Mechanisms of Ageing and Development 124 (2003) 887–895

mechanisms of ageing and development

www.elsevier.com/locate/mechagedev

Impact of psychological and endocrine factors on cytokine production of healthy elderly people

Clarice Luz [a,b,*], Fabiana Dornelles [a], Thales Preissler [a], Diego Collaziol [a], Ivana M. da Cruz [b], Moisés E. Bauer [a,c]

[a] *Instituto de Pesquisas Biomédicas, Hospital São Lucas da PUCRS, Pontifícia Universidade Católica do Rio Grande do Sul, Av. Ipiranga 6690, 2° andar – Caixa Postal 1429, 90610-000 Porto Alegre, RS. Brazil*

[b] *Instituto de Geriatria e Gerontologia, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil*

[c] *Faculdade de Biociências, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Brazil*

Received 11 March 2003; received in revised form 2 May 2003; accepted 22 May 2003

Abstract

Human ageing has been associated with immunological changes including blunted T-cell responses and increased production of pro-inflammatory cytokines. Here, we investigated the role of psychological and endocrine factors in the production of pro-inflammatory cytokines (tumor necrosis factor-α and interleukin (IL)-6) as well as soluble IL-2Rα, associated with T-cell activation. Forty-six elderly subjects (60–91 yrs) and 33 young adults (20–40 yrs) were recruited accordingly the SENIEUR protocol. The emotional status was measured by structured clinical interviews. Salivary cortisol levels (9, 12 and 22 h) and serum dehydroepiandrosterone (DHEA) were assessed by radioimmunoassays. The elderly were more stressed, depressed and anxious than the young subjects. Cortisol levels were increased whereas DHEA levels were significantly reduced in the elderly. Both groups showed equivalent production of pro-inflammatory cytokines as well as soluble IL-2Rα. Psychological scores were positively correlated to evening cortisol levels and negatively correlated to morning DHEA levels. No relationships were noted between psychological factors and cytokines studied. However, evening cortisol levels were found positively correlated to TNF-α and sIL-2Rα levels. These data indicate that healthy ageing is associated with significant distress and activation of the hypothalamic–pituitary–adrenal axis. Our data also suggest that there are complex psychoneuroendocrine relationships involved with cytokine production during ageing.

© 2003 Elsevier Ireland Ltd. All rights reserved.

Keywords: Ageing; Immunosenescence; Psychological stress; Psychoneuroimmunology; Cytokines; Hypothalamic–pituitary–adrenal axis

1. Introduction

There is a complex bi-directional communication between the nervous, endocrine and immune systems that can be demonstrated by the presence of shared neurotransmitters, hormones and cytokines (Blalock, 1989). Data produced by recent studies demonstrate that ageing may impair this psychoneuroimmunologic cross-talk (Straub et al., 2000; Martinez-Taboada et al., 2002). In particular, ageing has been associated with several immunological changes (immunosenescence) including thymic involution, changes in cell trafficking, impaired T-cell functions (Pawelec et al., 2002) and increased production of pro-inflammatory cytokines (Fagiolo et al., 1993; Gabriel et al., 2002). The latter are promptly produced by macrophages following stimulation with lipopolysaccharide (LPS, endotoxin). Also, altered cytokine levels and altered levels of cytokine antagonists might influence cytokine networks during ageing. Indeed, there is some evidence for increased serum levels of soluble IL-2 receptor (sIL-2R) in the elderly (Franze et al., 1996; Rea et al., 1996), which could contribute to decrease of interleukin (IL)-2 function. These data suggest that ageing may be associated with significant immunological activation in parallel with impaired T-cell functions.

* Corresponding author. Tel.: +55-51-3320-3000x2725; fax: +55-51-3320-3312.

E-mail address: mebauer@pucrs.br (C. Luz).

0047-6374/03/$ - see front matter © 2003 Elsevier Ireland Ltd. All rights reserved.

doi:10.1016/S0047-6374(03)00148-9

However, most immunological changes ascribed to immunosenescence can also be observed following chronic psychological stress (McEwen et al., 1997) or glucocorticoid therapy (Sapolsky et al., 2000). This led us to speculate that both psychological and endocrine factors could be involved during the immunosenescence. Psychological status may be such a risk factor for immunosenescence. Human ageing has been associated with several psychological and behavioural changes, including difficulty to concentrate, progressive cognitive impairments and sleep disturbances (Salzman and Shader, 1978). Although individually identified, these alterations may be associated with major depression. In addition, we have previously demonstrated that major depression (Bauer et al., 1995) or psychological stress (Bauer et al., 2000) is also known to dampen cell-mediated immunity. In addition, ageing of the endocrine system (endocrinosenescence) may be another risk factor for immunosenescence. Endocrinosenescence can be demonstrated by a substantial decline in several hormones, including growth hormone, testosterone, progesterone, aldosterone and dehydroepiandrosterone (DHEA). DHEA and its sulfated metabolite (DHEAS) are hormones secreted by the adrenal cortex in response to adrenocorticotropin. DHEA is the most abundant adrenal steroid and has been shown to have immunomodulatory properties as well. There is also some evidence suggesting that ageing is associated with significant activation of the hypothalamic–pituitary–adrenal (HPA) axis (Halbreich et al., 1984; Van Cauter et al., 1996; Deuschle et al., 1997; Heuser et al., 1998), resulting in increased production of cortisol and catecholamines that modulate several immune responses (Munck et al., 1984). Increased cortisol levels are also seen in demented patients (Maeda et al., 1991) or during chronic stress (Bauer et al., 2000). In particular, we have previously demonstrated that chronically stressed elderly subjects (caregivers of demented patients) had a blunted T-cell proliferation in association with increased cortisol levels (Bauer et al., 2000). Overall, these studies suggest that endocrinosenescence may be kept in close relationship with the immunosenescence.

Recent work suggests that cytokines and hormones could be considered as possible links between endocrinosenescence and immunosenescence (Straub et al., 2000). Indeed, it has been known for long that pro-inflammatory cytokines can readily activate the HPA axis during infection in animals (Besedovsky et al., 1977) or after administration in humans (Mastorakos et al., 1993). Another studies have linked the age-related decline in DHEA production to increased serum levels of IL-6 (Daynes et al., 1993; Straub et al., 1998). In addition, increased plasma TNF-α levels were correlated to major depression in the elderly (Vetta et al., 2001). However, we do not know how the extent of these changes may be related to altered psychological and HPA axis functions in the elderly.

In this study, we investigated whether strictly healthy ageing is associated with (i) psychological and endocrine alterations and (ii) assessed the LPS-induced production of early (TNF-α) and late (IL-6) pro-inflammatory cytokines as well as sIL-2R production in vitro. Finally, (iii) we analysed the impact of psychoneuroendocrine factors on cytokine production.

2. Materials and methods

2.1. Subjects

After receiving ethical approval from the University Committee (Pontifical Catholic University of Rio Grande do Sul, PUCRS, Porto Alegre, Brazil), written informed consent was obtained from all subjects. Forty-six non-institutionalised healthy elderly (31 females, 15 males), aged from 60 to 91 yrs (mean age 72.0 ± 8.5 yrs), were recruited from an existing database of 1118 socially active elderly subjects who had previously participated in research at the Institute of Geriatrics and Gerontology (PUCRS). All subjects were registered at the Office for Social Care in Gravataí (RS) and took part in the GENESIS Program for the study on the genetic–environmental interactions on human ageing. Thirty-three healthy young adults (18 females, 15 males), aged from 20 to 40 yrs (mean age 27.4 ± 6.7 yrs), also took part in this study and were all students or employees from the PUCRS.

All subjects were recruited according to the SENIEUR protocol (Ligthart et al., 1984) that defines rigorous criteria for selecting healthy individuals in immunogerontological studies. The health conditions were checked accordingly to accurate clinical investigations and to haematological and biochemical parameters. The exclusion criteria included: infections, acute or chronic inflammation, autoimmune diseases, heart disease, under nourishment, anaemia, leucopenia, clinical depression, caregiving, neurodegenerative disease, neoplasia and use of hormones (glucocorticoids) and drugs (alcohol, antidepressants, immunosuppressants, anticoagulants).

2.2. Psychological evaluation

The psychological status was assessed by means of structured clinical interviews. Depression was evaluated by a Geriatric Depression Scale (Yesavage et al., 1982) with cut-off point higher than five for the presence of depression symptoms (Shua-Haim et al., 2001). Anxiety was assessed by the Hamilton Anxiety Scale (HAM-A) with cut-off point higher than 20 for the presence of anxiety (Hamilton, 1967). Moreover, symptoms of stress

were monitored by 'Inventory of Stress Symptoms for Adults' (ISSL) (Lipp and Guevara, 1994). This scale includes a quadriphasic model for the study of stress that was based on Selye's model of stress (Selye, 1936). The Kuder–Richardson reliability coefficients for these scales were higher than 0.90. The ISSL is composed of four sections corresponding to the following phases of stress: alarm, adaptation, quasi exhaustion and exhaustion. The symptoms listed in the scale are specific for each phase of stress. The scoring was performed by means of three different stages related to the duration ($Q1 = $ last 24 h, $Q2 = $ last week and $Q3 = $ last month) and intensity of stress symptoms. The sum of all physical as well as psychological symptoms within each stage resulted in a score that is related to the presence of stress accordingly to the following criteria: $Q1 > 6$ or $Q2 > 3$ or $Q3 > 8$.

2.3. Collection of salivary samples and cortisol analysis

Salivary cortisol was measured as an objective marker of the HPA axis function. The assessment of cortisol in saliva has proven to be a valid and reliable reflection of the unbound hormone in the blood (Walker et al., 1978), with salivary cortisol concentrations corresponding to 5–10% of the levels present in the serum (Kahn et al., 1988). Salivary cortisol, which represents the free component of plasma cortisol, is not affected by alterations in protein binding (Kirschbaum and Hellhammer, 1994), remains stable in saliva for several days and has been used in the investigation of HPA disorders (Bauer et al., 2000, 2003). Furthermore, compared to venepuncture, the collection of saliva is a simple, non-invasive and stress-free procedure. Samples can be readily collected by participants without supervision or the assistance of medical staff. The assessment of cortisol in saliva is, therefore, a powerful tool for investigating HPA function during ageing.

Salivary samples were collected as previously described (Bauer et al., 2000). Participants were asked to collect three saliva samples with the help of cotton rolls over the course of the experimental day at 9 a.m., 12 p.m. and 10 p.m., always before meals and venepuncture. Sampling was performed across the day to assess some aspects of circadian pattern. Upon arrival in the laboratory, the samples were centrifuged and frozen at $-20\ ^{\circ}C$. Following defrosting, samples were centrifuged (1500 rpm/3 min) to allow precipitation of proteins and mucins. Salivary cortisol samples were then analysed by radioimmunoassay (RIA, DPC Medlab) using microtitre plates. The sensitivity of these assays was estimated in 0.1 nM/l. The intra- and inter-assay coefficients of variation were less than 10%. Results from each of the sampling times were expressed in nanomole per litre.

2.4. Collection of peripheral blood and isolation of mononuclear cells

Twenty millilitres of peripheral blood were collected by venepuncture in the morning (between 9 and 10 a.m.) and samples were stored into lithium–heparin tubes prior to analyses. Samples were always collected at the same time of day to minimize circadian variations. Peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over a Ficoll–Hypaque (Sigma) gradient ($900 \times g$, 30 min). Cells were counted by means of microscopy ($100 \times$) and viability always exceeded 95%, as judged from their ability to exclude trypan blue (Sigma).

2.5. Serum DHEA

Aliquots of peripheral blood were also collected without anticoagulant in order to measure serum DHEA. Hormone levels were assessed by a RIA kit (DSL-CPEI) within 4 h following blood collection. The sensitivity of these assays was estimated in 0.09 nM/l. The intra- and inter-assay coefficients of variation were less than 10%. Results from each of the sampling times were expressed in pg/ml.

2.6. Cell cultures and cytokines

To measure pro-inflammatory cytokines (i.e. TNF-α and IL-6), PBMCs (1.5×10^5 cells/ml) were stimulated with 0.2 μg/ml of endotoxin (LPS, *E. coli*, Sigma) in complete medium (RPMI-1640, supplemented with gentamicin 0.5%, glutamine 1%, hepes 1% and fetal calf serum 10%; all from Sigma) for 24 h at 37 °C in 5% CO_2. PBMCs were also stimulated with phytohemagglutinin 1% (PHA, Gibco) and cultured in complete medium for 96 h to assess the sIL-2Rα chain (p55) production. After the incubation, supernatants were transferred to eppendorf tubes, centrifuged ($900 \times g$, 15 min), collected and stored in freezer $-70\ ^{\circ}C$ prior to the analyses by Immulite™ chemiluminescent assays (DPC Medlab, SP, Brazil). Immulite is a solid-phase, two-site, chemiluminescent enzyme immunometric assay (Berthier et al., 1999). The solid phase, a polystyrene bead enclosed within an Immulite Test Unit, was coated with monoclonal murine antibody specific for the cytokines analysed. The supernatant and a protein/buffer matrix were simultaneously introduced into the Test Unit, and incubated for approximately 30 min at 37 °C with intermittent agitation. During this time, cytokine in the supernatant bound to the monoclonal antibody-coated beads. Unbound supernatant was then removed by a centrifugal wash. An alkaline phosphatase-labeled polyclonal sheep anti-cytokine antibody was introduced, and the Test Unit was incubated for another 30-min cycle. The unbound enzyme conjugate

was removed by a centrifugal wash. Substrate was added, and the Test Unit was incubated for further 10 min. The chemiluminescent substrate, a phosphate ester of adamantly dioxetane, underwent hydrolysis in the presence of alkaline phosphatase to yield an unstable intermediate. The continuous production of this intermediate resulted in the sustained emission of light, thus improving precision by providing a window for multiple readings. The bound complex—and thus also the photon output, as measured by the luminometer—was proportional to the concentration of cytokine in the sample. The cytokine concentration was calculated from the standard curve. The sensitivities for these assays are the following: TNF-α (0.7 pg/ml), IL-6 (5.0 pg/ml) and sIL-2R (5.0 pg/ml). The intra- and inter-assay coefficients of variation were less than 5%. The results are expressed in picogram per millilitre.

2.7. Statistical analysis

All variables were tested for normality of distribution by means of the Kolmogorov–Smirnov test. Cortisol data was analysed by repeated measures ANOVA that included one between-subjects variable (elderly versus young) and one within-subjects variable (cortisol levels). Multiple comparisons among group mean differences were checked with Tukey post hoc test. Differences between variables were also assessed by Student's t-test. Differences in proportions between groups were compared by means of χ^2-test. Relationships between variables were assessed by means of Pearson's product moment correlations. Data are expressed as mean ± S.E. in all figures and tables. All significance levels were two-tailed. A computer statistics package (SPSS 11.0, Chicago, USA) was used for statistical analyses in this study.

3. Results

3.1. Demographic data and psychological evaluation

Table 1 lists the demographic and psychological data for the subjects enrolled in this study. When elders were classified into decades of age, 20 subjects were between 60 and 70 yrs, 18 subjects were between 71 and 80 yrs and 8 subjects were older than 80 yrs. Most elderly (95.6%) and young subjects (81.0%) were Caucasian. The female/male ratio did not differ significantly between elderly and young subjects.

We observed that healthy ageing is associated with important psychological alterations. In particular, it was observed that elderly reported greater depression ($P = 0.005$), anxiety ($P = 0.001$) and stress ($P = 0.001$) than young adults (Table 1). Moreover, the majority of the elderly (76.1%) displayed stress symptoms in comparison to young adults (27.3%), $P < 0.0001$. Further analyses within the stressed subjects revealed that the majority of the elderly (67.4%) and young subjects (21.2%) were classified in the adaptation's stress phase, with only one elder (2.2%) classified in the exhaustion's stress phase, $P < 0.0001$. In addition, both groups presented similar stress symptoms (i.e. physical and psychological).

Table 1
Characteristics of the study sample

Factors	Young ($n = 33$)	Elderly ($n = 46$)
Age (yrs)	27.42 ± 1.16 (20–40 yrs)	72.00 ± 1.25**** (60–91 yrs)
Race	81.00% Caucasian	95.60% Caucasian
Gender	54.54% F 45.45% M	67.39% F 32.61% M
Weight (kg)	67.14 ± 2.27	68.64 ± 1.95
Height (m)	1.71 ± 0.01	1.58 ± 0.01****
Stress (Q1)	2.10 ± 0.32	4.09 ± 0.37****
Stress (Q2)	2.65 ± 0.50	4.98 ± 0.41***
Stress (Q3)	3.77 ± 0.67	6.22 ± 0.63**
Anxiety	3.29 ± 0.59	5.00 ± 0.35***
No anxiety: n (%)	21 (63.63%)	13 (28.26%)
Mild anxiety: n (%)	6 (18.18%)	10 (21.74%)
Moderate: n (%)	2 (6.06%)	12 (26.09%)
Severe anxiety: n (%)	4 (12.12%)	11 (23.91%)
Depression	17.94 ± 2.69	29.40 ± 1.87**
No depression: n (%)	26 (78.78%)	23 (50.00%)
Symptoms of depression: n (%)	6 (18.18%)	21 (46.65%)
Depression: n (%)	1 (3.03%)	2 (4.34%)

Stress scoring (Q1 = last 24 h, Q2 = last week and Q3 = last month). Statistical significances are indicated: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ and ****$P < 0.0001$ versus young subjects.

3.2. Endocrine evaluation

In this study we were able to assess HPA axis function by means of measuring salivary cortisol levels across the day. Salivary cortisol levels differed significantly over the three sampling times (Fig. 1A), $F(2, 142) = 46.72$, $P < 0.0001$. Cortisol peaked in the morning and presented a nadir at night, with a regular circadian pattern for both groups. Interestingly, cortisol levels were significantly elevated in the elderly compared to young adults, $F(1, 71) = 5.04$, $P = 0.02$ (Fig. 1A).

We also observed that elders had significantly lower serum DHEA levels compared to young subjects, $t = 5.34$, $P < 0.0001$ (Fig. 1B). Although young men presented significantly higher DHEA levels than young women (7.23 ± 0.59 versus 4.83 ± 0.75, $P < 0.05$), there were no gender-related differences in the elderly group.

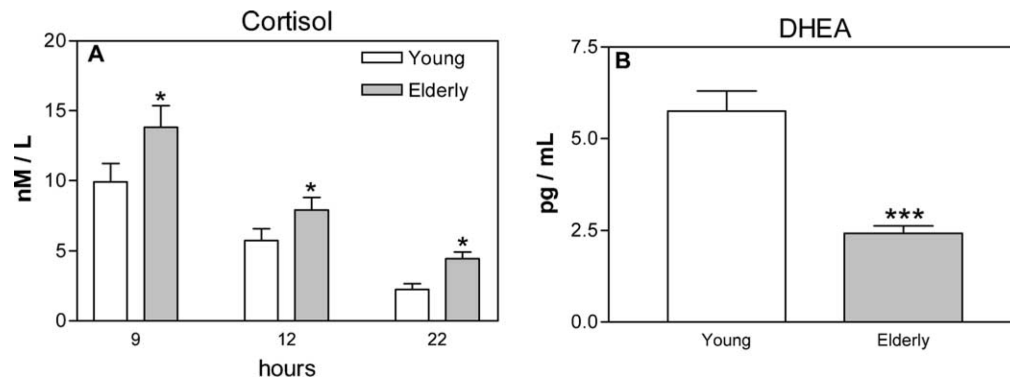

Fig. 1. Endocrine evaluation. Human ageing is characterised by higher salivary cortisol levels throughout the day (A) and reduced serum DHEA levels in the morning (B). Statistically significant differences are indicated: $*P < 0.05$ and $***P < 0.0001$.

3.3. Cytokines

Monocytes play a central role in innate immunity, participating during the processes of inflammation and in antigen presentation. We stimulated PBMCs with LPS, a potent monocyte activating agent, to evaluate the production of the pro-inflammatory cytokines. However, both TNF-α and IL-6 levels did not differ between young adults and elderly subjects (Fig. 2). Chronic T-cell activation leads to receptor shedding and shed sIL-2Rα may bind free IL-2, limiting its function. Increased serum sIL-2Rα levels were observed in the elderly and might be a clinical marker of strong antigenic stimulation. We thus stimulated PBMCs with PHA, a polyclonal mitogen that stimulates T-cells only, to evaluate sIL-2Rα production. In this study, however, the sIL-2Rα production was found to be the same between young and elderly subjects (Fig. 2).

3.4. Psychoneuroendocrine relationships on cytokine production

We further explored the complex relationships between psychological, endocrine and immune variables studied. We first investigated the impact of psychological factors on endocrine variables. Depression, anxiety and stress scores were found positively correlated to evening cortisol levels and negatively correlated to morning DHEA levels (Table 2).

The relationships between psychoneuroendocrine factors and cytokines were then investigated. No significant correlations were found between psychological factors and cytokines studied ($P > 0.17$). However, evening (22 h) cortisol levels were found positively correlated to TNF-α ($r = 0.34$, $P < 0.05$) and sIL-2Rα ($r = 0.29$, $P < 0.05$) levels (see Fig. 3). DHEA levels, however, were not found statistically correlated to any cytokine measured ($P > 0.14$).

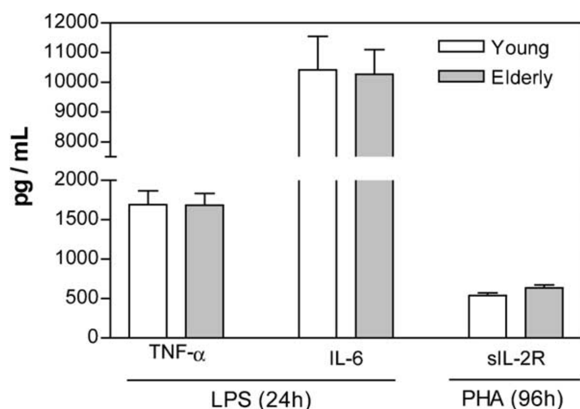

Fig. 2. Cytokine production. PBMCs were stimulated in vitro with either LPS and cultured for 24 h or PHA for 96 h. Cytokines were measured in the supernatants by quimioimmunoassays.

4. Discussion

There is a complex bi-directional communication between the nervous, endocrine and immune systems (Blalock, 1989). Although these systems are commonly studied separately by our fragmented Cartesian-based science, recent psychoneuroimmunologic research sheds some light into the importance of investigating systemic relationships. In this study, we investigated the impact of psychoneuroendocrine factors on cytokine production in vitro of strictly healthy (SENIEUR) elderly and young subjects. Ageing is associated with several

Table 2
Impact of psychological factors on neuroendocrine variables

Variables	Cortisol (9 h)	Cortisol (12 h)	Cortisol (22 h)	DHEA (9 h)
Depression	0.12 (0.30)	0.24 (0.04)	0.37 (0.001)	−0.43 (0.0001)
Anxiety	0.12 (0.29)	0.17 (0.16)	0.24 (0.04)	−0.42 (0.0001)
Stress (Q1)	0.21 (0.07)	0.22 (0.06)	0.37 (0.001)	−0.33 (0.005)
Stress (Q2)	0.10 (0.40)	0.15 (0.19)	0.39 (0.001)	−0.38 (0.001)
Stress (Q3)	0.22 (0.06)	0.19 (0.10)	0.33 (0.005)	−0.36 (0.002)

Stress scoring: Q1 = last 24 h, Q2 = last week and Q3 = last month. NS, non-significant. Pearson correlations are indicated (P).

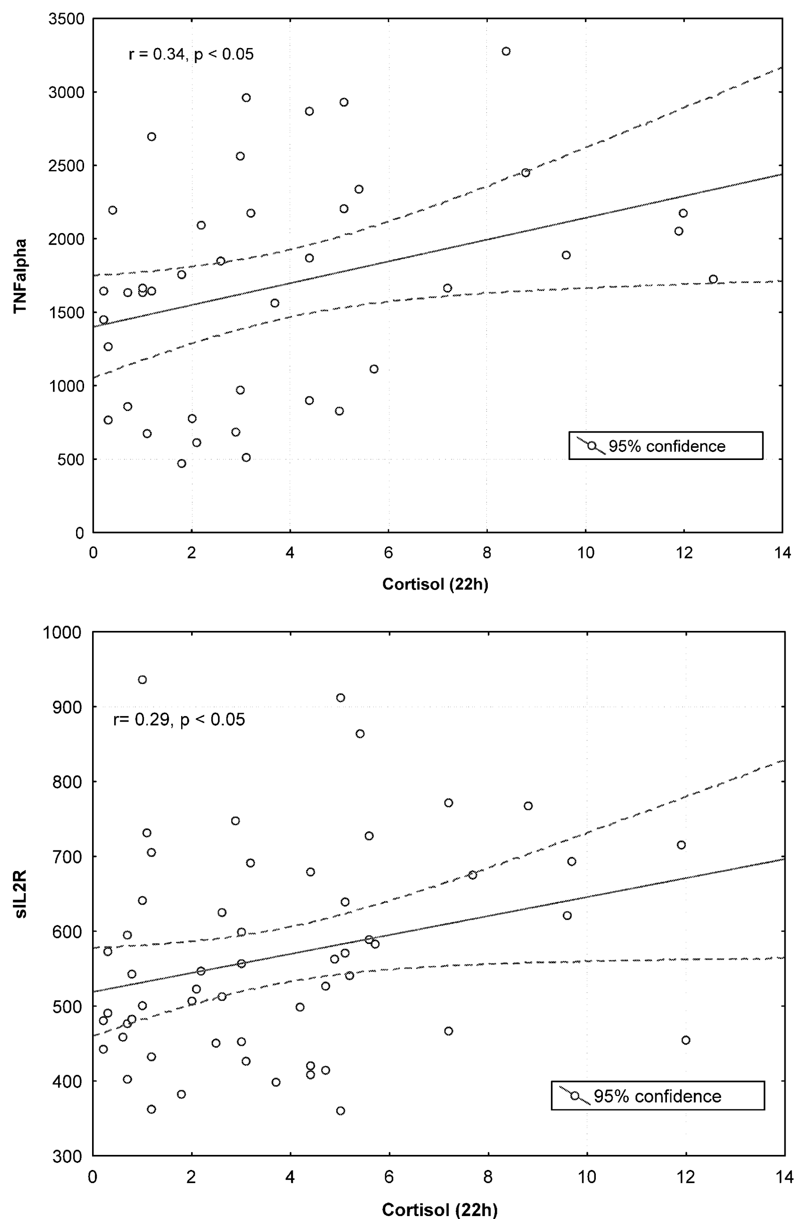

Fig. 3. Correlations between evening cortisol (22 h) and cytokines. The 95% confidence interval of the regression line, the correlation coefficients and the P values are indicated.

immune-related diseases including cardiovascular, infectious and cancer. Therefore, to control for age-related diseases that would interfere with our cytokine measurements, strictly healthy individuals were recruited by means of the SENIEUR protocol (Ligthart et al., 1984). However, we observed that SENIEUR elderly subjects were significantly more distressed than young adults. In particular, the healthy elderly were more depressed, anxious and stressed than young subjects. It is noteworthy to mention here, however, that the elderly subjects investigated in this study were not suffering from clinical depression or chronic stress. In fact, all were non-institutionalised and socially active individuals. The literature regarding age-related psychological changes is controversial and others did not find these changes (Nolen-Hoeksema and Ahrens, 2002). This could be due to methodological issues, since specific clinical interviews are required to assess depression in the elderly. Taken together, our data suggest that healthy ageing is accompanied with psychological changes and that the SENIEUR protocol is limited to evaluate these changes.

In parallel with psychological changes, we observed that healthy elderly subjects had increased salivary cortisol levels and reduced serum DHEA levels. These data are in partial agreement with previous studies (Halbreich et al., 1984; Straub et al., 1998) and suggest that adreno-senescence is characterised with significant

activation of the HPA axis in parallel with reduced serum DHEA levels. The HPA axis activation could be related to a degeneration of the limbic structures (hypothalamus and hippocampus) and may be responsible for a decreased sensitivity to cortisol and hyperactivation of the HPA axis during ageing (Sapolsky et al., 1986). Given the findings that even discrete HPA axis activation may impair cognitive function (Lupien et al., 1994) and induce sleep disturbances (Starkman et al., 1981), conditions frequently associated in the elderly, psychological or pharmacological strategies attenuating or preventing increased HPA function during ageing might be of considerable benefit for the elderly. However, there are studies in the literature reporting that cortisol levels are unchanged during healthy ageing (Waltman et al., 1991; Raff et al., 1999; Kudielka et al., 2000; Martinez-Taboada et al., 2002). These discrepancies could be related to small sample sizes, heterogeneity of subject populations in terms of gender distribution, age range, healthy status, differences in the analytical methods employed and psychological status. As regards to the latter, there is evidence suggesting that the stress-related HPA axis activation may produce the very same neurological changes in the limbic region via the direct action of cortisol in these areas. Therefore, it becomes difficult to dissociate these neuroendocrine changes observed in the elderly with those induced by psychological stimuli. For instance, we have previously demonstrated that chronically stressed elders had increased salivary cortisol levels compared to non-stressed elderly (Bauer et al., 2000). In this study, psychological scores were positively correlated to cortisol levels and negatively correlated to DHEA levels. Taken together, these data indicated that psychological factors may be implicated in activating HPA axis during healthy ageing. DHEA may antagonise several biological effects of glucocorticoids including neuronal protection to damaging effects of glucocorticoids (Svec and Porter, 1998). In addition, the fact that cortisol levels are higher and DHEA levels significantly reduced during ageing makes the neural environment more exposed to cortisol actions. This may lead to cognitive alterations and psychological disturbances as demonstrated by mental diseases (Michael et al., 2000).

No differences in LPS-induced production of pro-inflammatory cytokines were noted between young and elderly subjects. These data are in contrast to previous studies that found that ageing was associated with increased production of pro-inflammatory cytokines (Fagiolo et al., 1993; Straub et al., 1998; Gabriel et al., 2002) (Straub et al., 1998; Wolf and Kirschbaum, 1999). In addition, previous work has linked the age-related decline in DHEA production to increased serum levels of IL-6 (Daynes et al., 1993; Straub et al., 1998). This again was not found in our study. These apparent discrepancies could be explained by at least two methodological differences between this and previous studies: (a) whether cytokines were measured in serum or supernatants and (b) the psychological status of the elderly cohort. Levels of pro-inflammatory cytokines determined in serum may not necessarily reflect those found in vitro since cell types other than monocytes may also secrete them (e.g. endothelial cells). There are also soluble receptors circulating in serum that may antagonise their function and limit their detection. In this study, we have only assessed stimulated cytokine levels because recent work produced by our (Bauer et al., 2003) or other's laboratories (Beharka et al., 2001) revealed that unstimulated and stimulated cytokine levels are correlated in vitro. In addition, considering that our cohort of elderly subjects were significantly distressed, we hypothesize that this could have normalised the cytokines investigated in this study. However, the underlying mechanisms may be other than via HPA axis since cortisol levels were positively associated to TNF-α and sIL-2Rα levels. Although correlation does not prove causal relationship, it indicates that this line of evidence should merit further investigation. On the other hand, there is also some evidence of increased pro-inflammatory cytokines during major depression (Maes et al., 1995; Vetta et al., 2001). Therefore, it becomes difficult to dissociate the cytokine changes observed in the elderly with those induced by psychological stimuli. In addition, our cohort of elderly subjects produced similar sIL-2Rα levels following PHA stimulation compared to young adults. This result is contrasting with previous studies that have shown that ageing was associated with either increased serum sIL-2Rα levels (Franze et al., 1996; Rea et al., 1996) or increased production of sIL-2Rα in vitro (Liu et al., 1997; Rink et al., 1998) and will thus require further investigation.

In summary, we demonstrated here that healthy (SENIEUR) ageing is associated with significant psychological distress in parallel with higher cortisol levels and reduced DHEA levels. We demonstrated that it becomes difficult to dissociate these neuroendocrine changes observed in the elderly with those induced by psychological stimuli. No changes in cytokine production were observed in the elderly. Furthermore, we suggested that there are neuroendocrine interactions involved with the production of TNF-α and sIL-2Rα. Additional research is necessary to further explore these interactions in both healthy and pathological ageing.

Acknowledgements

The authors would like to acknowledge the excellent technical assistance of Ingrid Manfredi (Office for Social Care, Gravataí). We are grateful to the city hall of Gravataí for setting up special facilities for the recruitment of the elderly subjects. We are also indebted to

DPC-Medlab (São Paulo, Brazil) for kind donations of kits. The work presented in this manuscript was supported by grants from FAPERGS (M.E.B., Proc. 00/0168.9) and CNPq (M.E.B., Proc. 551180/01-3).

References

Bauer, M.E., Gauer, G.J., Luz, C., Silveira, R.O., Nardi, N.B., von Muhlen, C.A., 1995. Evaluation of immune parameters in depressed patients. Life Sci. 57, 665–674.

Bauer, M., Vedhara, K., Perks, P., Wilcock, G., Lightman, S., Shanks, N., 2000. Chronic stress in caregivers of dementia patients is associated with reduced lymphocyte sensitivity to glucocorticoids. J. Neuroimmunol. 103, 84–92.

Bauer, M., Papadopoulos, A., Poon, L., Perks, P., Lightman, S., Checkley, S., Shanks, N., 2003. Altered glucocorticoid immunoregulation in treatment resistant depression. Psychoneuroendocrinology 28, 49–65.

Beharka, A.A., Meydani, M., Wu, D., Leka, L.S., Meydani, A., Meydani, S.N., 2001. Interleukin-6 production does not increase with age. J. Gerontol. A Biol. Sci. Med. Sci. 56, B81–B88.

Berthier, F., Genin, C., Lambert, C., Bienvenu, J., 1999. Evaluation of an automated immunoassay method for cytokine measurement using the Immulite immunoassay system. Clin. Chem. Lab. Med. 37, 393–399.

Besedovsky, H., Sorkin, E., Felix, D., Haas, H., 1977. Hypothalamic changes during the immune response. Eur. J. Immunol. 7, 323–325.

Blalock, J., 1989. A molecular basis for bi-directional communication between the immune and neuroendocrine system. Physiol. Rev. 69, 1–32.

Daynes, R., Araneo, B., Ershler, W., Maloney, C., Li, G.-Z., Ryu, S.-Y., 1993. Altered regulation of IL-6 production with normal aging—possible linkage to the age-associated decline in dehydroepiandrosterone and its sulfated derivative. J. Immunol. 150, 5219–5230.

Deuschle, M., Gotthardt, U., Schweiger, U., Weber, B., Korner, A., Schmider, J., Standhardt, H., Lammers, C., Heuser, I., 1997. With aging in humans the activity of the hypothalamus–pituitary–adrenal system increases and its amplitude flattens. Life Sci. 61, 2239–2246.

Fagiolo, U., Cossarizza, A., Scala, E., Fanales-Belasio, E., Ortolani, C., Cozzi, E., Monti, D., Franceschi, C., Paganelli, R., 1993. Increased cytokine production in mononuclear cells of healthy elderly people. Eur. J. Immunol. 23, 2375–2378.

Franze, C., Di Stefano, F., Motta, M., Maugeri, D., Stivala, F., Favetta, A., Carnazzo, G., 1996. Comparison between serum interleukin-2 concentrations in healthy adult and elderly subjects. Arch. Gerontol. Geriatr. 61, 469–471.

Gabriel, P., Cakman, I., Rink, L., 2002. Overproduction of monokines by leukocytes after stimulation with lipopolysaccharide in the elderly. Exp. Gerontol. 37, 235–247.

Halbreich, U., Asnis, G., Zumoff, B., Nathan, R., Shindledecke, R., 1984. Effect of age and sex on cortisol secretion in depressives and normals. Psychiat. Res. 13, 221–229.

Hamilton, M., 1967. Development of a rating scale for primary depressive illness. Br. J. Soc. Clin. Psychol. 6, 278–296.

Heuser, I., Deuschle, M., Luppa, P., Schweiger, U., Standhadt, H., Weber, B., 1998. Increased diurnal plasma concentrations of dehydroepiandrosterone in depressed patients. J. Clin. Endocrinol. Metab. 83, 3130–3133.

Kahn, J., Rubinow, D., Davis, C., Kling, M., Post, R., 1988. Salivary cortisol: a practical method for evaluation of adrenal function. Biol. Psychiatry 23, 335–349.

Kirschbaum, C., Hellhammer, D.H., 1994. Salivary cortisol in psychoneuroendocrine research: recent developments and applications. Psychoneuroendocrinology 19, 313–333.

Kudielka, B., Schmidt-Reinwald, A., Hellhammer, D., Schurmeyer, T., Kirschbaum, C., 2000. Psychosocial stress and HPA functioning: no evidence for a reduced resilience in healthy elderly men. Stress 3, 229–240.

Ligthart, G.J., Corberand, J.X., Fournier, C., Galanaud, P., Hijmans, W., Kennes, B., Muller-Hermelink, H.K., Steinmann, G.G., 1984. Admission criteria for immunogerontological studies in man: the SENIEUR protocol. Mech. Ageing Dev. 28, 47–55.

Lipp, M., Guevara, A., 1994. Validação empírica do inventário de sintomas de stress. Estudos de Psicologia 11, 43–49.

Liu, J., Wang, S., Liu, H., Yang, L., Nan, G., 1997. The monitoring biomarker for immune function of lymphocytes in the elderly. Mech. Ageing Dev. 94, 177–182.

Lupien, S., Lecours, A., Lussier, I., Schwartz, G., Nair, N., Meaney, M., 1994. Basal cortisol levels and cognitive deficits in human aging. J. Neurosci. 14, 2893–2903.

Maeda, K., Tanimoto, K., Terada, T., Shintani, T., Kakigi, T., 1991. Elevated urinary free cortisol in patients with dementia. Neurobiol. Aging 12, 161–163.

Maes, M., Meltzer, H., Bosmans, E., Bergmans, R., Vandoolaeghe, E., Ranjan, R., Desnyder, R., 1995. Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. J. Affect. Disord. 34, 301–309.

Martinez-Taboada, V., Bartolome, M.J., Amado, J.A., Blanco, R., Garcia-Unzueta, M.T., Rodriguez-Valverde, V., Lopez-Hoyos, M., 2002. Changes in peripheral blood lymphocyte subsets in elderly subjects are associated with an impaired function of the hypothalamic–pituitary–adrenal axis. Mech. Ageing Dev. 123, 1477–1486.

Mastorakos, G., Chrousos, G.P., Weber, J., 1993. Recombinant IL-6 activates the hypothalamic–pituitary–adrenal axis in humans. J. Clin. Endocrinol. Metab. 77, 1690–1694.

McEwen, B.S., Biron, C.A., Brunson, K.W., Bulloch, K., Chambers, W.H., Dhabhar, F.S., Goldfarb, R.H., Kitson, R.P., Miller, A.H., Spencer, R.L., Weiss, J.M., 1997. The role of adrenocorticoids as modulators of immune function in health and disease: neural, endocrine and immune interactions. Brain. Res. Brain Res. Rev. 23, 79–133.

Michael, A., Jenaway, A., Paykel, E., Herbert, J., 2000. Altered salivary dehydroepiandrostenona levels in major depression in adults. Biol. Psychiatry 48, 989–995.

Munck, A., Guyre, P.M., Holbrook, N.J., 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr. Rev. 5, 25–44.

Nolen-Hoeksema, S., Ahrens, C., 2002. Age differences and similarities in the correlates of depressive symptoms. Psychol. Aging 17, 116–124.

Pawelec, G., Barnett, Y., Forsey, R., Frasca, D., Globerson, A., McLeod, J., Caruso, C., Franceschi, C., Fulop, T., Gupta, S., Mariani, E., Mocchegiani, E., Solana, R., 2002. T cells and aging, January 2002 update. Front. Biosci. 7, d1056–d1183.

Raff, H., Raff, J., Duthie, E., Wilson, C., Sasse, E., Rudman, I., Mattson, D., 1999. Elevated salivary cortisol in the evening of healthy elderly men and women: correlation with bone mineral density. J. Gerontol. A Biol. Sci. Med. Sci. 54, M479–M483.

Rea, I., Stewart, M., Campbell, P., Alexander, H., Crockard, A., Morris, T., 1996. Changes in lymphocyte subsets, interleukin 2, and soluble interleukin 2 receptor in old and very old age. Gerontology 42, 69–78.

Rink, L., Cakman, I., Kirchner, H., 1998. Altered cytokyne production in the eldely. Mech. Ageing Dev. 102, 199–209.

Salzman, C., Shader, R.I., 1978. Depression in the elderly. II. Possible drug etiologies; differential diagnostic criteria. J. Am. Geriatr. Soc. 26, 303–308.

Sapolsky, R.M., Krey, L.C., McEwen, B.S., 1986. The neuroendocrinology of stress and aging: the glucocorticoid cascade hypothesis. Endocr. Rev. 7, 284–301.

Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr. Rev. 21, 55–89.

Selye, H., 1936. A syndrome produced by diverse nocuous agents. Nature 138, 32.

Shua-Haim, J.R., Haim, T., Shi, Y., Kuo, Y.H., Smith, J.M., 2001. Depression among Alzheimer's caregivers: identifying risk factors. Am. J. Alzheimers Dis. Other Demen. 16, 353–359.

Starkman, M., Schteingart, D., Schork, M., 1981. Depressed mood and other psychiatric manifestations of Cushing's syndrome: relationship to hormone levels. Psychosom. Med. 43, 3–18.

Straub, R., Konecna, L., Hrach, S., Rothe, G., Kreutz, M., Scholmerich, J., Falk, W., Lang, B., 1998. Serum dehydroepiandrosterna (DHEA) and DHEA sulfate are negatively correlated with serum interleukin-6 (IL-6), and inhibits IL-6 secretion from mononuclear cells in vitro: possible link between endocrinesenescence and imunosenescence. J. Clin. Endocrinol. Metab. 83, 2012–2017.

Straub, R., Miller, L., Scholmerich, J., Zietz, B., 2000. Cytokines and hormones as possible links between endocrinosenescence and immunosenescence. J. Neuroimmunol. 109, 10–15.

Svec, F., Porter, J., 1998. The actions of exogenous dehydroepiandrosterone in experimental animals and humans. Proc. Soc. Exp. Biol. Med. 218, 174–191.

Van Cauter, E., Leproult, R., Kupfer, D.J., 1996. Effects of gender and age on the levels and circadian rhythmicity of plasma cortisol. J. Clin. Endocrinol. Metab. 81, 2468–2473.

Vetta, F., Ronzoni, S., Lupatelli, M., Novi, B., Fabbriconi, P., Ficconeri, C., Cicconetti, P., Russo, F., Bollea, M., 2001. Tumor necrosis factor-alpha and mood disorders in the elderly. Arch. Gerontol. Geriatr. (Suppl. 7), 442.

Walker, R., Riad-Fahmy, D., Read, G., 1978. Adrenal status assessed by direct radioimmunoassay of cortisol in whole saliva or paratid saliva. Clin. Chem. 24, 1460–1463.

Waltman, C., Blackman, M.R., Chrousos, G.P., Riemann, C., Harman, S.M., 1991. Spontaneous and glucocorticoid-inhibited adrenocorticotropic hormone and cortisol secretion are similar in healthy young and old men. J. Clin. Endocrinol. Metab. 73, 495–502.

Wolf, O.T., Kirschbaum, C., 1999. Actions of dehydroepiandrosterone and its sulfate in the central nervous system: effects on cognition and emotion in animal and humans. Brain Res. Rev. 30, 264–288.

Yesavage, J.A., Brink, T.L., Rose, T.L., Lum, O., Huang, V., Adey, M., Leirer, V.O., 1982. Development and validation of a geriatric depression screening scale: a preliminary report. J. Psychiatr. Res. 17, 37–49.