

Decreased Sensitivity to Dexamethasone in Lymphocytes from Patients with Alzheimer's Disease

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Cortisol levels in patients with Alzheimer's disease (AD) are relatively unaffected by a challenge with dexamethasone (DEX) *in vivo*. The present study demonstrates that DEX is less inhibitory for phytohemagglutinin (PHA)-induced T cell proliferation in AD patients as compared to age-matched controls. Since no significant differences were found between AD patients and age-matched controls with regard to the fraction of CD45RA⁺ or CD45RO⁺ CD4⁺ T cells nor the ability of peripheral blood mononuclear cells to produce IL-2 or IL-4, it is unlikely that the difference in DEX sensitivity is due to a changed lymphokine profile or a changed composition of the CD4⁺ T cell population. Sensitivity to DEX was negatively correlated with the ability to produce IL-2 and IL-4 in the controls but not in AD patients. This suggests that IL-2 and IL-4 synthesis in AD patients is less sensitive to regulation by glucocorticoids. © 1994 Academic Press, Inc.

INTRODUCTION

The pathogenesis of Alzheimer's disease (AD) is still largely unknown. The neuropathological hallmarks of this disease, namely the senile plaques, containing the β -amyloid protein (1), and the neurofibrillary tangles (2), have still not been proven to be causative in the pathogenesis of the disease, although a mutation found in the amyloid precursor protein (APP) gene (3) suggests that β -amyloid might cause AD in some cases. Evidence indicates that defects observed in AD are not confined to the central nervous system but can also be observed in other organ systems, i.e., the immune system. Compatible with an impaired immune system in AD, clinical data show that—compared to age-matched controls—life expectancy of AD patients is reduced; the primary cause of death is infections of the respiratory tract (4). Furthermore, there is evidence that treatment with anti-inflammatory drugs may reduce the rate of progression of the dementia in AD patients (5). Moreover, in contrast to vascular dementia, infection-related mortality in AD patients increases with the severity of dementia (6).

The fact that APP has been identified in phytohe-

magglutinin (PHA)-stimulated peripheral blood mononuclear cells (PBMC) of normal healthy individuals (7) also suggests that the deposition of the β -amyloid protein may be related to an immunological or inflammatory process. This is supported by the recent observation that Epstein-Barr virus-transformed B cell lines from an AD patient produce antibodies which are reactive with the β -amyloid protein (8).

In addition, proinflammatory cytokines like interleukin-1 (IL-1) and IL-6 may play a role in the amyloidogenesis in AD (9). Arguments in favor of this hypothesis are that microglial cells and astrocytes can produce IL-1 and IL-6, respectively (10, 11), and that IL-1 is able to increase APP mRNA expression in cultured human endothelial cells (12). However, plasma levels of IL-6, which are increased in various diseases with an inflammatory compound, are unchanged in AD patients (13).

With regard to the properties of the cells of the immune system in AD, several *in vitro* studies show a diminished proliferative response of PBMC (14, 15). However, other studies showed no differences with respect to this parameter (16–18).

In vivo studies indicate that the cortisol levels of AD patients are less inhibited by a dexamethasone (DEX) challenge as compared to controls, pointing to an imbalance of the hypothalamic-pituitary-adrenal axis (HPA) in AD (19–21). Since glucocorticoids are widely known for their potent immunosuppressive effect, we also studied whether the sensitivity of PBMC from AD patients to DEX was different from that of age-matched controls. Our present study shows that the suppressive effect of DEX on T cell proliferation is significantly less in AD patients as compared to controls and that this difference is unrelated to the capacity of the cells to produce IL-2 or IL-4.

MATERIALS AND METHODS

Patients

Patients ($n = 30$; mean age 69 years, 20 female and 10 male) and controls ($n = 30$; mean age 70 years, 19

female and 11 male) were derived from an epidemiological study of risk factors of clinically diagnosed early onset (diagnosis ≤ 70 years) AD (13). This population includes cases with familial as well as sporadic AD. AD was considered familial if there was at least one first degree relative with AD. All patients met the NINCDS-ADRDA criteria for probable AD (22). For this study, the clinical diagnosis of AD was confirmed by two independent experts (13).

Cell Cultures

PBMC were obtained by Percoll (Biochrom KG, Berlin, Germany) density centrifugation ($\rho = 1.077 \text{ g/cm}^3$) and cryopreserved in culture medium containing 20% (v/v) fetal calf serum (FCS; Seralab, Crawley Down, UK) and 10% (v/v) dimethyl sulfoxide, using a software-directed program for the cryopreservation of lymphocytes as described elsewhere (23). Per experiment, cell suspensions from an equal number of patients and controls were thawed by dropwise dilution (at least 10-fold) in ice-cold culture medium containing 20% FCS. The preservation time of PBMC from all donors used in this study was approximately 2 years. Viable cells ($\geq 80\%$) were enumerated by trypan blue exclusion. In agreement with Bom-van Noorloos *et al.* (23), we found no changes in the phenotype of the cells after this procedure, with the exception of a slight decrease in monocytes. With respect to functional data, a positive correlation was found between data obtained with cryopreserved cells as compared to fresh PBMC from identical donors (data not shown). The slight decrease in the percentage monocytes is probably responsible for the fact that peak responses with cryopreserved cells are found at Day 5 instead of a peak at Day 4 found with fresh PBMC. All cultures were performed in Iscove's modified Dulbecco's medium (Biochrom KG) supplemented with 10% FCS, penicillin (100 U/ml), streptomycin (100 $\mu\text{g/ml}$), L-glutamine (2 mM), and β -mercaptoethanol (50 μM).

PBMC (4×10^4 /well) were cultured in flat-bottom microtiter plates (Costar, Cambridge, MA). Cells were stimulated with 0.5 $\mu\text{g/ml}$ PHA (Murex, Dartford, UK) in the absence or in the presence of 10^{-7} M DEX (Sigma, St. Louis, MO). This PHA concentration was optimal for proliferative of PBMC, but suboptimal for the induction of IL-2. This approach allows a sensitive discrimination between individual AD patients and controls with respect to differences in DEX sensitivity of PBMC. Where indicated, exogenous human recombinant IL-2 (Cetus, Emeryville, CA; kindly provided by Dr. E. Braakman, Dr. Daniel den Hoed Cancer Center, Rotterdam, The Netherlands) was added to a concentration of 50 U/ml.

Cell proliferation was measured by adding 0.25 μCi methyl-tritium-thymidine ($[^3\text{H}]\text{TdR}$) (specific activity 2 Ci/mmol; Radiochemical Centre, Amersham, UK)

during the last 6 hr of culture. Cells were harvested onto glass fiber filter paper (Packard Instrument Company, Meriden, CT). Filters were counted using a Matrix 96 β -counter (Packard Instrument Co.). This procedure has an efficiency of about 20% as compared to standard liquid scintillation counting.

IL-2 production was induced by 10 $\mu\text{g/ml}$ PHA. For the induction of IL-4, anti-CD2 (CLB-T11.1/1 and CLB T11.1/2) and anti-CD28 (CLB-CD28/1) monoclonal antibodies (mAbs) were used in a 1:2000 dilution of murine ascites and 2 $\mu\text{g/ml}$ of purified antibody, respectively. These antibodies were a generous gift from Dr. R. A. W. van Lier from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (Amsterdam, The Netherlands). All supernatants were harvested at Day 3 and stored at -20°C until assayed.

IL-2 and IL-4 Assays

IL-2 was measured by ELISA, according to the instructions of the manufacturer (Genzyme, Cambridge, MA). Alternatively, IL-2 activity was assessed with the use of the CTLL-2 cell line. In the CTLL-2 bioassay, 5000 cells were cultured in the presence of serially diluted supernatants. Human recombinant IL-2 was used as a standard. During the last 4 hr of the 24-hr culture period, cells were pulsed with 0.25 μCi $[^3\text{H}]\text{TdR}$. IL-4 levels in the supernatant were assessed using an ELISA technique as described elsewhere (24) employing reagents generously provided to us by Dr. T. van der Pouw-Kraan, CLB, Amsterdam, The Netherlands. Plates were read using a Bio-Rad Microplate reader (Bio-Rad, Richmond, CA). Human recombinant IL-4 was used as a standard (Genzyme). The detection limit of the assay was 100 pg/ml.

Phenotypic Analysis

For phenotypic analysis, 5×10^4 cells were incubated with a saturating amount of FITC- or PE-conjugated mAbs. Thereafter, cells were washed and analyzed on a FACScan (Becton-Dickinson). Gates were set on the basis of forward and sideward scatter, including all viable leukocytes.

Seven thousand gated events were analyzed for green and red fluorescence. To determine the expression of CD45RA, CD45RO, or CD27 within the CD4^+ T cell population, only viable cells stained with anti-CD4-PE were included. The following reagents were used: 2H4-FITC (anti-CD45RA; Coulter, Hialeah, FL), UCHL-1-FITC (anti-CD45RO; DAKO, Glostrup, Denmark), and anti-CD27 (CLB-CD27/1), which was a kind gift from Dr. R. A. W. van Lier (CLB, Amsterdam, The Netherlands). Anti-CD3-FITC, anti-CD4-PE, anti-CD8-PE, and anti-CD14-FITC were all purchased from Becton-Dickinson.

Expression of Glucocorticoid Receptors (GCR)

PBMC were fixed and permeabilized as described elsewhere (25) with some modifications. Briefly, cells were washed twice with phosphate-buffered saline (PBS) to remove the FCS from the culture medium. Thereafter the cells were fixed (5 min, 4°C) with 2% paraformaldehyde in PBS. Subsequently, the cells were permeabilized by adding an equal volume of 0.4% Triton X-100 for an additional 5 min. Finally, the cells were washed twice with PBS containing 0.5% bovine serum albumin. Permeabilized PBMC (5×10^5) were incubated with 5 $\mu\text{g}/\text{ml}$ mAb number 7, a mouse antibody cross-reactive with the human glucocorticoid receptor [anti-GCR; kindly provided by Dr. A. C. Wikström, Karolinska Institutet, Huddinge, Sweden (26)]. Staining occurred with goat anti-mouse FITC (CLB, Amsterdam, The Netherlands) as a second step antibody. Double staining for CD4⁺ T cells was performed with a CD4PercP conjugate (Becton-Dickinson) in 5% (v/v) normal mouse serum. After each incubation step, cells were washed twice and 15,000 PBMC were analyzed on a FACScan (Becton-Dickinson). An irrelevant mouse IgG2a antibody was used as an isotype control. Results for each individual patient or control are expressed as relative fluorescence intensity, i.e., mean fluorescence intensity found with anti-GCR divided by the mean fluorescence intensity of the isotype control.

Statistics

Statistic analysis was performed using the two-tailed Mann-Whitney *U* test. Differences were considered statistically significant at a confidence level of 95% or higher ($P < 0.05$).

RESULTS

Decreased Sensitivity of T Cell Proliferation to DEX in AD Patients

In contrast to several other studies (14, 15), the present study does not confirm the observation that T cell proliferation in response to PHA (0.5 $\mu\text{g}/\text{ml}$) is impaired in AD patients. Stimulating PBMC from a well-defined panel of AD patients ($n = 30$) and a panel of age-matched controls ($n = 30$) led to a mean [³H]TdR incorporation by PBMC of 6504 and 6265 cpm, respectively ($P = 0.59$; data not shown).

Since AD patients are less sensitive to a DEX challenge *in vivo* (19–21), we studied whether this phenomenon was also true for the sensitivity of PBMC *in vitro*. As seen in Fig. 1A, the proliferative response of PBMC from AD patients to an optimal concentration of PHA (0.5 $\mu\text{g}/\text{ml}$) was less inhibited by 10^{-7} M DEX (32.7% of the control response on average) than that of controls (46.5% of the control response on average). Despite the heterogeneity of the response in both groups,

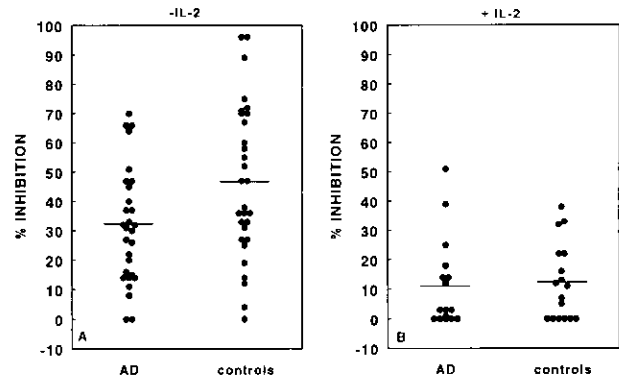


FIG. 1. PBMC of AD patients display a diminished sensitivity for dexamethasone. PBMC were stimulated with 0.5 $\mu\text{g}/\text{ml}$ PHA in the presence of 10^{-7} M DEX. For each individual donor the percentage inhibition of the response at Day 5 is shown (A). The effect of exogenous IL-2 (50 U/ml) on DEX-induced inhibition was studied with PBMC from 17 individuals of each group (B). Mean proliferative responses in the absence of DEX were 6265 (AD patients) and 6504 (controls) cpm in the absence of exogenous IL-2 and 7026 (AD patients) and 7457 (controls) cpm in the presence of exogenous IL-2. Background proliferation was less than 50 cpm.

this difference was significant ($P = 0.03$). The addition of a saturating amount of exogenous IL-2 (50 U/ml) to the cultures reduced the inhibitory effect of DEX and the differences between the two groups (Fig. 1B; $P = 0.90$). PBMC from familial and sporadic cases of AD were equally sensitive to DEX (data not shown).

As shown in Fig. 2—for a random selection of 11 patients and 12 controls—PBMC stimulated with a suboptimal PHA concentration (0.125 $\mu\text{g}/\text{ml}$) were almost completely inhibited by 10^{-7} M DEX (mean in-

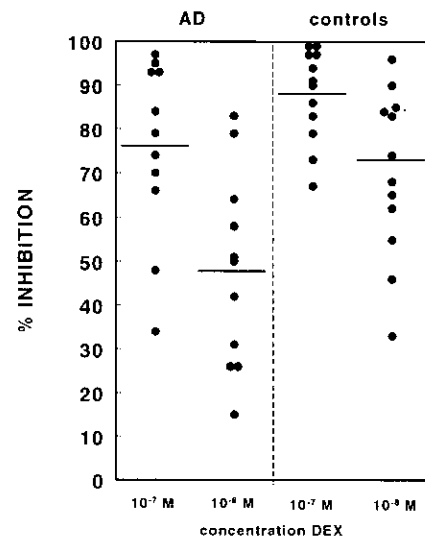


FIG. 2. Differences in dexamethasone sensitivity of PBMC from AD patients and controls at suboptimal PHA concentrations. PBMC from 11 AD patients and 12 controls were stimulated with 0.13 $\mu\text{g}/\text{ml}$ PHA. Mean proliferative responses in the absence of DEX were 3053 and 2465 cpm for AD patients and controls, respectively. The percentage of inhibition at Day 5 in the presence of 10^{-7} and 10^{-8} M DEX for each individual donor is shown.

inhibition 75.7 and 87.9% in AD and controls, respectively; $P = 0.12$). Partial inhibition was now found at 10^{-8} M DEX and a significant difference between the two groups was again found (mean inhibition 47.7 and 70.1% in AD and controls, respectively; $P = 0.02$).

We subsequently excluded the possibility that the difference in sensitivity to DEX between the two groups was because of a difference in growth kinetics of the cells. This was done for 15 patients and 14 age-matched controls, randomly chosen from the panel used in Fig. 1A. As can be seen in Fig. 3A there was no difference in growth kinetics of PBMC from AD pa-

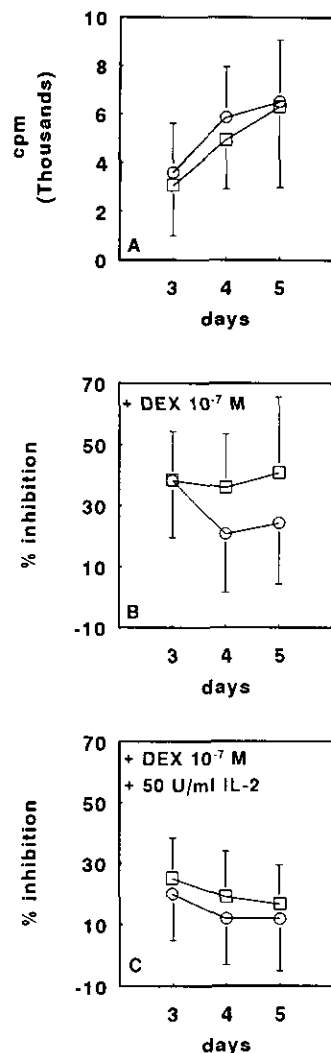


FIG. 3. Growth kinetics of PBMC from AD patients (○) and controls (□) are comparable. PBMC from 15 patients and 14 controls were stimulated with 0.5 μ g/ml PHA. The mean of the individual data is shown. Mean proliferative responses in the absence of exogenous IL-2 on Days 3, 4, and 5 are shown (A). The extent of inhibition by 10^{-7} M DEX in the absence (B) and in the presence (C) of 50 U/ml exogenous IL-2 is shown. The means of the proliferative responses in the presence of exogenous IL-2 on Days 3, 4, and 5 were 3372, 5192, and 6357 cpm for PBMC from AD patients and 3463, 4833, and 6255 cpm for PBMC from control donors. Proliferation in the absence of mitogen was less than 50 cpm.

tients and controls. As shown in Fig. 3B, the decreased sensitivity for DEX in AD was already evident at Day 4 of the culture ($P = 0.01$ versus $P = 0.04$ on Day 5). Again, the addition of a saturating amount of exogenous IL-2 (50 U/ml; Fig. 3C) to the cultures reduced the differences between the two groups.

Phenotype of PBMC from AD Patients and Controls

In the mouse, it has been demonstrated that DEX, while inhibiting IL-2 may enhance IL-4 production (27). In the human situation, IL-2 is the main product of naive $CD4^+CD45RA^+$ T cells whereas IL-4 is mainly produced by $CD4^+CD45RO^+$ T cells (28). We therefore evaluated whether the diminished DEX sensitivity of PBMC from AD patients was related to an altered composition of the $CD4^+$ T cell population. As depicted in Table 1, there were, however, no significant differences with regard to the fraction of $CD45RA^+CD4^+$ T cells or $CD45RO^+CD4^+$ T cells between the two groups, although there was a tendency toward a higher percentage of $CD4^+CD45RA^+$ T cells in the AD group. Moreover, there was no significant correlation in either group between the extent of inhibition by DEX and the percentage of $CD45RA^+$ or $CD45RO^+CD4^+$ T cells (data not shown). Also the percentage of $CD4^+$ T cells expressing CD27 (a marker absent from a subset of the $CD4^+CD45RO^+$ "memory" T cells) (29) was comparable in patients and controls.

In addition, no differences were observed with respect to the relative number of $CD3^+$ and $CD8^+$ T cells or $CD14^+$ monocytes in the PBMC of the two groups.

Comparable Expression of the GCR in $CD4^+$ T Cells from AD Patients and Age-Matched Controls

The lower sensitivity of PBMC in AD patients for DEX as compared to that of age-matched controls might be due to a difference in expression of the cytoplasmic GCR. Therefore, the level of GCR expression in $CD4^+$ T cells for 11 AD patients and 11 controls, randomly chosen from the panel in Fig. 1A, was deter-

TABLE 1
Phenotype of PBMC from AD Patients and Controls

Subjects	% of PBMC				% of $CD4^+$ T cells		
	CD3	CD3	CD8	CD14	CD45RA	CD45RO	CD27
AD	48.6	30.5	20.1	15.4	42.8	60.1	90.6
SD	13.9	9.6	11.1	7.5	18.3	16.1	5.9
<i>n</i>	31	31	31	31	31	31	22
Controls	47.7	27.4	22.0	13.6	36.8	63.4	87.4
SD	14.0	9.6	10.5	6.3	15.8	14.6	8.8
<i>n</i>	31	34	31	34	32	32	20
<i>P</i> value	0.87	0.33	0.46	0.43	0.14	0.36	0.27

Note. The relative number of cells in a total PBMC population. The percentage of $CD4^+$ T cells expressing $CD45RA^+$, $CD45RO^+$, and $CD27^+$ cells was calculated using gated $CD4^+$ (PE-positive) T cells. *n* and SD represent the number of donors tested and standard deviation, respectively.

mined by flow cytometry. Results are expressed as relative fluorescence intensity, i.e., the mean fluorescence intensity found with anti-GCR divided by the mean fluorescence intensity of the isotype control. As can be seen in Fig. 4, AD patients expressed comparable levels of GCR as controls (mean relative fluorescence intensity for AD and controls, 1.71 and 1.79, respectively; $P = 0.49$).

Diminished Sensitivity to DEX in AD Is Unrelated to IL-2 or IL-4 Production

Despite the fact that we did not find differences in the composition of the CD4⁺ T cells on the basis of their phenotype, PBMC from AD patients may differ from control cells with respect to the amount of IL-2 and/or IL-4 produced. Alternatively, the production of these cytokines may be differentially affected by DEX. Figure 5A shows that higher levels of IL-2 were detected in the patient group than in the control group (519 and 327 pg/ml in AD and controls, respectively), although this observation was not statistically significant ($P = 0.18$). The same tendency toward higher levels of IL-2 was observed when we determined the biological activity of the IL-2 present in the supernatants: in AD patients ($n = 20$) the mean IL-2 production was 25.5 U/ml and in controls it was 17.6 U/ml ($n = 21$; $P = 0.25$) (data not shown). In the presence of 10^{-7} M DEX, 6 out of 14 supernatants from patients showed detectable levels of IL-2 in the ELISA whereas only 2 out of 14 donors in the control group showed detectable amounts of IL-2. The mean inhibition of IL-2 production in AD patients and controls was 73 and 93%, respectively ($P = 0.18$).

Stimulation with PHA does not result in detectable

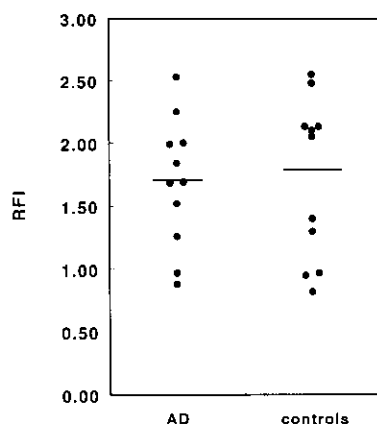


FIG. 4. Comparable expression of the glucocorticoid receptor in CD4⁺ T cells from AD patients and age-matched controls. For the measurement of the GCR, cells from 11 AD patients and controls were fixed and permeabilized (see Material and Methods) and indirectly stained with an anti-GCR antibody. The density of GCR in PBMC is depicted as the relative fluorescence intensity, i.e., the ratio between the mean fluorescence intensities found with the anti-GCR antibody and an irrelevant isotype-matched control antibody.

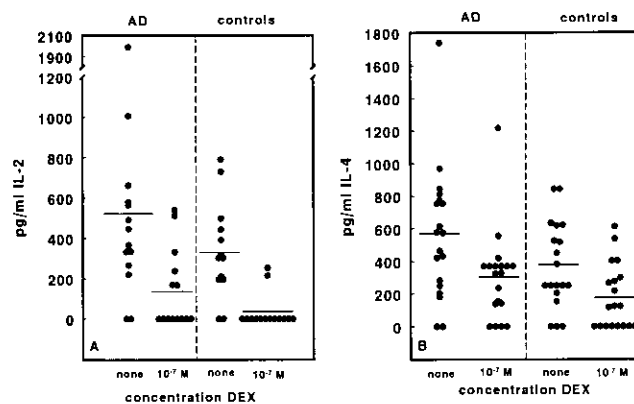


FIG. 5. IL-2 and IL-4 production in AD patients and controls are comparable and equally sensitive to DEX. For the measurement of IL-4, PBMC from 19 AD patients and controls were stimulated with the combination of anti-CD2 and anti-CD28 (see Materials and Methods). For the measurement of IL-2, PBMC from 14 patients and 14 controls were stimulated with $10 \mu\text{g/ml}$ PHA. Supernatants were harvested at Day 3 and the interleukins were determined by ELISA. Background values for IL-2 and IL-4 were lower than 100 pg/ml.

levels of IL-4 in the supernatant of PBMC (data not shown). We therefore stimulated PBMC with a combination of anti-CD2 and anti-CD28. As can be seen in Fig. 5B, the mean IL-4 production was 561 and 371 pg/ml in AD patients and controls, respectively. Again, these differences between the two groups were not significant ($P = 0.15$). When the cultures were performed in the presence of 10^{-7} M DEX, the mean inhibition of IL-4 in AD patients and controls was 50 and 61%, respectively ($P = 0.26$).

Donors that produced high levels of IL-2 or IL-4 in the absence of DEX also produced the highest levels in the presence of DEX ($P < 0.005$ for both IL-2 and IL-4 using linear regression).

By comparing the extent of inhibition of IL-2 and IL-4 in the total population (pooled data from AD patients and controls), it appeared that at a concentration of 10^{-7} M DEX the IL-2 production was more sensitive to DEX than the IL-4 production (data from Fig. 5 were used; mean inhibition of IL-2 and IL-4 production was 83.1 and 55.3%, respectively). Thus, although DEX does not enhance IL-4 production (as described for the mouse), it may affect the production of IL-2 and IL-4 differently.

Since DEX inhibits the production of IL-2 as well as IL-4, it might be that the extent of inhibition by DEX in the proliferative response is correlated with the extent of interleukin production. As can be seen in Fig. 6, this was indeed the case in the control group (Figs. 6A and 6B), i.e., high producers of IL-2 or IL-4 were relatively insensitive to DEX.

In contrast, there was no relation between IL-2 or IL-4 production and DEX sensitivity in the AD group (Figs. 5C and 5D). This suggests that the diminished sensitivity to DEX in AD is not exclusively related to an effect on the synthesis of IL-2 or IL-4.

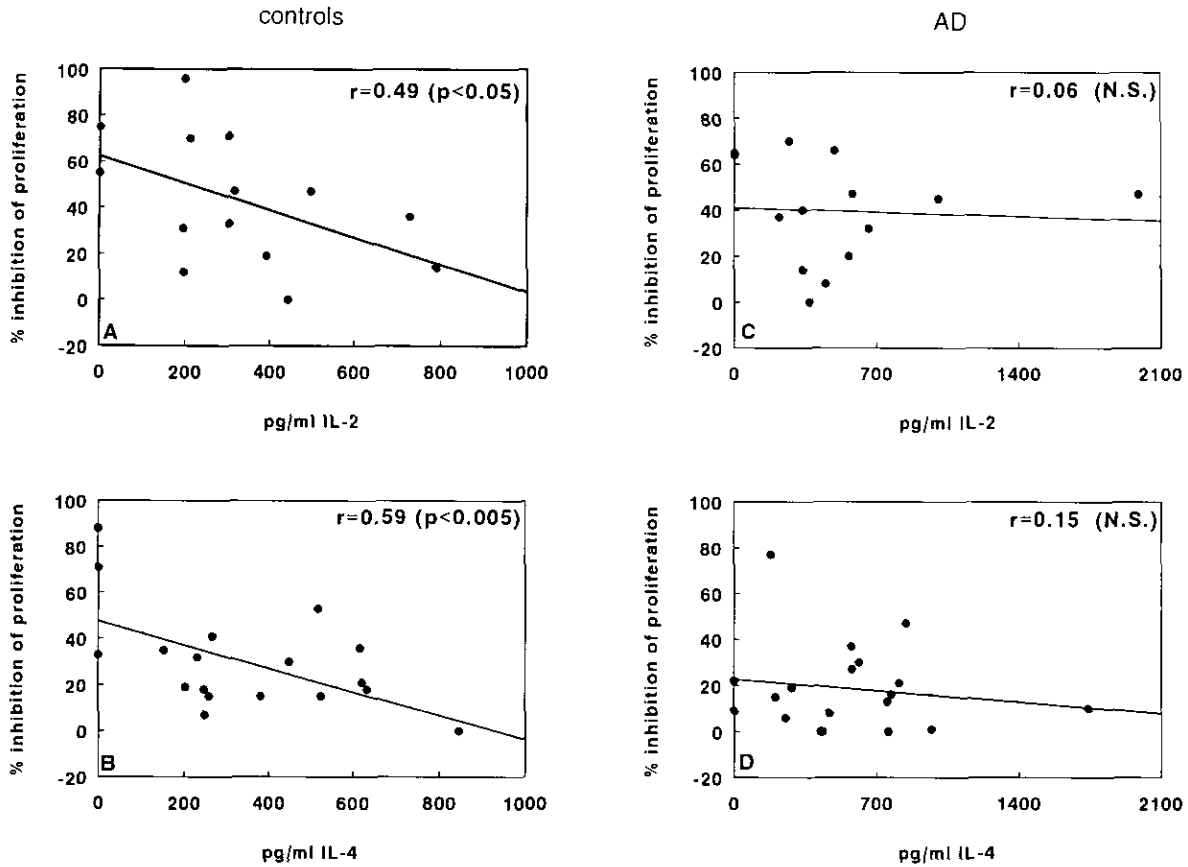


FIG. 6. Inhibition of proliferation by dexamethasone correlates with the production of IL-2 and IL-4 in controls, but not in AD patients. Inhibition of proliferation by DEX in the PHA response was plotted against the amount of IL-2 produced by the same cells in the absence of DEX for each individual control (A) and AD patient (C). In a similar fashion, DEX-mediated inhibition of the anti-CD2/anti-CD28 response was plotted against the amount of IL-4 produced by controls (B) and AD patients (D). Lymphokine data were derived from the experiments shown in Fig. 4. Correlation coefficients and P values were obtained through linear regression.

DISCUSSION

The present study investigated the possibility that the functioning of the immune system in AD patients is altered as a consequence of a disturbed HPA axis. Previous *in vivo* studies have indicated that cells from the pituitary gland in these patients, as compared to controls, are less sensitive to the influence of glucocorticoids, in that cortisol levels in plasma are less decreased by an *in vivo* challenge with DEX, although this appeared not to be specific for Alzheimer's disease (19–21).

Our experiments show that the same is true for the sensitivity of T cells *in vitro*. This might be caused by the fact that these cells were already suppressed *in vivo*, e.g., by occupancy of the intracytoplasmic GCR. However, no evidence was obtained for a suppressed T cell population *in vivo*, since proliferative responses of T cells from AD patients and controls *in vitro* were comparable, and cytokine production was higher in AD patients. The fact that some reports (14, 15) show a diminished proliferative response in AD might in our view be caused by the lack of uniformity in AD with respect to diagnosis and the heterogeneity among AD patients.

A second possibility is that a diminished DEX sensitivity of T cells in AD is related to an altered composition and function of the CD4⁺ T cell population. Daynes and Araneo (27) showed in the murine system that DEX can enhance IL-4 production while inhibiting IL-2 production, indicating a differential effect of this hormone in IL-2- and IL-4-producing cells. Given the fact that IL-4 is produced by CD45RO⁺ CD4⁺ T cells and not by CD45RA⁺ CD4⁺ T cells (28), it might be that the effect of DEX is dependent on the phenotype and functional characteristics of the CD4⁺ T cells in the human situation. In this regard it is worthwhile to remark that the process of aging is accompanied by a decrease in the relative number of CD4⁺CD45RA⁺ T cells (30, 31), most likely as a consequence of thymic involution (32). Moreover, patients with Down's syndrome—who frequently develop AD at a young age—have lower numbers of CD4⁺CD45RA⁺ T cells in their peripheral blood (33). However, we were not able to show a statistically significant difference in phenotype between AD patients and controls: comparable numbers of naive and memory CD4⁺ T cells were found. Also, no significant difference was found between the two groups with regard to IL-2 and IL-4 production.

Our data did not show that DEX stimulates IL-4 production by human PBMC as was found in the murine system by Daynes and Araneo (27). It might, however, be that IL-2 and IL-4 production by human CD4⁺ T cells is differentially affected by DEX: 10⁻⁷ M DEX caused an 83% inhibition of IL-2 production and a 55% inhibition of IL-4 production. It should be taken into account that different activation pathways were used for the induction of these lymphokines and that the effect of DEX may depend on the activation pathway.

The reasons for the lower sensitivity of the proliferative response in AD are as yet unclear, but are possibly related to quantitative differences in the expression of the GCR. This possibility would explain the lack of correlation between the extent of inhibition by DEX and interleukin production in AD, in contrast to the significant correlation that was found in controls. However, the expression of the GCR receptor, as determined by immunofluorescence, was comparable between patients with early onset AD and controls (Fig. 4). Therefore, it might be that the affinity of the GCR in PBMC from AD patients is lower. Alternatively, the production of growth factors different from IL-2 and IL-4 may play a role in the lower sensitivity to DEX in AD patients.

That both PBMC and cells from the pituitary gland in AD are less sensitive to DEX points to a more general phenomenon. More insight into the mechanism of the diminished DEX sensitivity may provide valuable information concerning the etiology of the disease. PBMC are a useful and easily accessible tool to study such putative generalized cell biological defects in AD.

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