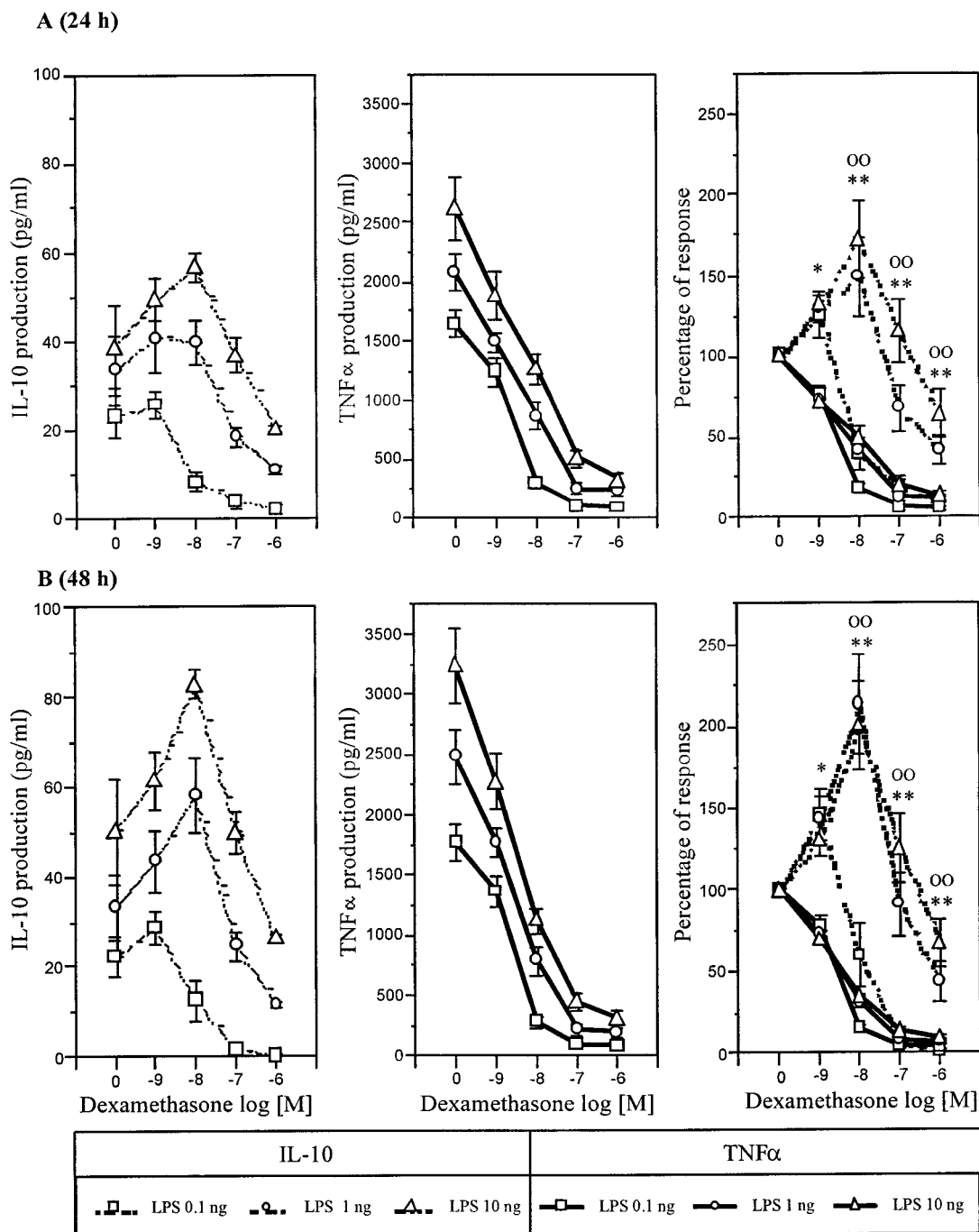We used the same TNFα and IL-10 concentrations that were observed in LPS-stimulated whole-blood cell cultures

FIG. 1. Dexamethasone effects on the LPS-stimulated secretion of TNFα or IL-10 in whole-blood cell cultures from male normal volunteers. Data are expressed as mean ± SEM. Modulation is presented as mean absolute cytokine levels (*left* and *middle* panels) and as mean percent baseline (*right* panels) after an incubation of 24 h (A) or 48 h (B). The statistical comparisons of cytokine levels are shown only for the mean percent baseline data (*right* panels). Dexamethasone suppressed TNFα in a dose-dependent fashion. By contrast, its effect on IL-10 secretion was biphasic, producing a stimulation at the lower doses employed and an inhibition at the higher doses. At each of the three concentrations of LPS stimulation employed, the dexamethasone-induced modulation at 10^{-9}–10^{-6} mol/L was significantly different between TNFα and IL-10 (*, $P < 0.05$; **, $P < 0.001$). A LPS dose-dependent modulation of the IL-10 secretion by dexamethasone from 10^{-8}–10^{-6} mol/L was observed: IL-10 was strongly suppressed after 0.1 ng LPS stimulation by the higher doses of dexamethasone (10^{-8}–10^{-6} mol/L), whereas the IL-10 secretion was biphasic after 1 ng and 10 ng LPS stimulation (oo, $P < 0.001$).

(1 ng/mL and 50 pg/mL, respectively) (Fig. 1) and investigated whether TNFα or IL-10 preincubation could change the sensitivity to dexamethasone assessed by dexamethasone-mediated IL-6 inhibition in whole-blood cell cultures from 10

healthy subjects. The whole-blood cell cultures exposed to TNFα or IL-10 did not produce IL-6. After LPS stimulation, however, whole-blood cell cultures not exposed to either cytokine secreted more IL-6 (399.8 ± 29.2 pg/mL) than when
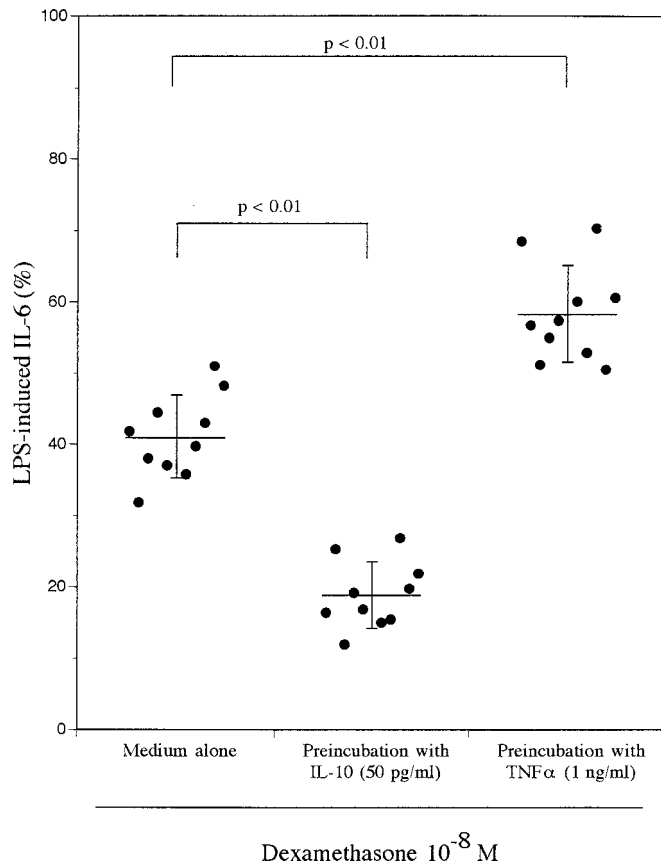
FIG. 2. Low doses of dexamethasone suppressed LPS-induced IL-6 secretion by whole-blood cell cultures pretreated with TNFα or IL-10. The former diminished ($P < 0.01$), whereas the latter improved ($P < 0.01$), the ability of dexamethasone to suppress IL-6 secretion, changes compatible with decreased or increased sensitivity to glucocorticoids, respectively.

exposed to TNFα (155.7 ± 24.2 pg/mL, $P < 0.01$) or IL-10 (232.6 ± 44.8 pg/mL, $P < 0.01$). Preincubation with TNFα was associated with a lower IL-6 secretion than preincubation with IL-10 ($P < 0.01$). A longer pretreatment, up to 48 h before LPS stimulation, was associated with poor IL-6 secretion and did not enable us to assess corticosensitivity. The dexamethasone-mediated IL-6 inhibition was stronger after preincubation with IL-10 (18.9 ± 1.5%, $P < 0.01$) and weaker after preincubation with TNFα (58.5 ± 2.1%, $P < 0.01$) than with medium alone (41.1 ± 1.83%).

TNFα and IL-10 modulate dexamethasone-induced IL-1 Ra secretion by a human monocytic cell line (Fig. 3)

We examined whether TNFα and IL-10 preincubation could change the sensitivity to dexamethasone assessed by dexamethasone-induced IL-1 Ra secretion in the human monocytic cell line U937. In the baseline condition, IL-1 Ra secretion was increased after both TNFα (16.4 ± 0.9 pg/mL, $P < 0.01$) and IL-10 (10.1 ± 0.6 pg/mL, $P < 0.01$) preincubation; no significant difference in the IL-1 Ra level was observed after these two preincubations. After PMA stimulation, however, dexamethasone-induced IL-1 Ra secretion at 10^{-6} mol/L was higher with IL-10 preincubation (55.7 ± 3.5
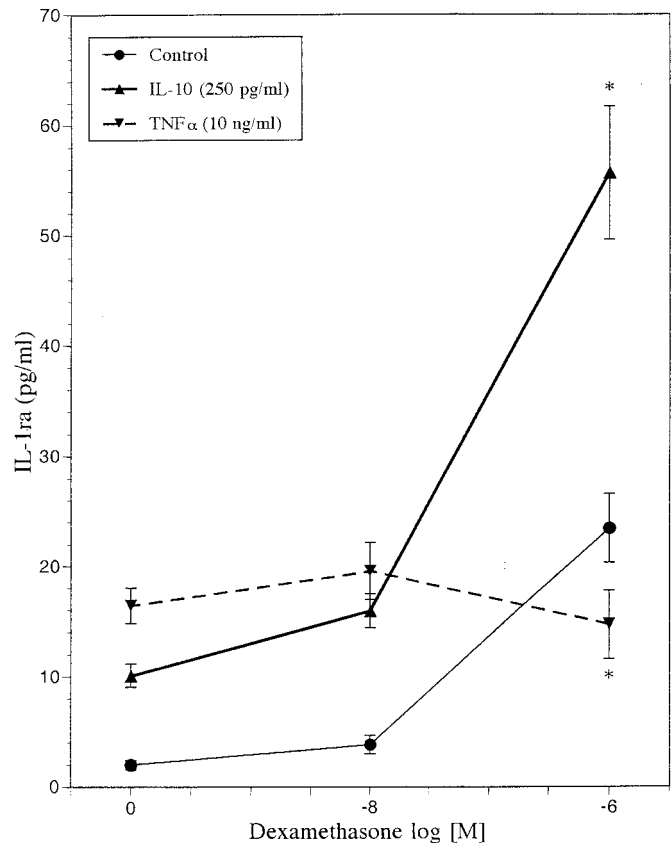

FIG. 3. Dexamethasone caused a dose-dependent stimulation of IL-1Ra in human monocytic U937 cells stimulated with PMA. This response was decreased by preincubation with TNFα (*, $P < 0.05$) but was enhanced by preincubation with IL-10 (*, $P < 0.05$). Results are from three independent experiments.

pg/mL, $P < 0.05$) and weaker with TNFα preincubation (14.7 ± 1.7 pg/mL, $P < 0.05$) than with medium alone (23.5 ± 3.1 pg/mL). No significant difference was observed between the IL-1 Ra responses of plus and minus clones of U937 cells.

TNFα decreases, and IL-10 increases, the GR concentration (but not binding affinity) in a human monocytic cell line (Fig. 4)

To assess the effect of TNFα and IL-10 on the GR number and binding affinity, we used dexamethasone radioligand binding in the human monocytic cell line U937. This was done to avoid the high variability observed in studies of isolated peripheral blood mononuclear cells. U937 cells were cultured, in the absence or presence of TNFα or IL-10, for up to 48 h; and dexamethasone binding was assessed. No change in proliferation rate was observed between the different preincubations (data not shown). Both TNFα and IL-10 failed to induce a significant modulation of the GR number at time periods less than 48 h. However, 48-h exposure to TNFα resulted in a 60% decrease of the GR number (4,834 ± 246 sites/cell *vs.* 11,709 ± 544 sites/cell, $P < 0.001$) without any change of the binding affinity (11.4 ± 1.5 nmol/L *vs.* 16.1 ± 1.8 nmol/L), whereas exposure to IL-10 resulted in a 50% increase of the GR numbers (17,734 ± 87 sites/cell *vs.* 11,709 ± 544 sites/cell, $P < 0.001$), also without any change

FIG. 4. Saturation analyses and Scatchard plots (*inset*) of [^{3}H]-dexamethasone binding in human monocytic U937 cells pretreated with TNFα or IL-10. The former decreased (*, $P < 0.001$), whereas the latter increased (*, $P < 0.001$) the concentration of dexamethasone binding sites. Results are from three independent experiments.
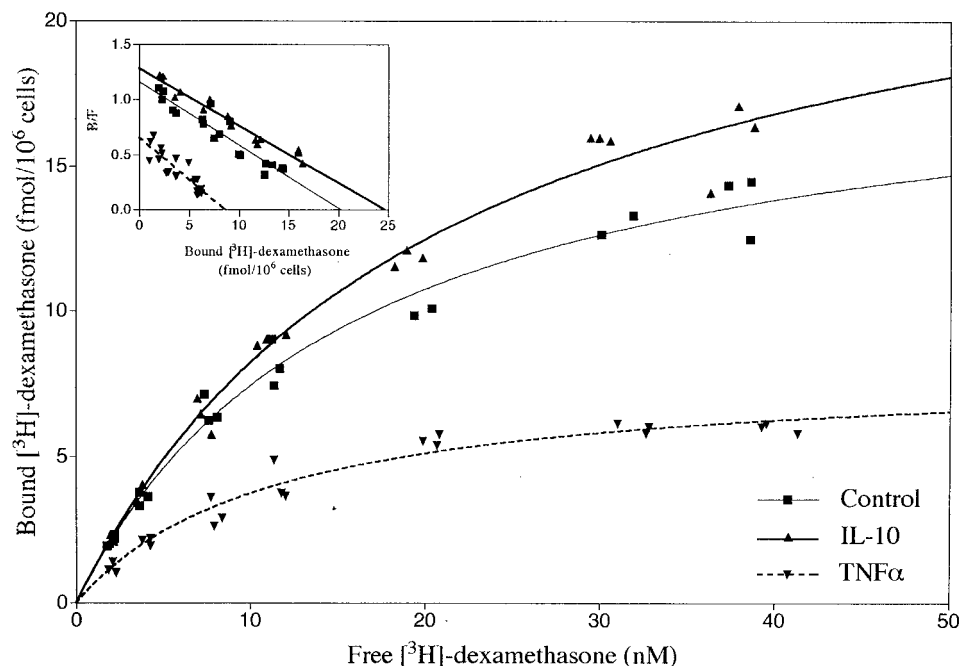
TABLE 1. The effect of IL-10 and TNFα on dexamethasone binding sites in U937 cells

	Binding sites/cell (Bmax)	K_d (nM)
Control (n = 3)	11,709 ± 544	16.1 ± 1.8
IL-10 preincubation (n = 3)	17,734 ± 87[a]	21.5 ± 2.4
TNFα preincubation (n = 3)	4,834 ± 246[a]	11.4 ± 1.5

[a] Bmax, $P < 0.001$.

of the binding affinity (21.5 ± 2.4 nmol/L *vs.* 16.1 ± 1.8 nmol/L). The results are summarized on Table 1.

Discussion

Although glucocorticoids clearly suppress proinflammatory cytokines, they have been reported not to change (16, 19), to increase (17), or to decrease IL-10 secretion (18, 21). An *in vivo* increase of plasma IL-10 by exogenous glucocorticoids was previously reported both in human and murine endotoxemia (22, 23). Here, we observed a biphasic effect of glucocorticoids on IL-10 secretion by LPS-stimulated whole-blood cell cultures. Depending on the amplitude of LPS stimulation, glucocorticoids at low doses increased, and at high doses decreased, IL-10 secretion; in contrast, TNFα secretion was always suppressed by dexamethasone in a classic sigmoidal fashion. Thus, the degree of monocyte/macrophage activation modulates the effect of dexamethasone on IL-10 secretion, whereas the direction of the TNFα response to glucocorticoids remains unchanged. cAMP-elevating drugs increase IL-10 and decrease TNFα secretion (24), and cAMP-responsive elements have been described in both the IL-10 and TNFα promoters (25, 26). The GR interacts with cAMP-responsive element-binding protein, and this interaction may play a role in the differential modulation of IL-10 and TNFα secretion by glucocorticoids (27).

IL-1Ra is a receptor-level natural antagonist of IL-1 that was isolated from monocytes and monocytic cell lines (28).

Glucocorticoids inhibit IL-1 Ra secretion by LPS-stimulated human peripheral monocytes (29) but potentiate the IL-1β secretory response of monocytic cells to PMA (30). The PMA-differentiated monocytic cell line U937 produces IL-1Ra (31). Here, we demonstrated that dexamethasone increased IL-1 Ra secretion by these cells, providing an additional explanation for an indirect antiinflammatory action for glucocorticoids.

Preincubation with TNFα or IL-10 modulated the sensitivity to dexamethasone in both normal leukocytes present in whole-blood cell cultures and U937 cells. Both TNFα and IL-10 inhibited IL-6 and enhanced IL-1 Ra secretion in the absence of dexamethasone; however, their effects were in opposite directions in the presence of dexamethasone. Thus, whereas IL-6 was more inhibited by TNFα than by IL-10 at the baseline state, dexamethasone-mediated IL-6 inhibition was less prominent after TNFα incubation than after IL-10 incubation. Similarly, whereas IL-1 Ra secretion was slightly more stimulated by TNFα than by IL-10 at the baseline state, in the presence of dexamethasone, it was blocked by TNFα and dramatically enhanced by IL-10 preincubation.

IL-10 increased, and TNFα decreased, the GR concentration of U937 cells without modifying its binding affinity for dexamethasone. Previously, some cytokines (such as IFNγ) were shown to increase sensitivity of cells to glucocorticoids by increasing the receptor concentration (6), whereas other cytokines (such as IL-2, IL-4, and IL-13) decreased sensitivity by decreasing its binding affinity (7, 8). MIF, the macrophage migration inhibitory factor, is regulated by glucocorticoids like IL-10; however, unlike IL-10, it blocks dexamethasone-mediated inhibition of cytokine production by LPS-stimulated monocytes (10). Our study suggests that IL-10 may enhance glucocorticoid action by increasing the GR concentration, whereas TNFα may exert the opposite effect by decreasing the GR concentration. Changes in the receptor may be one of several mechanisms for altering tissue sensitivity

to glucocorticoids (3). Indeed, the modulation of dexamethasone response by cytokines may also be related to NF-κB, which is involved in the transcription of many proinflammatory cytokines, including IL-6, and is highly expressed in inflamed tissues (5). Glucocorticoids and IL-10 inhibit NF-κB activation, whereas TNFα activates NF-κB (32–34). The opposite effect on NF-κB modulation exerted by TNFα and IL-10 may also provide an additional explanation for the opposite effects of these cytokines on glucocorticoid sensitivity described here.

In Crohn's disease, blocking TNFα with humanized antibody (cA2) or administering IL-10 were found to be useful adjunctive therapies (35, 36). Because TNFα decreases, and IL-10 increases, sensitivity to dexamethasone, this study provides evidence for another potential beneficial indirect effect of these cytokine therapies. This also suggests that the balance of TNFα/IL-10 secretion during the course of an inflammatory disease may determine changes in sensitivity to glucocorticoids. Thus, a patient with a high or low TNFα/IL-10 ratio would, respectively, be less or more sensitive to endogenous and exogenous glucocorticoids and more or less susceptible to develop corticosteroid-resistant and -sensitive inflammatory disease.

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