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Decreased Corticosensitivity in Quiescent Crohn's Disease

An *Ex Vivo* Study Using Whole Blood Cell Cultures

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Corticosensitivity influences the degree and the duration of an inflammatory reaction by altering target cell responses to endogenous and/or exogenous glucocorticoids. Indeed, different clinical responses to glucocorticoids have been observed among patients with Crohn's disease, suggesting different degrees of corticosensitivity in these subjects. The purpose of this study was to compare the corticosensitivity of patients with quiescent Crohn's disease to that of healthy subjects (HS). Nineteen patients with quiescent Crohn's disease and 14 HS were studied; all patients were steroid-free for at least six months; 7 of the 19 were corticosteroid-dependent (CSD) and treated with nonglucocorticoid immunosuppressants at the time of the study. Corticosensitivity was measured by the inhibition of LPS-induced cytokine secretion in whole blood cell cultures treated with increasing concentrations (10^{-9} to 10^{-6} M) of dexamethasone. Tumor-necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) were measured using specific immunoassays. Crohn's disease patients had a markedly decreased dexamethasone-mediated inhibition of TNF-α ($P < 0.01$), IL-6 ($P < 0.001$), and IL-1β ($P < 0.01$) compared to healthy subjects, with a shift of the dexamethasone dose–response curve to the right. No significant differences in the basal and LPS-stimulated secretion of the three cytokines were observed between CSD and non-CSD patients, and both subgroups of patients had similar degrees of dexamethasone-mediated cytokine inhibition. We conclude that patients with Crohn's disease have a significant decrease in the corticosensitivity of their leukocytes. This may be related to a specific genetic/constitutional background and/or could be acquired, due to inflammation-related endocrine and/or immune factors.

KEY WORDS: glucocorticoids; cytokines; corticosensitivity; Crohn's disease.

Increasing evidence supports an important role of the hypothalamic–pituitary–adrenal (HPA) axis in the pathogenesis and course of inflammatory diseases (1). The end-effector of the HPA axis, glucocorticoids, restrain the immune and inflammatory responses (2, 3). Thus, the inflammatory response of inflammatory disease-susceptible Lewis and -resistant Fischer rats is inversely related to the magnitude of their HPA axis response to inflammatory mediators (4, 5), while the glucocorticoid receptor antagonist RU 486 renders Fischer rats susceptible to streptococcus cell wall-induced arthritis (4).

Glucocorticoids are used as first line antiinflamma-

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tory and immunosuppressive drugs in many inflammatory and autoimmune diseases (6). Different clinical responses to glucocorticoids have been observed among patients suffering from such diseases, and patients with Crohn's disease have been classified as corticosteroid-sensitive (when a good response to treatment is observed), corticosteroid-dependent (CSD) (when glucocorticoids are needed to keep the inflammatory disease quiescent), and corticosteroid-resistant (CSR) (when they are not responsive to glucocorticoid treatment). The prevalence of corticosteroid dependency in Crohn's disease is about 15–35% (7–9), while corticosteroid resistance is observed in less than 10% of the patients (9, 10).

Corticosensitivity depends not only on the glucocorticoid receptor (GR) number and affinity, but also on pre- and postreceptor mechanisms of GR activation, including the interactions of GR with cytoplasmic and nuclear factors and specific DNA-responsive elements (11, 12). Only a few clinical studies have been carried out on corticosensitivity and none in Crohn's disease. Familial glucocorticoid resistance was studied by Chrousos et al (13) and Lamberts et al (14) and was shown to be of genetic etiology and related to a decrease of GR affinity for its ligand or to a decrease in GR number per cell (13, 14). Sher et al (15) described two populations of corticosteroid-resistant (CSR) asthma patients: type 1 is defined by decreased GR affinity binding, and type 2 is defined by decreased GR number per cell. Type 1 CSR asthma was shown to be reversible and secondary to inflammation, while type 2 CSR asthma appeared to be genetically and/or constitutionally determined (15). Finally, Schlaghecke et al reported a 50% decrease of GR number/cell in patients with rheumatoid arthritis (16); however, inexplicably, this did not appear to influence the *in vitro* corticosensitivity of these patients (17).

Whole blood cell culture is an appropriate *ex vivo* technique to analyze corticosensitivity within a controlled environment, by studying the inhibition of cytokine secretion by graded concentrations of glucocorticoids (18). The aim of this work was to compare corticosensitivity between patients with quiescent Crohn's disease and healthy control subjects.

MATERIALS AND METHODS

Patients and Controls. Patients with Crohn's disease (10 women and 9 men) and healthy volunteers (7 women and 7 men) served as blood donors. The mean age was 35 years in patients with Crohn's disease (range: 20–53 years) and 33 years in controls (range: 24–44 years). The clinical characteristics of patients with Crohn's disease are summarized in Table 1. All patients with quiescent Crohn's disease were followed in the Gastroenterology Division at the University Hospital of Liège, Belgium. The diagnosis of Crohn's disease was made by classical clinical, radiological, and endoscopic criteria. Seven of the 19 patients were corticosteroid-dependent. The corticosteroid dependency was defined either by two successive relapses following glucocorticoid discontinuation or by two successive relapses at dose tapering after a successful treatment of a flare-up with glucocorticoids (7, 8, 19). Treatment with immunosuppressive drugs (azathioprine or methotrexate) allowed the complete withdrawal of glucocorticoids in these patients. Nine non-CSD patients were treated with 5-aminosalicylic acid (5-ASA). All patients studied were in clinical remission and corticosteroid-free for at least six months. Three groups were defined: non-corticosteroid-dependent (non-CSD) patients; corticosteroid-dependent (CSD) patients; and healthy controls (HS). Data from the interleukin-1β (IL-1β) assay of four CSD patients and two non-CSD patients were not included. Similarly, data from the tumor necrosis factor-α (TNF-α) assay of one CSD patient and one non-CSD patient were not included because of absence of stimulation by LPS.

Table 1. Clinical Characteristics of Corticosteroid-Dependent (CSD) and Non-Corticosteroid-Dependent (Non-CSD) Patients with Crohn's Disease

	Non-CSD patients (N = 12)	*CSD patients (N = 7)*
Mean age [yr (range)]	37(20–53)	31(24–48)
Female/male	6/6	4/3
Disease duration [months (mean, range)]	144(8–284)	96(30–156)
Crohn's disease activity index	<150	<150
Disease location		
Small bowel only	2/12	0/7
Colonic only	1/2	5/7
Ileocolonic	9/12	2/7
Disease type		
Inflammatory	4/12	7/7
Fibrostenotic	8/12	1/7
Fistulizing	1/12	0/7
Treatment		
5-ASA	9/12	0/7
Azathioprine (2 mg/kg/day)	0/12	6/7
Methotrexate (25 mg/week)	0/12	1/7

Whole Blood Cell Cultures. The blood was treated as previously described (18). Blood samples were collected in apyrogenic heparinized tubes provided by Biosource/Medgenix (Fleurus, Belgium). The white blood cell count was in the normal range in CD patients. The blood was processed immediately and diluted with 1/10 RPMI 1640 supplemented with 2 mM glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin (Biowitthaker), and was then distributed in 2-ml wells. RPMI 1640 medium was tested for apyrogenicity. LPS (endotoxin from *Salmonella enteritidis*; Sigma, St. Louis, Missouri) was added at a final concentration of 25 pg/ml. A synthetic glucocorticoid, dexametha-

TABLE 2. BASAL AND LPS-STIMULATED CYTOKINE LEVELS IN CORTICOSTEROID -DEPENDENT (CSD) AND NON-CORTICOSTEROID -DEPENDENT (NON-CSD) PATIENTS WITH CROHN'S DISEASE AND HEALTHY SUBJECTS*

	Cytokine (pg/ml)		
	Non-CSD patients	*CSD patients*	*Healthy subjects*
TNF-α basal	60 ± 24 ($N = 11$)	104 ± 84 ($N = 6$)	35 ± 11 ($N = 14$)
TNF-α after LPS	204 ± 65 ($N = 11$)	248 ± 160 ($N = 6$)[a]	420 ± 123 ($N = 14$)
IL-6 basal	188 ± 56 ($N = 12$)	344 ± 260 ($N = 7$)	102 ± 42 ($N = 14$)
IL-6 after LPS	417 ± 106 ($N = 12$)	658 ± 447 ($N = 7$)[a]	815 ± 178 ($N = 14$)
IL-1β basal	42 ± 13 ($N = 10$)	122 ± 98 ($N = 3$)	17 ± 7 ($N = 14$)
IL-1β after LPS	87 ± 23 ($N = 10$)	167 ± 124 ($N = 3$)	135 ± 33 ($N = 14$)

* Data expressed as mean ± SEM. CSD patients vs HS: [a] $P < 0.05$.

sone, was added at a final concentration ranging from 10^{-9} to 10^{-6} M in separate wells. Plates were incubated at 37°C in a 5% CO_2 athmosphere; the incubation time was 24 hr for LPS-stimulated whole blood cell cultures (18, 20). The contents of the wells were then collected and centrifuged at 900*g* for 10 minutes. Supernatants were recovered and frozen at −20°C. Blood was drawn from women during the proliferative phase of their cycle.

Immunoassays. TNF-α, IL-1β, and IL-6 were measured with specific immunoassays from Biosource/Medgenix without any cross-reactivity. Immunoassays were performed according to the manufacturer's directions. The detection limit was 3 pg/ml for TNF-α, 2 pg/ml for IL-1β, and 2 pg/ml for IL-6. Plates were read at 460 nm by a microplate ELISA reader from Medgenix Diagnostics. Absorbancy was transformed to cytokine concentration using a standard curve computed by Medgenix ELISA software. Plasma cortisol (RIA, Immunotech, Marseille, France) and cortisol-binding globulin (RIA, Radim, Liege, Belgium) were measured with the routine laboratory methods. The blood was collected from each individual between 3 and 6 PM. The blood was then processed immediately for whole blood cell cultures. Plasma obtained at the same time was frozen at −20°C until assay. Once all the plasma samples from the patients and the healthy subjects were collected, cortisol and cortisol binding globulin concentrations were measured in a single batch.

Statistical Analyses. Statistical analyses were carried out using the SAS software package (SAS Institute Inc., Cary, North Carolina). The relative changes in cytokine production were computed for each dose of dexamethasone. A log transformation was used for both the absolute values of cytokine production and for the percentage of the response in each individual to normalize the distribution of the data. A generalized linear mixed model with random effects (SAS Proc Mixed) was fitted to study the dexamethasone general effect and dose effect on cytokine secretion. All results were considered to be significant at the 5% critical level ($P < 0.05$). The SAS Proc Mixed is an ANOVA for repeated measures, as is appropriate where the observations in the same subject are not independent of each other. This method adjusts the standard error as a function of the covariance structure of the observations.

The ED_{50} was calculated as the dose of dexamethasone necessary to produce a 50% inhibition in the secretion of each of the three cytokines for each individual, using the SAS Proc Mixed program. The statistical comparisons were between the means of the ED_{50}s in the three groups of subjects with the Kruskal-Wallis and the Wilcoxon tests.

Other statistical comparisons within the same group or between the three groups were performed using nonparametric (Kruskal-Wallis and Wilcoxon tests) and parametric tests (ANOVA and Student *t* tests after a logarithmic transformation).

RESULTS

Cytokine Secretion in Whole Blood Secretion from CD Patients and Healthy Subjects

There was a significant difference in cytokine secretion between basal and LPS-induced levels of cytokines in the non-CSD patients ($P < 0.001$ for TNF-α, IL-6, and IL-1β), in CSD patients ($P < 0.001$ for TNF-α and IL-6, $P < 0.01$ for IL-1β), and in healthy subjects ($P < 0.001$ for TNF-α, IL-6, and IL-1β) (Table 2).

Basal cytokine levels in whole blood cell cultures were similar in both groups of patients with Crohn's disease and healthy subjects. LPS-stimulated IL-6 and TNF-α levels were significantly different between CD patients and healthy subjects ($P < 0.05$ and $P < 0.05$, respectively) and between CSD patients and healthy subjects ($P < 0.05$ and $P < 0.05$, respectively). No difference was observed between non-CSD patients and healthy subjects (Table 2).

Inhibition of Cytokine Secretion by Dexamethasone in Whole Blood Cell Cultures from CD Patients and Healthy Subjects

The results of the concentration-dependent effect of dexamethasone on TNF-α, IL-6, and IL-1β secretion in whole blood cell cultures in the three groups are shown in Figures 1–3. In the top panels of these figures, the mean percentages of inhibition of cytokine production are presented, while in the bottom panels the means of the logarithmically transformed absolute cytokine levels are shown.

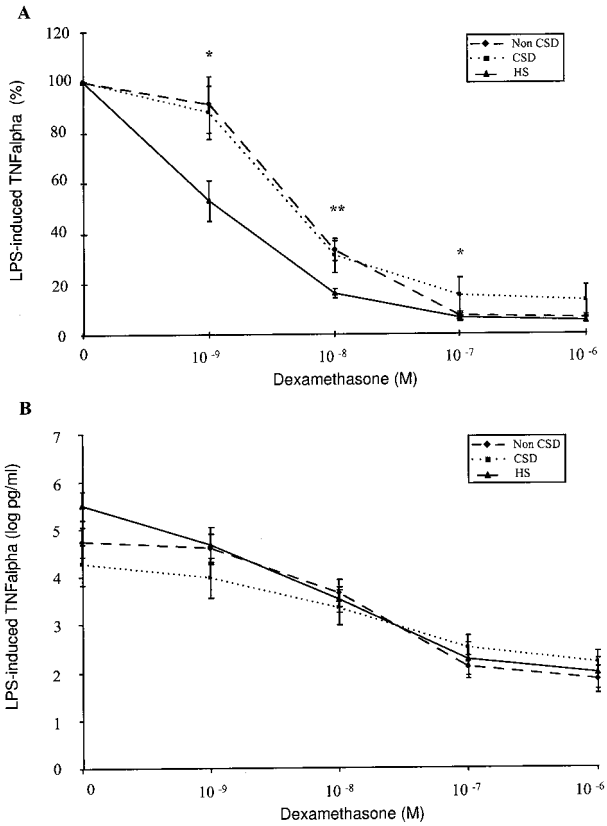

Fig 1. Effect of dexamethasone on LPS-induced TNF-α secretion in whole blood cell cultures from 17 patients with Crohn's disease (11 non-CSD and 6 CSD) and 14 HS. Statistical analyses are described in Results. Data are expressed as the mean ± SEM: inhibition is presented as mean percent baseline (A, top panel) or in mean of logarithmically transformed absolute cytokine levels (B, bottom panel). HS: healthy subjects; CSD: corticosteroid-dependent; Non-CSD: non-corticosteroid-dependent. CD patients vs HS: $*P < 0.05$, $**P < 0.01$.

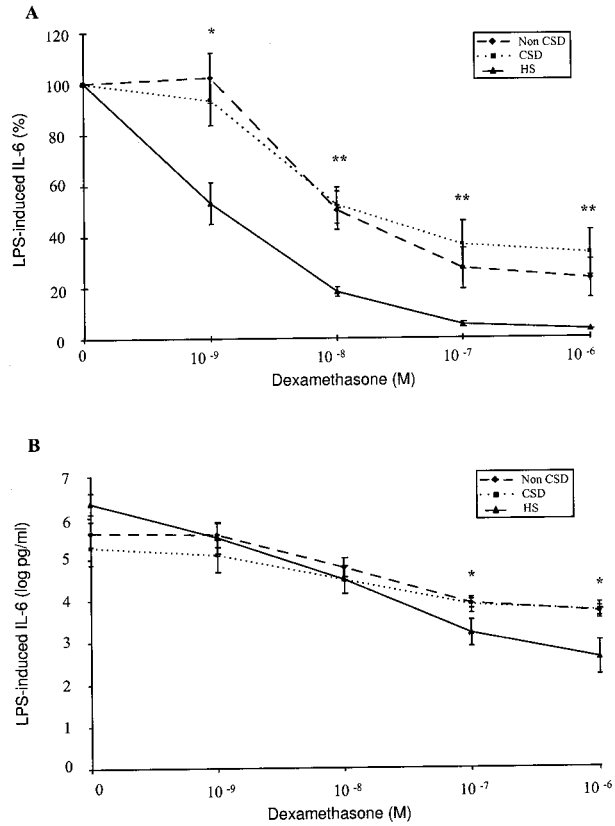

Fig 2. Effect of dexamethasone on LPS-induced IL-6 secretion in whole blood cell cultures from 19 CD patients (12 non-CSD and 7 CSD) and 14 healthy subjects. Statistical analyses are described in Results. Data are expressed as the mean ± SEM: inhibition is presented as mean percent baseline (A, top panel) or in mean of logarithmically transformed absolute cytokine levels (B, bottom panel). HS: healthy subjects; CSD: corticosteroid-dependent; Non-CSD: non corticosteroid-dependent. CD patients vs HS: $*P < 0.01$, $**P < 0.001$.

TNF-α. There was a significant inhibition of TNF-α secretion by dexamethasone from 10^{-8} to 10^{-6} M in healthy subjects ($P < 0.001$), in non-CSD patients ($P < 0.001$), and in CSD patients ($P < 0.001$). While there was a significant inhibition of TNF-α secretion by dexamethasone at 10^{-9} M in healthy subjects ($P < 0.001$), no inhibition was observed in non-CSD or CSD patients.

IL-6. There was a significant inhibition of IL-6 secretion by dexamethasone from 10^{-8} to 10^{-6} M in healthy subjects ($P < 0.001$) and in non-CSD ($P < 001$) and in CSD patients ($P < 0.001$). While there was a significant inhibition of IL-6 secretion by dexamethasone at 10^{-9} M in healthy subjects ($P < 0.001$), no inhibition was observed in non-CSD or CSD patients.

IL-1β. There was a significant inhibition of IL-1β secretion by dexamethasone from 10^{-8} to 10^{-6} M in healthy subjects ($P < 0.001$) and in non-CSD (from $P < 0.01$ to $P < 0.001$) and in CSD patients (from $P < 0.06$ to $P < 0.001$). While there was a significant inhibition of IL-1β secretion by dexamethasone at 10^{-9} M in healthy subjects ($P < 0.05$), no inhibition was observed in non-CSD or CSD patients.

Plasma Cortisol and Cortisol-Binding Globulin Levels in CD Patients and Healthy Subjects

Plasma cortisol (112.6 ± 6.5 vs 120.6 ± 12.2 μg/dl, respectively) and cortisol-binding globulin (28.1 ± 1.3 vs 25 ± 1.3 μg/ml, respectively) levels were similar in CD patients and healthy subjects. Plasma cortisol (120 ± 10.1 vs 104.5 ± 7.5 μg/dl, respectively) and cortisol-binding globulin (25.3 ± 2.0 vs 31.3 ± 2.8 μg/ml) were also similar in non-CSD and CSD patients.

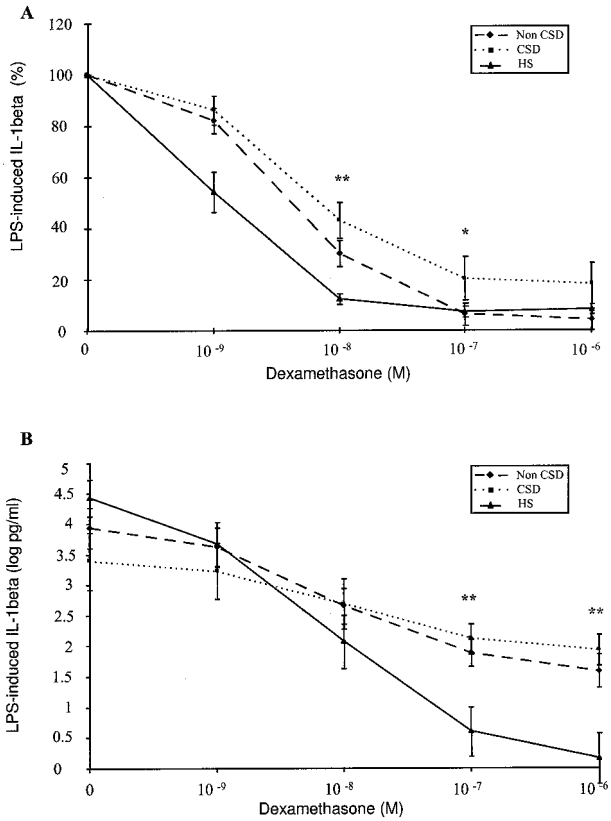

Fig 3. Effect of dexamethasone on LPS-induced IL-1β secretion in whole blood cell cultures from 13 CD patients (10 non-CSD and 3-CSD) and 14 healthy subjects. Statistical analyses are described in Results. Data are expressed as the mean ± SEM: inhibition is presented as mean percent baseline (A, top panel) or in mean of logarithmically transformed absolute cytokine levels (B, bottom panel). HS: healthy subjects; CSD: corticosteroid-dependent; Non-CSD: non corticosteroid-dependent. CD patients vs HS: $*P < 0.05$, $**P < 0.01$.

Assessment of Corticosensitivity in CD Patients and Healthy Subjects

The top panels of Figures 1–3 show the mean percentage of inhibition of cytokine production by dexamethasone in the three groups, taking into account the *O*-dexamethasone concentration (cytokine level after LPS stimulation), which is the reference for each individual (Figures 1A, 2A, and 3A).

TNF-α. A significant difference between all patients with Crohn's disease and controls was observed in the general pattern of TNF-α inhibition by dexamethasone ($P < 0.01$). A significant difference also was observed between all patients and controls in the rate of TNF-α inhibition by dexamethasone at 1 nM ($P < 0.05$), 10 nM ($P < 0.01$) and 100 nM ($P < 0.05$). There was a slight, but nonsignificant, difference at 1 μM of dexamethasone between all patients and controls ($P < 0.052$) (Figure 1A).

A difference was observed in the rate of TNF-α inhibition between non-CSD patients and controls by dexamethasone at 1 nM and 10 nM ($P < 0.01$). A similar difference was observed in the rate of TNF-α inhibition between CSD patients and controls by dexamethasone at 1 nM and 10 nM ($P < 0.01$) and from 100 nM to 1 μM ($P < 0.05$) (Figure 1A).

IL-6. A significant difference between all patients and controls was observed in the general pattern of IL-6 inhibition by dexamethasone ($P < 0.001$). A significant difference in IL-6 inhibition also was observed between all patients and controls at 1 nM ($P < 0.01$) and from 10 nM to 1 μM of dexamethasone ($P < 0.001$) (Figure 2A).

There was a difference in the rate of IL-6 between non-CSD patients and controls by dexamethasone at 1 nM ($P < 0.01$) and from 10 nM to 1 μM ($P < 0.001$). A difference also was observed between CSD patients and controls by dexamethasone at 1 nM ($P < 0.05$) and from 10 nM to 1 μM ($P < 0.001$) (Figure 2A).

IL-1β. A significant difference between all patients with Crohn's disease and controls was observed in the general pattern of IL-1β inhibition by dexamethasone ($P < 0.01$). A difference between all patients and controls also was observed in the rate of IL-1β inhibition by dexamethasone at 10 nM ($P < 0.01$) and at 100 nM ($P < 0.05$). There was a slight, but nonsignificant, difference at 1 nM between all patients and controls ($P = 0.092$) (Figure 3A).

A significant difference in the rate of IL-1β inhibition was observed between non-CSD patients and controls by dexamethasone at 10 nM ($P < 0.01$). There was a significant difference in the rate of IL-1β inhibition by dexamethasone between CSD patients and controls at 10 nM ($P < 0.01$) and at 1 μM ($P < 0.05$). There was a slight, but nonsignificant, difference at 1 nM of dexamethasone between non-CSD patients and CSD patients versus controls ($P = 0.15$ and $P = 0.16$, respectively) (Figure 3A).

Interestingly, no significant difference between non-CSD and CSD patients was observed in the dexamethasone general and dose effect on cytokine secretion (Figures 1A, 2A, and 3A).

The IC_{50}s of dexamethasone effects on the secretion of the three inflammatory cytokines are reported on Table 3. There was a global significance between patients with Crohn's disease and HS for TNF-α ($P < 0.01$), IL-1β ($P < 0.01$), and IL-6 ($P < 0.001$). There were no significant differences between non-CSD patients and CSD patients for each of the three cytokines.

TABLE 3. COMPARISON OF IC_{50} OF DEXAMETHASONE INHIBITION ON THREE PROINFLAMMATORY CYTOKINES BETWEEN CORTICOSTEROID -DEPENDENT (CSD) AND NON-CORTICOSTEROID - DEPENDENT (NON-CSD) PATIENTS WITH CROHN'S DISEASE AND HEALTHY SUBJECTS*

	IC_{50}		
	Non-CSD patients (10^{-9} *M*)	*CSD patients* (10^{-9} *M*)	*Healthy subjects* (10^{-9} *M*)
TNF-α	8 ± 6.5[a]	6.3 ± 2.3[a]	2.7 ± 2.4
IL-6	54 ± 138.4[b]	25.4 ± 28.9[a]	2.9 ± 2.6
IL-1β	12.2 ± 17.8[a]	17.2 ± 19.4[a]	2.7 ± 2.3

* Data are expressed as mean ± SD. [a] $P < 0.001$; [b] $P < 0.001$ vs healthy subjects.

The bottom panels of Figures 1–3 show the means of logarithmically transformed absolute cytokine levels in the three groups. A shift to the right of the sigmoidal dose–response curve of cytokine inhibition by dexamethasone was observed in Crohn's disease patients in comparison to healthy subjects (Figures 1B, 2B, and 3B).

There was no significant difference in the inhibition of TNF-α secretion by dexamethasone between CD patients and healthy subjects or between the three groups (Figure 1B). There was a significant difference in the inhibition of IL-6 secretion by dexamethasone at 100 nM and 1 μM between CD patients and healthy subjects ($P < 0.01$ and $P < 0.01$, respectively). A significant difference in the inhibition of IL-6 secretion also was observed at 1 μM of dexamethasone between non-CSD and healthy subjects and between CSD patients and healthy subjects ($P < 0.01$) (Figure 2B). There was a significant difference in the inhibition of IL-1β secretion by dexamethasone at 100 nM and 1 μM between CD patients and healthy subjects ($P < 0.01$ and $P < 0.01$, respectively). A significant difference also was observed at 100 nM and 1 μM between CSD patients and healthy subjects ($P < 0.05$ and $P < 0.01$, respectively) (Figure 3B).

DISCUSSION

The corticosensitivity of patients with Crohn's disease and control subjects was evaluated in this study by the degree of inhibition of cytokine secretion in whole blood cell cultures by graded concentrations of dexamethasone. The data suggested a significant decrease in the corticosensitivity of patients with Crohn's disease. There was no difference between CSD patients and non-CSD patients, however. All of our patients in this study were in clinical remission (Crohn's disease activity index <150) and corticosteroid-free for at least six months. Previous studies on steroid-sensitive and steroid-resistant asthma were carried out in patients concurrently receiving glucocorticoid therapy (15, 21, 22). Glucocorticoids normally induce a down-regulation of GR, and this could lead to a decrease in corticosensitivity. Thus, a study of corticosensitivity in patients treated with exogenous glucocorticoids may reflect treatment rather than disease effect. The complete abstention from glucocorticoids for at least six months in non-CSD patients and in CSD patients treated with immunosuppressants allowed us to avoid this potential pitfall.

Interleukin-1β, IL-6, and TNF-α are important cytokines of innate immunity and mediate both specific and nonspecific inflammatory responses. These cytokines are also important mediators of inflammation in the intestinal mucosa of patients with inflammatory bowel diseases. Enhanced secretion of such cytokines by peripheral blood mononuclear cells (PBMCs) was reported in patients with Crohn's disease earlier (23). We did not confirm this, but rather demonstrated that the basal production of inflammatory cytokines *ex vivo* was not different from that of controls and that after LPS stimulation, the production of TNF-α and IL-6 was, in fact, decreased in CSD patients compared to those of healthy subjects. This could be explained by the different methodologies used and the type of subject populations studied. All the CSD patients were treated with immunosuppressants.

The decreased PBMC corticosensitivity of patients with Crohn's disease might be related to the inflammatory state itself. Indeed, corticosensitivity was earlier shown to be modulated by cytokines (24). Some proinflammatory cytokines, such as IL-1β, IL-6, TNF-α and interferon-γ (IFN-γ), increased (24–26), while other mostly antiinflammatory cytokines, such as IL-4 and IL-13, decreased sensitivity to glucocorticoids, possibly glucocorticoid receptor number and affinity changes (27, 28). Glucocorticoids are potent inhibitors of NF-κB, a pivotal transcription factor for the expression of many cytokine genes in chronic inflammatory diseases (29, 30). NF-κB functions as an intracellular amplification factor that exacerbates chronic inflammatory processes and itself inhibits the activity of the ligand-bound glucocorticoid receptor (31). Excessive NF-κB induction thus could prevent glucocorticoid suppression and could contribute to a decrease in corticosensitivity (32).

The susceptibility to develop an inflammatory disease could also be related to a genetically and/or constitutionally determined decrease in corticosensitivity. Type 2 CSR asthma was shown to be related to a primary decrease of GR number per cell (15).

Hyposensitivity could lead to an hyperimmune state and to susceptibility to inflammatory and autoimmune diseases (11, 12). A primary decrease in corticosensitivity could result in a relative inability of endogenous glucocorticoids to modulate the mucosal immune response to bacterial or alimentary luminal antigens, superantigens, and nonspecific immunostimulants. This study suggests that decreased corticosensitivity in patients with quiescent Crohn's disease might be a factor favoring further relapses.

A potential effect of the treatment with 5-ASA or azathioprine could not be excluded. Whether such a treatment could directly influence corticosensitivity is, however, unclear. The pattern and degree of dexamethasone-mediated inhibition were the same in quiescent CSD and quiescent non-CSD patients. On one hand, this could mean that corticosteroid dependency in Crohn's disease is not related to a decrease of corticosensitivity. On the other hand, the same corticosensitivity in both groups might be due to the immunosuppressants improving the corticosensitivity of CSD patients with Crohn's disease (33).

Recently, the activity of the HPA axis itself was shown to be altered in inflammatory diseases such as rheumatoid arthritis (34). Chikanza et al reported that rheumatoid arthritis patients had normal cortisol levels and a normal response to exogenous corticotropin releasing factor but a reduced response to increased levels of circulating IL-1 and IL-6 induced by surgical stress, compared to patients with osteoarthritis and chronic osteomyelitis also undergoing a major operation (34). Similarly, early untreated patients with rheumatoid arthritis had paradoxically normal ACTH and cortisol levels, which would not have been predicted by their pain, fever, and high levels of inflammatory cytokines (35). In our patients, hypercortisolism could be associated with a decrease in corticosensitivity via homologous down-regulation of the GR. The HPA axis of patients with Crohn's disease has not been studied extensively as yet; however, our normal baseline measurements of cortisol and cortisol-binding globulin in this study do not corroborate the idea of a major change in the activity of the HPA axis in Crohn's disease, yet further studies need to be done to rule out subtle or dynamic changes of their axis.

In conclusion, a decrease of corticosensitivity in whole blood cell cultures was observed in patients with Crohn's disease compared to healthy controls. The alteration of corticosensitivity in Crohn's disease could be genetic/constitutional and/or acquired in response to endocrine and/or immune factors related to the inflammatory disease. Further studies are needed to characterize the complex interactions between the HPA axis and the immune system in inflammatory bowel diseases.

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