CD4 T Lymphocytes from Patients with Chronic Fatigue Syndrome Have Decreased Interferon-γ Production and Increased Sensitivity to Dexamethasone

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A disturbed hypothalamus–pituitary–adrenal gland axis and alterations at the immune system level have been observed in patients with chronic fatigue syndrome (CFS). Glucocorticoids are known to modulate T cell responses; therefore, purified CD4 T cells from CFS patients were studied to determine whether they have an altered sensitivity to dexamethasone (DEX). CD4 T cells from CFS patients produced less interferon-γ than did cells from controls; by contrast, interleukin-4 production and cell proliferation were comparable. With CD4 T cells from CFS patients (compared with cells from controls), a 10- to 20-fold lower DEX concentration was needed to achieve 50% inhibition of interleukin-4 production and proliferation, indicating an increased sensitivity to DEX in CFS patients. Surprisingly, interferon-γ production in patients and controls was equally sensitive to DEX. A differential sensitivity of cytokines or CD4 T cell subsets to glucocorticoids might explain an altered immunologic function in CFS patients.

Chronic fatigue syndrome (CFS) is a disease of unknown origin characterized by severe disabling fatigue with a duration of >6 months and a reduction in normal activity of at least 50% [1]. Several causes have been held responsible for the disease, including viral infections, a disturbed hypothalamus–pituitary–adrenal gland (HPA) axis and an altered immune function [1, 2]. However, there is no convincing evidence for a role of viral or other infections; for example, titers of antibodies specific for Epstein-Barr virus–related antigens do not differ between patients and controls [3]. The observation that CFS patients have lower levels of free cortisol in their urine and reduced evening plasma cortisol levels in conjunction with elevated levels of plasma adrenocorticotropic hormone [4] is in support of a disfunctioning HPA axis. As far as the immune system is concerned, several functional abnormalities have been reported in CFS, including a decreased NK cell activity, a reduced mitogenic response of lymphocytes, and alterations in cytokine production [2, 5, 6]. Although it is well established that various hormones related to the HPA axis have immunomodulatory properties, which are not exclusively immunosuppressive in nature [7], it is unknown to what extent immunologic aberrations in CFS are due to an altered activity of the HPA axis.

To our knowledge, this study is the first to compare properties of purified CD4 T cells from CFS patients with those of cells from healthy controls who were matched for age and sex. The study of these cells instead of unseparated peripheral blood mononuclear cells (PBMC) provides a more accurate approach to the comparison of Th1 cell activity with Th2 cell activity. Th1 cells regulate cellular immunity by the secretion of cytokines such as interferon-γ (IFN-γ), whereas Th2 cells regulate humoral immunity by cytokines such as interleukin (IL)-4. Of interest, the balance between Th1 and Th2 cells, which appears to be important in the development of various diseases [8], is suggested to be regulated by glucocorticoids [9]. Therefore, we studied to what extent immunologic alterations in CFS were accompanied by an altered sensitivity of CD4 T cells to dexamethasone (DEX).

Materials and Methods

Subjects. Eighteen white CFS patients fulfilling the criteria of Fukuda et al. [1] and living within the postal code area of Leiden were recruited from the Dutch Myalgic Encephalomyelitis Patient Association. To exclude any known causes of fatigue, an internist contacted each patient's general practitioner and any other specialist who had been consulted. Known causes of fatigue were determined by anamnesis, physical examination, and laboratory investigation, including thyroid function tests and tests for autoantibodies or paraproteins. The duration of symptoms ranged from 1–3 years. Each patient was accompanied by a race- and sex-matched healthy control. Ages for controls differed by not more than 4 years from those of CFS patients. The mean age was 36.7 ± 13 years for the CFS group and 40.5 ± 13 years for the controls. Patients and controls were not using medication; vitamins and homeopathics were allowed.

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Informed consent was obtained from study participants.

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Table 1. Functional responses of peripheral blood mononuclear cells (PBMC) and CD4 T cells from CFS patients compared with responses of cells from age- and sex-matched controls.

	PBMC*				Negatively selected CD4 T cells*				Positively sorted CD4 T cells†			
	CFS	Controls	*n*	*P*‡	CFS	Controls	*n*	*P*‡	CFS	Controls	*n*	*P*‡
Proliferation (cpm ± SD)	4335 ± 1062	4261 ± 796	16	NS	4293 ± 1837	4522 ± 2868	16	NS	4381 ± 3373	5095 ± 3152	14	NS
IL-4 production (pg/mL ± SD)	590 ± 416	536 ± 371	11	NS	841 ± 507	934 ± 563	14	NS	532 ± 98	516 ± 136	13	NS
IFN-γ production (U/mL ± SD)	3032 ± 2578	4007 ± 2514	11	NS	1258 ± 1266	3276 ± 2787	9	.011	392 ± 194	899 ± 311	12	.034

* Stimulated with combination of anti-CD2 and anti-CD28.
† Stimulated with phytohemagglutinin.
‡ Wilcoxon rank sum test for matched pairs.

At study entry, blood was obtained for additional examinations. Differential blood counts did not reveal abnormalities. By use of flow cytometry, it was established that the percentages of CD14 monocytes, CD4 T cells, CD8 T cells, and CD19 B cells did not differ from control values. As a highly sensitive approach to exclude the presence of inflammatory processes, we established plasma levels of C-reactive protein by ELISA, using polyclonal antibodies (DAKO, Copenhagen): Values for patients were similar to those for controls (median, 0.5 and 0.7 mg/L, respectively). In routine practice, a cutoff of 5 mg/L is used to detect inflammation. No other signs or symptoms of disease developed within the 6-month study period. Only 1 patient was able to resume work within this time.

Isolation of cells. PBMC were isolated from EDTA-blood by Histopaque-1077 (Sigma, St.Louis) density centrifugation and cryopreserved to enable batchwise analysis of patients and controls at a later time. CD4 T cells were enriched to a purity of at least 85% by the depletion of CD8 T cells, B cells, NK cells, and monocytes, as described previously [10]. Additional experiments were done with CD4 T cells obtained by positive selection (>95% purity), using anti-CD4 microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) according to the manufacturer's instructions; the percentage of CD14 monocytes in this fraction was 4% on average.

Cell cultures. Cultures of PBMC and CD4 T cells were done in Iscove's Modified Dulbecco's Medium (Gibco, Paisley, UK) supplemented with 10% fetal calf serum (Sebak, Aidenbach, Germany), 100 U/mL penicillin, 100 μg/mL streptomycin, 2 m*M* L-glutamine, and 50 μ*M* β-mercaptoethanol. Cells were cultured at a density of 40,000 cells/well in a volume of 200 μL in flat-bottomed 96-well microtiter plates (Costar, Cambridge, MA) and stimulated with 0.5 μg/mL phytohemagglutinin (Murex, Dartford, UK) or with a cocktail of two anti-CD2 and one anti-CD28 antibodies [10]. DEX (Sigma) was added to a final concentration of 10^{-6}, 10^{-7}, or 10^{-8} *M*.

Supernatants of CD4 T cell cultures were harvested at day 3. Proliferation was measured at day 4 by the addition 18.5 KBq (0.5 μCi) methyl-[^{3}H]thymidine with a specific activity of 74 GBq/mmol (Radiochemical Centre, Amersham, UK) during the last 6 h of culture. Supernatants of PBMC cultures were harvested on day 4, and proliferation was measured on day 5. The culture conditions described above and time points to measure cytokines and proliferation were previously established to represent suboptimal conditions for cryopreserved cells; they were used to allow the detection of stimulatory and inhibitory effects of DEX. Supernatants were stored at −20°C until assay. Labeled cells were harvested onto glassfiber filters (Canberra Packard, Meriden, CT), which were counted by use of a beta-counter (Matrix 96; Canberra Packard).

IL-4 and IFN-γ assays. Levels of IFN-γ and IL-4 were determined by ELISA as previously described [11]. For the IFN-γ ELISA, we used mouse anti-human IFN-γ (clone MD-2) as capture antibody, biotinylated mouse anti-human IFN-γ (clone MD-1) as a detecting antibody, and human recombinant IFN-γ as a reference standard (reagents were provided by Peter van der Meide, Biomedical Primate Research Centre, Rijswijk, The Netherlands). For the IL-4 ELISA, we used mouse anti-human IL-4 (clone 8D4-8) as a capture antibody, biotinylated rat anti-human IL-4 (clone MP4-25D2) as detecting antibody, and human recombinant IL-4 as a reference standard (reagents were purchased from Pharmingen, San Diego).

Statistical analysis. Statistical analysis was done using the Wilcoxon rank sum test for matched pairs. Differences with a confidence level of ⩾95% were considered statistically significant ($P < .05$).

Results

Decreased Th1 activity in CD4 T cells from CFS patients. It has been shown, on the basis of cultures of unseparated PBMC or whole blood cells, that CFS patients have decreased levels of IFN-γ, TNF-α, and IL-1 production in response to phytohemagglutinin or lipopolysaccharide [2, 5]. IFN-γ can be released by CD4 T, CD8 T, or NK cells. To establish whether CFS is accompanied by an altered activity of Th1 or Th2 cells within the immunoregulatory $CD4^+$ T cell compartment, we compared cytokine production by PBMC with that by enriched CD4 T cells. To ensure optimal T cell stimulation, we stimulated cells with a combination of anti-CD2 and anti-CD28 antibodies. As shown in table 1, on average, anti-CD2– and anti-CD28–stimulated PBMC from CFS patients had re-

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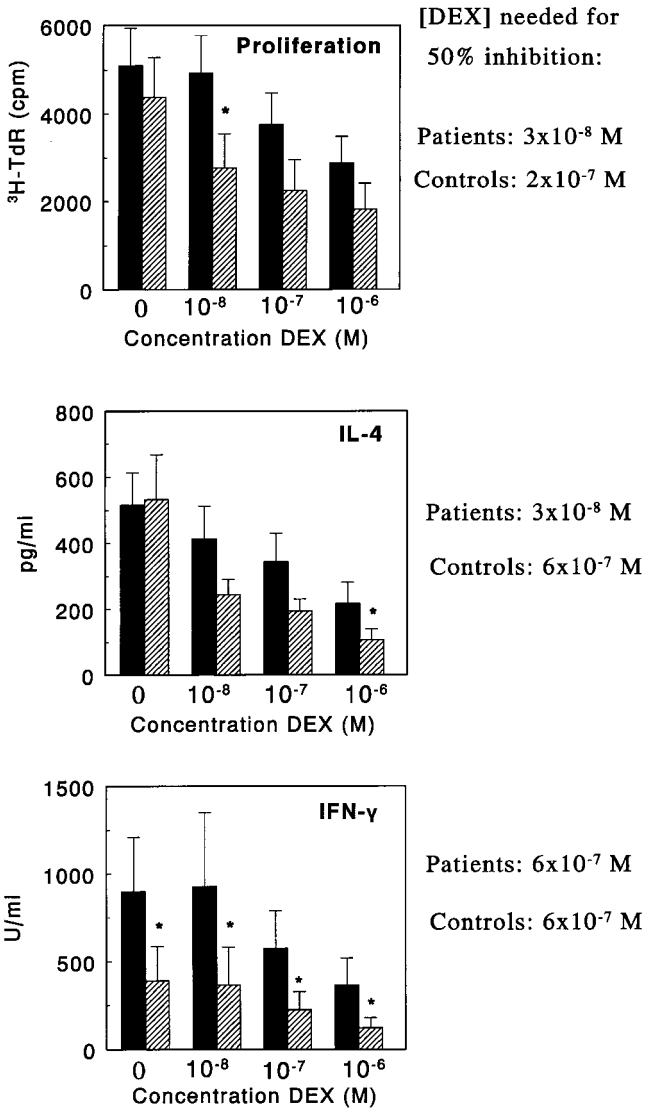

Figure 1. Positively selected CD4 T cells from CFS patients have increased sensitivity to dexamethasone. Cells were stimulated with phytohemagglutinin. Measurements of cytokine production and proliferation were done at culture days 3 and 4, respectively. Results are means ± SE of proliferation for 14 patients (hatched bars) and controls (black bars), of interleukin-4 production for 13 patients and controls, and of interferon-γ production for 12 patients and controls. * $P <$ .05, Wilcoxon rank sum test for matched pairs. DEX, dexamethasone.

duced production of IFN-γ; however, this difference appeared not to be significant. Furthermore, no differences were detected in proliferation or IL-4 production. However, when the functional properties of the enriched CD4 T cells were studied, it was found that the cells from CFS patients versus controls had significantly ($P < .02$) decreased levels of IFN-γ production.

CD4 T cells obtained by negative selection combine the advantage of not carrying a possibly modulatory antibody with the disadvantage of a relatively low purity (although >85%). This still leaves the possibility that an unidentified contaminating cell population could contribute to cytokine production. Therefore, we examined whether our results could be confirmed using positively sorted CD4 T cells. Moreover, we used phytohemagglutinin as a suboptimal monocyte-dependent system that is highly sensitive to DEX (see below), in contrast to the anti-CD2/anti-CD28 system [10]. As shown in table 1, phytohemagglutinin-stimulated positively sorted CD4 T cells from CFS patients produced significantly less IFN-γ than did cells from age- and sex-matched controls ($P < .05$). Again, no significant difference was observed with regard to proliferation or IL-4 production.

CD4 T cells from CFS patients have increased sensitivity to DEX. CFS patients have been shown to have a disturbed HPA axis resulting in low levels of cortisol [4]. Since a disturbed HPA axis may be reflected in the sensitivity of lymphocytes for DEX [11], we examined the possibility that CFS patients also have an altered sensitivity to glucocorticoids. As shown in figure 1 (top), the proliferative response of positively selected CD4 T cells from CFS patients is more sensitive to DEX than are responses of cells from matched controls. On average, 2×10^{-8} *M* DEX was needed for 50% inhibition of proliferation of CD4 T cells from CFS patients. At least a 10-fold higher DEX concentration was required for a comparable effect in controls. Likewise, IL-4 production by CD4 T cells from CFS patients was more sensitive to DEX (figure 1, middle). On average, 3×10^{-8} *M* DEX was needed to achieve 50% inhibition of IL-4 production by CD4 T cells from the patients, whereas a 20-fold higher concentration was required for a comparable effect in the controls.

Figure 1 (bottom) shows that CD4 T cells from CFS patients produced less IFN-γ than did control cells. Surprisingly, IFN-γ production by CD4 T cells from both groups was equally sensitive to DEX: 50% inhibition of the initial IFN-γ production was achieved by the addition of 6×10^{-7} *M* DEX. No correlations were found between DEX sensitivities and the extent of IL-4 or IFN-γ production (data not shown).

Discussion

In the past few years, it has become clear that various immunologic diseases are related to an altered activity of Th1 or Th2 cells [8]. To establish whether previously reported immunologic aberrations in CFS are related to an altered activity of one of these subsets, we studied the characteristics of purified CD4 T cells. Both negatively selected CD4 T cells (>85% pure) and positively selected CD4 T cells (>95% pure) from CFS patients had a reduced IFN-γ production compared with that for controls. This difference was selective for IFN-γ production; IL-4 production and cell proliferation were normal.

The difference in cytokine production between the CFS group and the control group was not related to a difference in the composition of the CD4 T cell compartment. Our patient group did not differ from the control group with respect to the fraction of naive and memory CD4 T cells (patients: 50.5% ±

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12.8% CD45RA vs. 55.1% ± 18.9% CD45RO; controls: 51.8% ± 9.9% CD45RA vs. 50.1% ± 12.8% CD45RO cells; data not shown). It is therefore likely that the reduced IFN-γ production reflects a selective reduction in the activity of Th1 cells in CFS rather than a shift from Th1 to Th2. This is consistent with a report demonstrating a decrease in delayed-type hypersensitivity (which is mediated by Th1 cells) in CFS [12].

Since CFS patients have a dysfunction of their HPA axis that results in low levels of cortisol [4] and in view of the idea that glucocorticoids may selectively suppress Th1 cells [9], it would have been expected that patients would produce more IFN-γ in vitro due to less suppression in vivo. Our observation of an even lower IFN-γ production in CFS might be due to suppression of IFN-γ in vivo as a consequence of an increased sensitivity of the CD4 T cells for glucocorticoids (e.g., as a consequence of an increased number or affinity of glucocorticoid receptors). Although such an increased sensitivity could indeed be demonstrated using proliferation and IL-4 production as a read-out, this was not found for IFN-γ. This is possibly due to the fact that in patients (as opposed to controls) this cytokine is already suppressed in vivo and that relatively high DEX concentrations are needed in vitro to inhibit IFN-γ even further. Alternatively, a different mechanism might be involved. It is well established that the production of IFN-γ and the induction of Th1 responses is dependent on IL-12 [13] and that IL-10 can inhibit the production of IFN-γ through the inhibition of IL-12 [14]. Preliminary data we obtained in whole blood cultures indicate that IL-10 levels are increased in CFS, suggesting that this cytokine may also contribute to a lower IFN-γ production in CFS patients.

It is expected that more insight into the cause of CFS can be obtained by determining to what extent an altered HPA axis is responsible for the reported immunologic aberrations in CFS.

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