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Mental deterioration correlates with response of natural killer (NK) cell activity to physiological modifiers in patients with short history of Alzheimer's disease

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Abstract

Natural killer (NK) cell activity of peripheral blood mononuclear (PBM) cells was measured in 16 subjects with mild to moderate senile dementia of Alzheimer's type (sDAT) chosen for short history of disease and no medication, and in 17 age- and sex-matched controls. Levels of cytotoxicity at baseline and after PBM cell exposure to modifiers either negative (cortisol 10^{-6} M) or positive (rIL-2 650 IU/ml and rIFN-γ 100 UI/ml, respectively) were related to indices of hypothalamic–pituitary–adrenal (HPA) function and Gottfries Bråne Rating Scale (GBS) score for mental deterioration. Spontaneous NK cell activity was not significantly different in sDAT subjects vs controls. In vitro inhibition by cortisol was lower in sDAT ($P<0.05$); cytokine-induced changes were greater (rIL-2, $P<0.02$; rIFN-γ, $P<0.05$). Percent negative or positive variations from baseline significantly correlated with GBS scores ($P<0.05$ or less). Serum cortisol and cortisol/DHEAS molar ratio at 0800 h were significantly higher in sDAT ($P<0.05$ and $P<0.02$, respectively). Cortisol/DHEA ratio positively correlated with GBS

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scores ($P<0.02$). Moreover, the ratios of incremental area of response ACTH/cortisol and β-endorphin/cortisol after 1 μg/kg ovine-corticotrophin-releasing hormone (o-CRH) positively correlated with percent increase of NK cell activity after rIL-2 ($P<0.01$). Data indicate that patients with mild cognitive impairment and short history of sDAT show abnormal responsiveness of NK cell activity to physiological modifiers while maintaining normal spontaneous activity. Furthermore, data are compatible with partial glucocorticoid resistance at the immune level. Progressing sDAT longitudinal studies are needed to address: i) the clinical applicability of these abnormalities as prognostic factors; ii) the role played by pro-opiomelanocortin (POMC)-derived peptides and adrenal androgens in the control of NK cell activity.

Keywords: Cortisol; Cytokines; POMC-derived peptides; DHEA; Dementia; NK cell activity

1. Introduction

Natural killer (NK) cells are granular lymphocytes that were first identified in mice because of their ability to rapidly lyse certain virus-infected or tumor cell targets (Trinchieri, 1989). They lack the surface markers of either T- cells or B-cells, and were called "natural" killer cells since they have innate cytotoxic properties. In addition to their lytic activity, in recent years other functions of NK cells have been appreciated, first their capacity to produce cytokines, including interferon (IFN)-γ, granulocyte-macrophage-colony stimulating factor (GM-CSF), interleukins (IL-3, IL-5, IL-10, IL-12 and IL-13) (Seaman, 2000). Through the release of cytokines, NK cells regulate the differentiation and the activity of other cells, particularly cells of the immune system. Cytokines are also essential for most aspects of the biology of NK cells. IL-12 and IFN-γ are key molecules not only for achieving phenotypic characteristics, but also for tuning cytotoxicity in blood and tissues (Wolf et al., 1994; Biron and Gazzinelli, 1995).

Hormones are known to be important regulators of NK cells (Angeli et al., 1992). Peptide and steroid hormones of the hypothalamic–pituitary–adrenal (HPA) axis exert differential effects, both in vitro and in vivo (Chiappelli et al. 1991, 1996; Gatti et al., 1993; Bedesowsky and Del Rey, 1996; Masera et al., 1999). Our previous studies in psychiatric diseases, such as anorexia nervosa, demonstrated that in vivo response of the HPA axis to physiological challengers significantly correlates to in vitro responsiveness of NK cells to IL-2 (Staurenghi et al., 1997).

Dehydroepiandrosterone (DHEA) is the most abundant Δ_5 steroid hormone in humans. Most of the circulating hormone is in the form of sulfate ester (DHEAS), but a number of studies have shown that DHEA, rather than DHEAS, is responsible for most actions (Kroboth et al., 1999; Steel, 1999). Various metabolic, immune and cognitive effects, have been attributed to this steroid (Khorram et al., 1997). Low concentrations of DHEAS have been associated with physical frailty, decline in muscle mass, loss of sleep and senescence. As for immune effects, DHEA appears to up-regulate host immune response against infections and counteract stress-induced immunesuppression (Loria, 1997). Specifically, a number of studies raise the question whether this hormone has anti-glucocorticoid activities (Kroboth et al., 1999).

Senile dementia of Alzheimer's type (sDAT) is a neurodegenerative disorder and the most common cause of dementia in the elderly. Alterations in the HPA system have been observed in sDAT. They eventually lead to excess glucocorticoid secretion as a consequence of dysregulation of the system at the hypothalamic, the pituitary and the adrenal levels (Rasmuson et al., 1998; Weiner et al., 1997). Besides upregulated cortisol levels that may have deleterious effects on the brain, the well-known age-related decline of circulating DHEAS has been credited in the pathogenesis of sDAT (Mao and Barger, 1998; Herbert, 1998).

Inconsistent results have been published upon NK cell cytotoxicity in sDAT (Araga et al., 1991; Solerte et al., 1999). In patients with advanced disease, the number of NK effectors and the levels of activity have been reported to be elevated, reduced or normal, as well as responses to cytokines (Gasparini et al., 1998).

In the present study we focused on the degree of mental deterioration, HPA function and NK cell activity of peripheral blood mononuclear (PBM) cells in subjects affected by mild sDAT and with a short history of disease.

2. Materials and methods

Sixteen subjects affected by sDAT were enrolled. Diagnosis met the criteria of the National Institute of Neurological and Communicative Disorders and Stroke and Alzheimer's disease and Related Disorders Association (NINCDS–ADRDA) (McKhann et al., 1984) for "probable" sDAT. All cases were outpatients. All subjects showed brain atrophy and no sign of cerebral infarction or leukoaraiosis, as determined by computer tomography or magnetic resonance imaging scan. The duration of disease was less than three years (26±3 months). Clinical assessment was performed using the Mini-Mental State Examination (Folstein et al., 1975), the Global Deterioration Scale (GDS) (Reisberg et al., 1982) and the Gottfries Bråne Dementia Rating Scale (GBS) (Gottfries et al., 1982). None of them was under psychotropic or immunosuppressive medication. The 21-item version of the Hamilton Rating Scale for Depression (Hamilton, 1960) was used to exclude from the study any subject with major depression (score over 14).

Seventeen healthy age- and sex-matched subjects were recruited from the general population as controls. None of them had any abnormality on physical examination, and routine blood laboratory tests proved to be normal following biochemical and microbiological analysis.

Subjects with sDAT and controls were in good general medical health, were free of any medication and no diseases (e.g. respiratory and urinary tract infections) known to affect immune function were observed two months before the inclusion in the study. Demographic and clinical data for subjects and controls are summarized in Table 1.

All subjects enrolled in the study gave written informed consent. Procedures followed agree with the ethical methods of the institutional responsible committee on human experimentation. All subjects were non smokers. Between 0800 h and 2000 h subjects were allowed to eat, sleep and nap ad libitum.

Table 1
Clinical feature of 16 sDAT and 17 control subjects

	sDAT	Controls[a]
Age (mean±SEM)	66.9±1.9	66.7±1.9
Gender (male/female)	10/6	11/6
Body mass index (kg/m^2; mean±SEM)	25.2±1.5	25.5±0.9
Duration of illness (months±SEM)	26.8±2.8	NA
HRSD (score±SEM)	8.3±0.5	8.2±0.5
MMSE (score±SEM)	19.6±0.6	29.6±0.2
Hachinski ischemic score (score±SEM)	3.2±0.2	NA
GDS (score±SEM)	3.6±0.1	1.0±0.0
GBS (score±SEM)	60.9±4.9	NA

[a] NA: non-applicable.

2.1. NK cell activity

2.1.1. Tissue culture media and target cells

The medium RPMI 1640 (HyClone Europe Ltd., Cramlington, UK) enriched with 10% heat-inactivated fetal bovine serum (HyClone Europe Ltd., Cramlington, UK), 1% glutamine and 50 μg/ml gentamicin (complete medium), was used routinely for all cultures. The human myeloblastoma cell line K562 was the source of sensitive targets for measurements of NK cytotoxicity. The cell line was maintained in our laboratory in suspension culture flasks at 37°C in a 5% CO_2 incubator. All targets used had a >90% viability measured by a trypan blue dye exclusion procedure.

2.1.2. Materials

Cortisol (Sigma, St. Louis, USA) was initially dissolved in 95% ethanol and then diluted in distilled water to 1 mM stock solution, and stored at 4°C for up to one month. For use in experiments, the steroid was promptly diluted in complete medium at the final concentration of 10^{-6} M. Ethanol was added (in final concentrations 100 μg/ml^{-1} μg/ml) in control studies and did not exert any effect on cell viability or on NK activity. rIFN-γ (Sigma, St. Louis, USA) and rIL-2 (Eurocetus, Emeryville, USA), were diluted in complete medium and used at the concentration of 650 IU/ml/7×10^6 PBM cells and 100 IU/ml/7×10^6 PBM cells, respectively.

2.1.3. Separation of PBM cells

PBM cells were obtained from heparinized venous blood samples drawn at 0800 h from subjects with sDAT and from controls. PBM cells were immediately separated by Ficoll–Hypaque density gradient centrifugation (Boyum, 1976). The resultant preparations contained more than 98% mononuclear cells. 75–80% of them were lymphocytes identified by May Grünwald–Giemsa staining.

2.1.4. Treatment of effector cell preparations

After separation, PBM cells were washed three times, counted and resuspended to a density of 7×10^6 cells/ml in complete medium. Effector cells were incubated for 20 hours with buffer or modifiers (cortisol: 10^{-6} M; rIFN-γ: 650 IU/ml; rIL-2: 100 IU/ml) at 37°C in a humidified atmosphere of 95% air and 5% CO_2 before being assayed for cytotoxicity. After incubation PBM cells were washed twice, assessed for viability by the trypan blue dye exclusion method and then assayed for cytotoxicity. The effect on cell viability of any tested compound was negligible.

2.1.5. Cytotoxicity assay

NK activity was measured by a non radiometric direct 4-h cytotoxicity assay, using K562 cells as targets. Target cells were mixed with PBM cells to give different effector (E) to target (T) cell ratios (25:1, 12.5:1, 6.25:1, 3.125:1). The assay procedure is based on the kinetic measurement by an automatic PC-aided microtiter plate reader (UV-Max™ provided with Soft-Max™ software, Molecular Devices Corporation, Menlo Park, USA) of the amount of the lactatedehydrogenase (LDH) released in the supernatant of lysed target cells, according to the method of Korzeniewski and Callewaert (1983). Data on NK activity of PBMC incubated with or without modifiers were expressed as Lytic Units (LU)/10^7 cells according to Pross et al. (1981).

2.2. HPA function

We performed a stimulation test with ovine CRH and a standard single dose suppression test with dexamethasone for the subsequent determination of cortisol. Basal DHEAS levels were also measured.

CRH test was performed as follows: after an overnight fast, subjects were placed at bed rest with an indwelling catheter inserted in one arm and maintained open by a saline infusion. Ovine CRH (o-CRH; Clinalfa AG, Laufelfingen, Switzerland) was administered as a bolus of 1 μg/kg body weight intravenously at time 0'. Blood samples were collected at −15', 0', 10', 20', 30', 45', 60', and 90'. The mean values of the −15' and 0' determinations were considered as basal levels. Serum cortisol, DHEA and DHEAS were determined by RIA (Sorin Biomedica, Saluggia, Italy), and ACTH and β-EP were measured by IRMA using commercially available kits (Allégro® Kits, Nichols Institute, provided by Instrumentation Laboratory, Milan, Italy). Intra- and interassay variability coefficients in our laboratory were respectively as follows: ACTH, 3.5% and 7%; β-EP, 4.4% and 9%; cortisol, 3.8% and 5.9; DHEA, 7.3% and 7%; DHEA-S, 9.4% and 9.6%.

Patterns of HPA response along the time course of the test (105 minutes) were expressed as net increment at peak, percent increment above baseline and percent incremental area under the curve (AUC) for each considered analyte; as the ratio of percent responses of POMC-derived peptides to the corresponding responses of cortisol; as the ratio of percent responses of cortisol to the corresponding responses of DHEA.

A dexamethasone suppression test (DST) was performed by administering orally

a dose of 1 mg dexamethasone as tablets at 2300 h, with blood drawn at 0800 h for the determination of serum cortisol levels. The next day blood samples were taken at 0800 h and 1600 h. The DST was performed in all subjects one week after the o-CRH-stimulation test, during an outpatient control session. The degree of inhibition was expressed as the percent decrease below baseline. Serum cortisol levels >5 μg/dl at 0800 h and 1600 h after dexamethasone were regarded as indicative of non-suppression.

2.3. Statistical analysis

Parametric tests were used in those cases in which sample data verified the three assumptions of normal distribution, independence of measurements and homogeneity of variance. Only when these assumptions were violated, were non-parametric tests employed. Statistical significance of differences in the values of cytotoxicity recorded under different conditions by the same PBM cell preparation was determined by paired Student's *t*-test (or Wilcoxon's rank sign test when appropriate). Differences between levels of cytotoxicity recorded under the same experimental condition in subjects and controls were statistically validated by unpaired two sample *t*-test (or rank sum test, when appropriate). Correlations between psychiatric and endocrine or immune variables were evaluated by Spearman's rank correlation test (two-tailed). In all tests $P<0.05$ was regarded as significant. Statistical analysis was performed using Statistix (NH Analytical Software, USA) softwares. All results are presented as means±SEM.

Outliers were not eliminated from data sets.

3. Results

Both in controls and in sDAT subjects, levels of spontaneous NK cell activity showed a wide interindividual variability. No gender differences were found. Although being higher, mean spontaneous NK cell activity was not significantly different in AD subjects vs controls. After exposure to cortisol, mean values of NK cell cytotoxicity were different in the two groups: percent reduction resulted significantly lower in AD ($P<0.05$) (Fig. 1A). On the other hand, cytokine-induced cytotoxicity was higher in sDAT subjects; statistical significance was attained for percent increase above base levels with P values <0.02 for rIL-2 and <0.05 for IFN-γ (Fig. 1B and C). In sDAT, percent changes of NK cell cytotoxicity after exposure of PBM cells to cortisol negatively correlated with GBS score, whereas correlation was in the opposite sense after exposure to boosting cytokines (Fig. 2A–C).

As far as HPA function is concerned, all sDAT subjects but one were normal suppressors (i.e. post-dexamethasone serum cortisol levels at 0800 h and 1600 h <5 μg/d). NK cell responses to modifiers and GBS score of the single non-suppressor are symbolized in Fig. 2. Controls were all normal suppressor to DST.

Subjects suffering from sDAT had serum cortisol levels at 0800 h significantly higher than controls ($P<0.05$). Concomitant levels of ACTH, β-EP and DHEAS

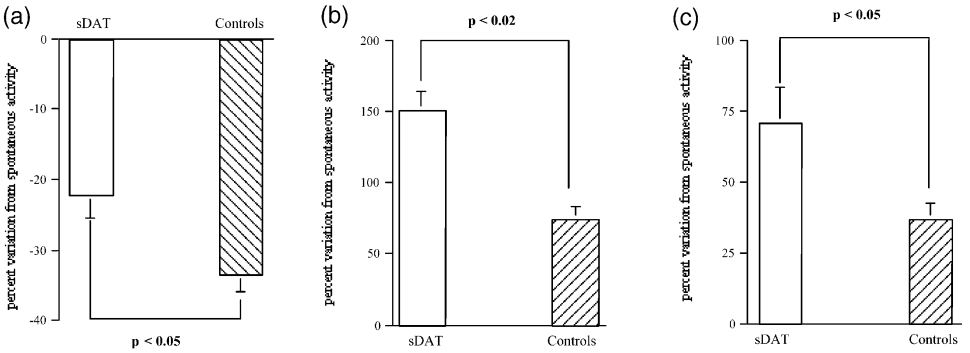

Fig. 1. Mean±SEM percent changes from spontaneous activity, taken as 100, of cytotoxic activity of PBMC after incubation for 20 h with cortisol (10^{-6} M) (a) or rIL-2 (100 IU/ml) (b) or rIFN-γ (650 IU/ml) (c).

were not different (Table 2). Cortisol/DHEAS molar ratio was significantly higher in sDAT ($P<0.02$). Net increment at peak, percent increments and percent incremental areas under the curve (AUC) after o-CRH are presented in Table 2. Percent incremental AUC of DHEA results were significantly lower in sDAT ($P<0.02$). The incremental AUC ACTH/cortisol or β-EP/cortisol positively correlated with percent increase of NK cell activity after IL-2 ($P<0.01$) (Fig. 3). Also in controls, higher ACTH and β-EP levels after o-CRH paralleled higher levels of NK cytotoxicity after IL-2 or IFN-γ but significance of the above mentioned correlations was not attained (data not shown). The only variable that correlated with GBS score was the ratio of incremental AUC cortisol/DHEA ($P<0.02$) (Fig. 4).

4. Discussion

sDAT is a multifactorial process characterized by heterogeneous patterns of cognitive deterioration. As for other immune functions, natural cytotoxicity of PBM cells has been found to be elevated, reduced or comparable to controls in patients with a long history of disease and severe disability (Araga et al., 1991; Solerte et al., 1999). Discrepancies among reported data on NK cell function plausibly argue for a number of variables both endogenous (genomics; stage of disease) and exogenous (therapy). The present series of patients was selected for having mild cognitive impairment and no therapy. Mean levels of the spontaneous cytotoxicity were higher than in controls, although significance was not attained. In our protocol, a non-radiometric assay on K562 cells was applied. Therefore, the present data are not strictly comparable to those obtained with ^{51}Cr-release from target cells (Araga et al., 1991). Furthermore, our results deal with mixed mononuclear cells and not with purified NK cells. The use of a whole mononuclear cell population is expected to provide more valuable information with regard to in vivo activity, since cell-to-cell regulatory mechanisms are intact and several sources of soluble modulators are present. Accordingly, one

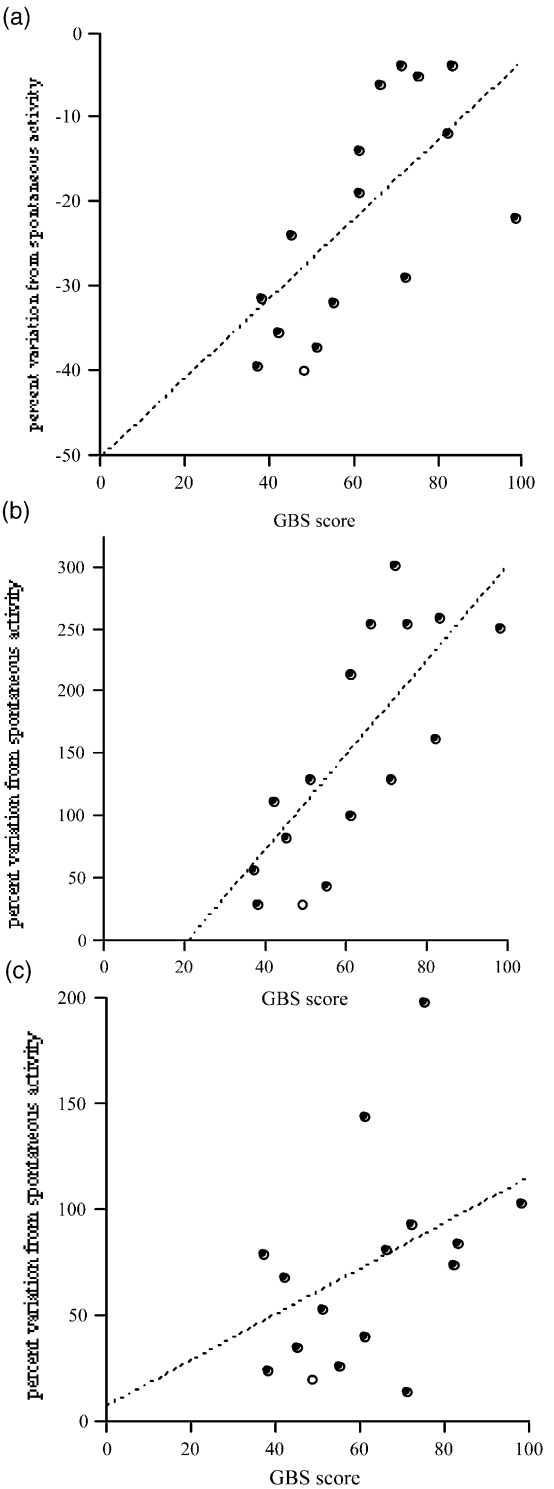
(a)
percent variation from spontaneous activity
0
-10
-20
-30
-40
-50
0
20
40
60
80
100
GBS score
(b)
percent variation from spontaneous activity
300
250
200
150
100
50
0
0
20
40
60
80
100
GBS score
(c)
percent variation from spontaneous activity
200
150
100
50
0
0
20
40
60
80
100
GBS score

Table 2
HPA axis function in sDAT and controls

	sDAT	Controls	*P*
Baseline			
ACTH 0800 h (pg/ml)	21.8±2.5	29.7±4.5	NS
β-EP 0800 h (pg/ml)	15.0±2.1	21.5±3.1	NS
Cortisol 0800 h (μg/dl)	13.3±0.7	10.2±1.2	<0.05
DHEAS 0800 h (μg/dl)	135.7±24.9	181.9±24.2	NS
Cortisol/DHEAS 0800 h (nmol/l/μmol/l)	188.1±42.4	70.7±12.1	<0.02
After o-CRH			
ACTH: net increment (pg/ml)	66.2±12.4	40.1±8.2	NS
β-EP: net increment (pg/ml)	35.8±47.1	19.4±10.1	NS
Cortisol: net increment (μg/dl)	9.7±0.7	12.1±1.7	NS
DHEA: net increment (ng/ml)	6.0±1.6	7.5±1.6	NS
ACTH: % increment	285.8±40.6	231.0±40.8	NS
β-EP: % increment	185.4±31.4	131.2±21.6	NS
Cortisol: % increment	161.7±6.3	143.8±23.2	NS
DHEA: % increment	119.4±45.5	194.0±35.6	NS
ACTH: % increment AUC	148.7±22.4	121.4±19.1	NS
β-EP: % increment AUC	98.7±14.7	68.0±12.9	NS
Cortisol: % increment AUC	82.0±3.9	73.3±11.2	NS
DHEA: % increment AUC	78.6±19.1	131.2±25.9	<0.02

could state that in early stages of sDAT baseline natural cytotoxicity of PBM cells does not change (Solerte et al., 1999). However, the susceptibility of NK cell activity to in vitro inhibition by 10^{-6} M cortisol was significantly lower in subjects than controls. Percent cortisol-dependent changes ranged widely, from minimal to about 40% less than baseline. When we plotted these changes against GBS scores, a negative correlation was apparent. In other words, mental deterioration paralleled glucocorticoid resistance of NK effectors.

We used one standardized neuropsychological rating scale that was chosen for easier applicability to our cases. The GBS rating scale covers a wide range of cognitive abilities known to be vulnerable to the effects of progressive neurodegenerative changes associated with sDAT. It is a reliable tool in distinguishing even slight differences among individuals. The use of a standardized rating scale makes possible immediate perception of impairment with respect to conspicuous deviations in the overall score profile. To add reliability, we carefully evaluated every GBS performance with respect to premorbid levels of functioning, on the basis of school achieve-

Fig. 2. Correlations between cognitive impairment (GBS score) and percent variations of NK cell activity after exposure of PBM cells to either cortisol (a), rIL-2 (b) or rIFN-γ (c). Validation of data was performed using Spearman's rank correlation test ($r=-0.73$, 0.78 and 0.55 for cortisol, rIL-2 and rIFN-γ, respectively; $P<0.01$ for cortisol and rIL-2; $P<0.05$ for rIFN-γ). Open circle (○) represents the non-suppressor subject.

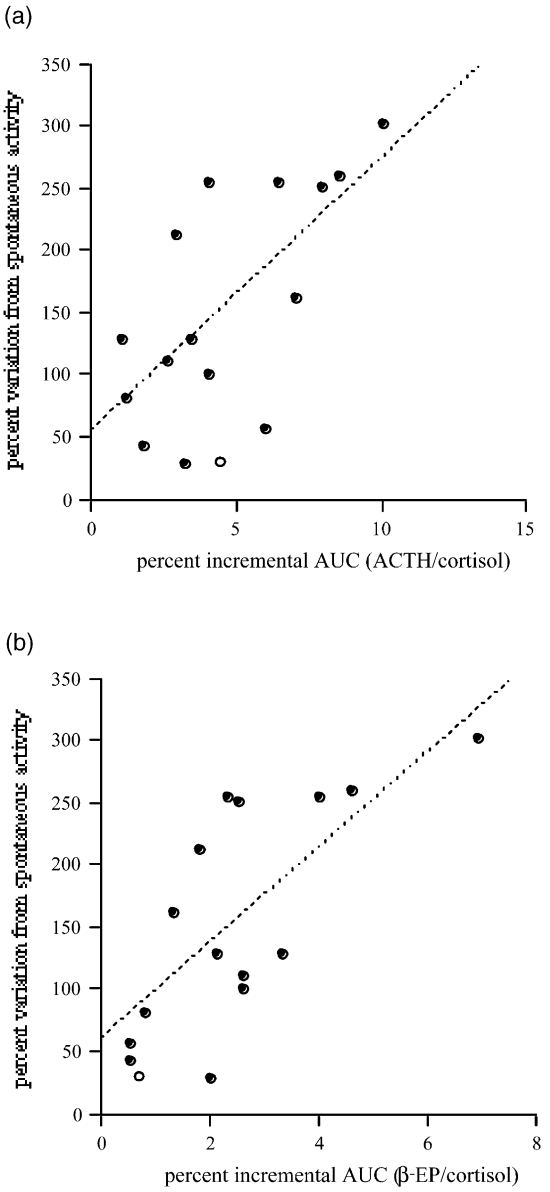

Fig. 3. Correlations between IL-2-induced percent enhancement of NK activity and responses to in vivo stimulation with o-CRH expressed as percent incremental area under the curve (AUC) ratios of ACTH/cortisol (a) or of β-EP/cortisol. Validation of data was performed using Spearman's rank correlation test (r=0.65 and 0.74; $P<0.05$ and $P<0.01$, respectively). Open circle (○) represents the non-suppressor subject.

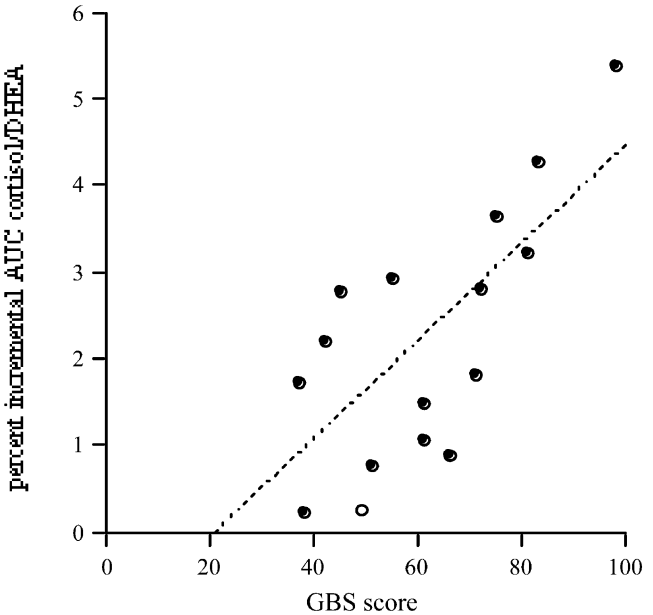

Fig. 4. Correlation between cognitive impairment (GBS score) and responses to in vivo stimulation with o-CRH expressed as percent incremental AUC ratios of cortisol/DHEA. Validation of data was performed using Spearman's rank correlation test (r=0.66; P<0.02). Open circle (○) represents the non-suppressor subject.

ments, job records, specific areas of learning and memory, attention and concentration, and interviews with family members.

All cases but one showed suppressed serum cortisol levels in the morning after 1 mg dexamethasone administration at 2300 h. Available data indicate that a half or more of subjects with advanced sDAT are "non suppressor" to such a standard DST (Martignoni et al., 1990; Carr et al., 1997). Abnormal suppressibility of CRH and ACTH production, hence of cortisol levels in the blood stream, has been attributed not only to severity of brain damage, but also to duration of illness. Pertinently, Huizega et al. (1998) have suggested the use of a lower dexamethasone dose in precocious sDAT to demonstrate subtle disturbances of the glucocorticoid feedback. Our data agree with the view that peripheral glucocorticoid resistance (at the immune level) may precede the corresponding resistance at the brain level (Seeman and Robbins, 1994). In our cases, the degree of inhibition of NK cell activity by physiological concentrations of cortisol clearly showed a different impact of the disease on mental performance. In addition, no subject showed evident symptoms of depression, anxiety or panic attacks. Would our observation be further substantiated, a simple, cost-effective immunological test in the peripheral blood could be of value to assess precociously those subjects at higher risk of rapid mental impairment. In fact, DST, which more directly explores glucocorticoid information to the brain, is not a reliable predictor of advancing dementia (Swanwick et al., 1998). A recent study has put

forward further caveats, since serum cortisol levels apparently correlated with severity of dementia at certain stages of the disease, but the relationship lost stability at follow up analyses (Weiner et al., 1997).

In our study, cortisol/DHEAS molar ratio in the peripheral blood at 0800 h and DHEA response to o-CRH were different in sDAT and controls. A vast literature has focused on neuroprotective effects of DHEA. DHEA and DHEAS were found to improve long-term memory and diminish amnesia in experimental animals (Roberts et al., 1987; Wolkowitz et al., 1999). In aged humans, the administration of DHEA reportedly improved cognitive performance (Morales et al., 1994). Very recently, Bernardi et al. (2000) demonstrated that the DHEA response to CRH is significantly lower in demented individuals than in controls. Unfortunately, those authors did not search for correlations with the degree of mental deterioration. Our results are in substantial agreement with previous findings, but also highlight for the first time that DHEA response to o-CRH is even lower in cases with more apparent mental impairment. Since cortisol response to o-CRH did not differ in our patients and controls, and cortisol/DHEA ratio after o-CRH stimulation negatively correlated with GBS scores, the defective adrenal androgen response to stress, hence to rapid rise of ACTH secretion, could be a further sign of advancing sDAT. In this view, our results also agree with Ferrari et al. (2000) who found a significant increase of cortisol/DHEA molar ratio when going from healthy elderly to demented patients. Furthermore, Murialdo et al. (2001) have very recently described a direct correlation between mean 2400 h cortisol levels and severity of dementia in patients diagnosed with mild to very severe sDAT assessed by GDS. We suggest that these neuroendocrine changes may be already clearly established at the early stages of disease.

We also noticed hyper-responsiveness of peripheral NK cells to boosting cytokines, such as IFN-γ and IL-2. The percent increase of cytotoxicity after exposure of PBM cells to these cytokines was positively correlated with GBS scores, on the one hand, and indices of POMC-derived peptide secretion on the other. Both in vitro and in vivo studies support the view that at least two POMC-derived peptides, β-EP and ACTH, enhance NK cell susceptibility to IFN-γ and IL-2 and counteract cortisol-dependent inhibition (Gatti et al., 1993; Masera et al., 1999). We have previously reported on the relationship between circulating β-EP and ACTH and IL-2-dependent enhancement of NK cytotoxicity in clinical conditions characterized by hyperactivity of the HPA system (Staurenghi et al., 1997; Masera et al., 1999). Production of β-EP and ACTH by lymphocytes has been also convincingly demonstrated (Smith et al., 1986; Blalock, 1999). Glucocorticoids apparently down-regulate gene expression of immune POMC similarly to pituitary POMC (Smith et al., 1986). One could speculate that in sDAT, NK cell activity, at least in the earlier years of disease, has higher susceptibility to boosting cytokines also as an indirect consequence of impaired glucocorticoid action. In fact, immune POMC-derived products have paracrine and autocrine, but not endocrine effects (Buzzetti et al., 1989; Olsen et al., 1992).

In conclusion, we found that sDAT patients with mild cognitive impairment and short history of illness show abnormal responsiveness of NK cell activity to either negative or positive physiological modifiers, while maintaining normal spontaneous activity. The patterns of response are compatible with partial glucocorticoid resist-

ance at the immune level. Since the extent of negative or positive changes from baseline activity were found to be correlated with the course of disease, as expressed by the GBS score, longitudinal studies are needed to explore whether changes in responsivity of NK cells to modifiers remain the same while the disease is progressing or change direction in more severe cases.

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