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# Healthy Aging Is Associated with Unaltered Production of Immunoreactive Growth Hormone but Impaired Neuroimmunomodulation

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## Key Words

Aging · Glucocorticoids · Immunosenescence · Lymphocytes · Psychoneuroimmunology

## Abstract

**Background:** Both endocrine and immune systems are continuously remodeled during aging. **Objective:** Here, we investigated to what extent adrenal and somatosenescence are associated reciprocal changes in the immune system during strictly healthy aging. **Methods:** Forty-six elderly subjects and 33 young adults were recruited according to the health criteria of the SENIEUR protocol. Peripheral blood mononuclear cells were isolated and stimulated in vitro with lipopolysaccharide or phytohemagglutinin to assess the production of immunoreactive growth hormone (GH). Peripheral sensitivity to steroids was assessed in vitro by dexamethasone-, cortisol- or dehydroepiandrosterone (DHEA)-induced inhibition of T-cell proliferation. DHEA and GH levels were measured by radioimmunoassays. **Results:** Healthy elderly had lower salivary DHEA and serum GH levels (somatosenescence). They presented reduced T-cell sensitivity to dexamethasone but similar cellular sensitivities to cortisol and DHEA. Their cells produced similar levels of immunoreactive GH compared to the cells of young adults. **Conclusions:** These data indicate that healthy aging is associated with adrenal and somatosenescence as well as impaired

neuroendocrine immunoregulation at the level of the lymphocyte. In addition, somatosenescence may not be associated with a reciprocal decline in immunoreactive GH.

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## Introduction

Aging is associated with several physiological alterations including changes in the immune system (immunosenescence). However, it remains controversial whether these changes are a cause or an effect of underlying diseases in humans. Strenuous efforts have been made to circumvent this problem by separating ‘disease’ from ‘aging’, as exemplified by the application of the SENIEUR protocol [1], which defines rigorous criteria for selecting healthy individuals to immunogerontological studies. When diseased subjects are excluded, immunosenescence involves thymic involution, lower lymphocyte counts (e.g. naïve T cells), a switch from Th1 to Th2 cytokines, impaired humoral responses to new antigens and blunted T-cell proliferation [2]. The clinical consequences of T-cell senescence include increased susceptibility to infectious diseases, neoplasias and autoimmune diseases [3]. Aging is also associated with chronic low-grade inflammation characterized by increased pro-inflammatory cytokines [4–6] that account for certain phe-

notypic changes at advanced age [7], particularly those that resemble chronic inflammatory disease (decreased lean body mass, osteopenia, low-grade anemia, decreased serum albumin and cholesterol, and increased inflammatory proteins). The age-associated rise in IL-6 has been linked to lymphoproliferative disorders, multiple myeloma, osteoporosis, and Alzheimer's disease [8]. However, this altered morbidity is not evenly distributed and may be influenced by other immune-modulating factors. These data led us to consider that other factors may potentially contribute to the heterogeneity of these changes, including neuroendocrine pathways. Therefore, the understanding of the interplay between the immune, endocrine and nervous systems in the elderly is of paramount importance.

In addition to immunosenescence, the endocrine system also undergoes important changes during aging. Both human and animal studies have demonstrated a decline in growth hormone (GH), sex hormones and dehydroepiandrosterone (DHEA) with aging [9]. DHEA is the major secretory product of the human adrenal. The hormone is uniquely sulfated (DHEAS) before entering the plasma, and this prohormone is converted to DHEA and its metabolites in various peripheral tissues [10]. Serum DHEA levels decrease after the 2nd decade of life, reaching about 5% of the original level in the elderly [11]. However, it remains to be established whether this hormonal change can also be observed when stress-free collection procedures are employed (e.g. salivary samples). DHEA and its metabolites have been reported to have immunomodulatory properties, including increased mitogen-stimulated IL-2 production [12, 13], diminished TNF- $\alpha$  or IL-6 production [5, 14], inhibition of natural killer cell differentiation [15] or rodent mitogen-induced lymphocyte proliferation [16]. Furthermore, DHEA has been proposed to exert restoring effects on immunosenescence, including an important adjuvant effect on the immunization of aged mice with recombinant hepatitis B surface antigen [17] or influenza [18]. However, DHEA treatment did not produce any beneficial immune response to influenza vaccination in elderly subjects [19]. Although many workers have tested adrenal function in the elderly, the effect of aging on salivary DHEA production and steroid immunomodulation has not been compared between elderly people of a defined health status and young subjects.

Previous studies have demonstrated that serum GH levels are significantly reduced during aging [20] – a process known as somatosenescence. However, GH is not exclusively produced by the pituitary gland; human im-

mune cells are also able to secrete several neuropeptides including GH [21, 22]. Immunoreactive GH has several immunoenhancing characteristics and may thus be important in modulating both humoral and cellular immune function [21, 23]. However, it remains to be established whether reduced peripheral GH levels (i.e. somatosenescence) are paralleled by altered production of immunoreactive GH.

The measurement of peripheral hormones may not be sufficient to finally determine the functional hormonal action in target tissues. Glucocorticoid (GC) immunomodulation is orchestrated by specific binding of GCs on two distinct cellular receptors: mineralocorticoid (MRs) and glucocorticoid receptors (GRs). Although MRs have higher affinity for circulating GCs than GRs, most (if not all) effects on the immune system are mediated via GRs [24]. The presence of these receptors indicates that the immune system is prepared for hypothalamic-pituitary-adrenal axis activation and the subsequent elevation in endogenous GCs. However, it has been suggested that chronically elevated cortisol levels may produce a state of acquired steroid resistance enabling lymphocytes to respond with less intensity to GCs. This phenomenon has previously been observed during major depression [25, 26] and chronic stress [27–29].

We have recently demonstrated that healthy aging was associated with significant psychological distress and increased salivary cortisol (~45%) levels in parallel with changes in T-cell subsets [30] and unaltered production of pro-inflammatory cytokines [31]. It was also observed that cortisol levels were positively correlated to cytokines [31]. Here, we further investigated the neuroendocrine/immunoregulation during healthy aging. We hypothesized that higher cortisol levels observed during aging [30] would render peripheral lymphocytes more resistant to GCs. This was achieved by investigating the ability of GCs (dexamethasone and cortisol) and DHEA to suppress T-cell proliferation *in vitro*. We also hypothesized that lower serum GH levels would be paralleled by reciprocal blunted production of immunoreactive GH by peripheral blood mononuclear cells (PBMCs).

## Materials and Methods

### Subjects

Forty-six non-institutionalized healthy elderly (31 females), aged 60–91 years (mean  $\pm$  SD, 72.00  $\pm$  8.51 years), were recruited from an existing database of 1,118 community-dwelling elderly subjects who had previously participated in research at the Institute of Geriatrics and Gerontology (Pontifical Catholic Univer-

sity of Rio Grande do Sul, PUCRS, Porto Alegre, Brazil). All subjects were recruited from local community centers and registered at the Office for Social Care in Gravataí (Brazil). This elderly population corresponded both ethically and socioeconomically to the general population of our State (Rio Grande do Sul, Brazil). All subjects took part in the GENESIS Program for the study on the genetic-environmental interactions on human aging. Thirty-three healthy young adults (18 females), aged 20–40 years (mean  $\pm$  SD, 27.40  $\pm$  6.70 years), also took part in this study; they were students or employees from the PUCRS.

Recruitment of the subjects was in accordance with the SENIEUR protocol [1] that defines rigorous criteria for selecting strictly healthy individuals to immunogerontological studies. The health status was assessed by thorough clinical investigations, and hematological and biochemical parameters. Exclusion criteria included: infections, acute or chronic inflammation, autoimmune diseases, cardiac disease, undernourishment, anemia, leukopenia, mood disorders, caregiving, neurodegenerative diseases, neoplasias and use of hormones (steroids) and drugs (e.g. alcohol, antidepressants, immunosuppressants, and anticoagulants).

#### *Procedures*

Subjects reported to the laboratory between 7 and 8 a.m. and were promptly examined by a geriatrician. After the clinical examination, subjects were asked to collect the first saliva sample (9 a.m.), and blood was immediately drawn for the immunological analyses. Before leaving the laboratory, subjects were asked to collect the second saliva sample (12 a.m.) and were instructed to collect the third sample (8 p.m.) at home. The latter was kept in the fridge and returned to the laboratory within 1 week. The study protocol was approved by both scientific and ethics committees (PUCRS), and written informed consent was obtained from all subjects.

#### *Nutritional Analyses*

Nutritional status was assessed in this investigation because it is known to influence immune function [32]. The assessments consisted of both body mass index (BMI: weight/height<sup>2</sup>) and serum proteins (total serum proteins, albumin, vitamin B<sub>12</sub>, folic acid, and ferritin). These parameters have previously been used as markers of the nutritional status in gerontological studies [33], as well as in previous work exploring the effects of stress on the immune system [27]. Albumin was also measured for its function as a major serum transport protein for DHEA (85% is bound to albumin) [34], and alterations ascribed to this carrier may thus change the active free albumin levels present in the tissue. Serum vitamin B<sub>12</sub> and folic acid were measured by electrochemiluminescence kits (Eleclys 2010; Roche). Ferritin was assessed by chemiluminescence kits (Immulite I; DPC-Medlab, São Paulo, Brazil). Albumin was measured by the standard enzymatic method of Biuret (540 nm) in combination with staining procedures (kit GT-Laboratories, Buenos Aires, Argentina). Measurement of these variables allowed us to examine the extent to which any observed immune impairment could be explained by these factors.

#### *Collection of Salivary Samples and Measurements of DHEA*

The assessment of steroids in saliva has proven to be a valid and reliable reflection of the unbound hormone in the blood [35], with salivary cortisol and DHEA concentrations reflecting 5–10% of the levels present in serum [25, 36]. Participants were

asked to collect three saliva samples with the help of cotton swabs over the course of the experimental day at 9, 12 and 20 h, always before meals and venipuncture. Sampling was performed during the whole day to assess some aspects of the circadian rhythm. Upon arrival at the laboratory, the samples were centrifuged and frozen at  $-20^{\circ}\text{C}$ . Salivary DHEA was analyzed by a modified radioimmunoassay (RIA; DPC-Medlab) according to previous work [37]. The sensitivity of this assay was 0.031 nM. The intra- and interassay coefficients of variation were less than 10%; DHEA results at each sampling time were expressed in nanomoles per liter.

#### *DHEA and GH Assessments*

Aliquots of peripheral blood were collected without anticoagulant for serum DHEA measurements. Hormonal levels were assessed by RIA kits for DHEA (Diagnostic Systems Laboratories, Webster, Tex., USA) and GH (DPC-Medlab). The sensitivity of these assays was  $\sim$ 0.031 nM. The intra- and interassay coefficients of variation were less than 10%. Results were expressed in picograms per milliliter.

#### *Collection of Peripheral Blood and Isolation of Mononuclear Cells*

Twenty milliliters of peripheral blood were collected by venipuncture in the morning (between 9 and 10 h) and samples stored into lithium heparin tubes prior to analyses. Samples were always collected at the same time of day to minimize circadian variations. PBMCs were isolated by density gradient (Ficoll-Hypaque; Sigma, St. Louis, Mo., USA) centrifugation for 30 min at 900 g. Cells were counted using a microscope (magnification:  $\times$ 100), and viability always exceeded 95%, which was assessed by trypan blue exclusion (Sigma).

#### *Cell Cultures and Steroid Sensitivity Assays*

To assess the production of immunoreactive GH, PBMCs ( $1.5 \times 10^5$  cells/ml) were stimulated with 0.2  $\mu\text{g/ml}$  of lipopolysaccharide (*Escherichia coli*, Sigma) or phytohemagglutinin (PHA) 1% (Gibco, Gaithersburg, Md., USA) in complete medium (RPMI-1640, supplemented with gentamicin 0.5%, glutamine 1%, HEPES 1%, and fetal calf serum 10%; all from Sigma) for 24 and 96 h, respectively, at  $37^{\circ}\text{C}$  in 5% CO<sub>2</sub>. Supernatants were collected by aspiration at the end of the culture period, and immunoreactive GH was measured by RIA.

Peripheral sensitivity to hormones was estimated by functional assays developed to measure the ability of steroids to suppress T-cell proliferation in vitro. T-cell proliferation was evaluated by incubating PBMCs ( $1.5 \times 10^5$  cells/well) with PHA (2, 1 and 0.5%, Gibco) in complete culture medium for 96 h at  $37^{\circ}\text{C}$  in 5% CO<sub>2</sub> atmosphere. Dexamethasone (selective GR agonist) and cortisol (which binds to both GRs and MRs) were added in duplicate (50  $\mu\text{l/well}$ ; both water-soluble substances purchased from Sigma) to mitogen-stimulated lymphocyte cultures. GC concentrations were used in a range ( $10^{-9}$  to  $10^{-4}$  M) that free GCs would reach at rest ( $10^{-8}$  M), during stress ( $10^{-6}$  M), or under pharmacological treatment ( $10^{-5}$  M) in vivo [38, 39]. Only two optimal DHEA concentrations ( $10^{-6}$  and  $10^{-5}$  M) were used due to the unresponsiveness of lower concentrations and toxicity of higher doses (i.e.  $> 10^{-5}$  M). One optimum PHA concentration (1%) was used for all steroid cultures. Fifty microliters of culture medium was added to all stimulated cultures. In spontaneous (without lectin)

**Table 1.** Characteristics of the participants

Variable	Young adults (n = 33)	Elderly (n = 46)	p value
Age, years	27.42 ± 1.16	72.00 ± 1.25	<0.0001
Caucasians, %	81.00	95.60	NS
Females, %	54.54	67.39	NS
Weight, kg	67.14 ± 2.27	68.64 ± 1.95	NS
Height, m	1.71 ± 0.01	1.58 ± 0.01	<0.0001
BMI	22.89 ± 0.63	27.18 ± 0.83	0.0001
TSP, g/dl	7.31 ± 0.12	7.05 ± 0.15	NS
Albumin, g/dl	3.92 ± 0.11	3.80 ± 0.09	NS
Vitamin B <sub>12</sub> , pg/ml	495.31 ± 47.66	516.87 ± 29.73	NS
Folic acid, ng/ml	5.02 ± 0.46	7.00 ± 0.44	0.003
Ferritin, ng/ml	112.30 ± 16.61	179.67 ± 21.34	0.01

TSP = Total serum proteins; NS = nonsignificant. Means ± SE.

cultures, mitogen was replaced by 150 µl of culture medium. Data are presented as percentage of basal proliferation (PHA 1% without steroids).

#### Cell Proliferation/Viability Assay

The proliferative responses were determined by a modified colorimetric assay [40]. In the last 4 h of the culture, 100 µl of the supernatant was gently discarded and 30 µl of freshly prepared 3-(4,5-diamethyl 2-thiazolyl) 2,5 diphenyl-2H-tetrazolium (Sigma) solution (5 mg/ml in RPMI-1640) was added to each well. The dehydrogenase enzymes in metabolically active cells convert this substrate to formazan, producing a dark blue precipitate. The cell cultures were incubated for 4 h at 37°C in 5% CO<sub>2</sub> atmosphere. After complete removal of the supernatant, 100 µl of dimethyl sulfoxide (Sigma) was added to each well. Optical density (OD) was determined using a BioRad ELISA plate reader at wavelengths of 570 and 630 nm. Proliferation/viability was expressed as OD of stimulated – OD of nonstimulated cultures.

#### Statistical Analysis

All variables were tested for normality of the distribution by means of the Kolmogorov-Smirnov test. Salivary DHEA levels were log-transformed to correct for skewed distributions. Proliferation and salivary DHEA data were analyzed by repeated measures ANOVA that included one between-subject variable (elderly vs. young) and one within-subject variable (DHEA, mitogen or steroid levels). Multiple comparisons among levels were checked with the Bonferroni post hoc test. Differences between demographic and nutritional variables were assessed by Student's t test, and differences in proportions between groups were tested by means of the  $\chi^2$  test. The area under the curve (AUC) of hormonal data and in vitro dexamethasone responses were estimated using the trapezoidal rule. The dexamethasone concentration that provided 50% inhibition (IC<sub>50</sub>) of lymphocyte proliferation was estimated by non-linear regression (Prism 4.0, Graphpad Software, San Diego, Calif., USA). A sigmoidal dose-response equation was chosen to fit the data ( $R^2 > 0.95$ ). Data are expressed as means ± SE in all figures and tables. A statistical software (SPSS 11.5; SPSS, Chicago, Ill., USA) was used for the analyses.

## Results

### Demographic Data and Nutritional Analyses

This study included a screening for strictly healthy subjects to control for diseases that may interfere with neuroendocrine and immunological analyses. All subjects recruited fulfilled the rigorous criteria of the SENIEUR protocol [1] for the selection of healthy individuals. Based on these criteria, 4.11% from the 1,118 community-dwelling strictly healthy elders were recruited, and demographic and nutritional data are summarized in table 1. The majority of the elderly (95.6%) and young subjects (81.0%) were Caucasians, and the female/male ratio ( $\chi^2 = 1.35$ ,  $p = 0.25$ ) or smoking habits ( $\chi^2 = 5.51$ ,  $p = 0.14$ ) did not differ between groups.

In order to ensure that only strictly healthy individuals were selected, the nutritional status was also investigated in this study. The elderly subjects had an elevated BMI ( $27.18 \pm 0.84$ ) compared to young adults ( $22.89 \pm 0.63$ ;  $p = 0.0001$ ). No statistically significant differences were noted for total serum proteins, vitamin B<sub>12</sub> or albumin levels between the elderly and young controls (table 1). In contrast, elderly subjects had significantly higher folic acid and ferritin levels compared to young adults. Nevertheless, both variables were found within the normal reference range (folic acid: 3–17 ng/ml and ferritin: 9–370 ng/ml). In addition, a complete hematological analysis revealed no age-related changes (i.e. lymphocyte counts or hemoglobin levels for example) [30].

### DHEA Assessment

In this study, we assessed the adrenal function by means of reliable measurements of salivary (free) DHEA

during the day (fig. 1). The elderly had significantly lower salivary DHEA levels compared to young adults [ $F(1,64) = 31.91, p < 0.0001$ ]. Accordingly, we observed that elders presented significantly lower AUC DHEA levels compared to young adults ( $3.75 \pm 0.40$  vs.  $8.15 \pm 0.86$  nmol/h, respectively;  $t = 5.28, d.f. = 67, p < 0.0001$ ). In addition, there was a significant interaction between

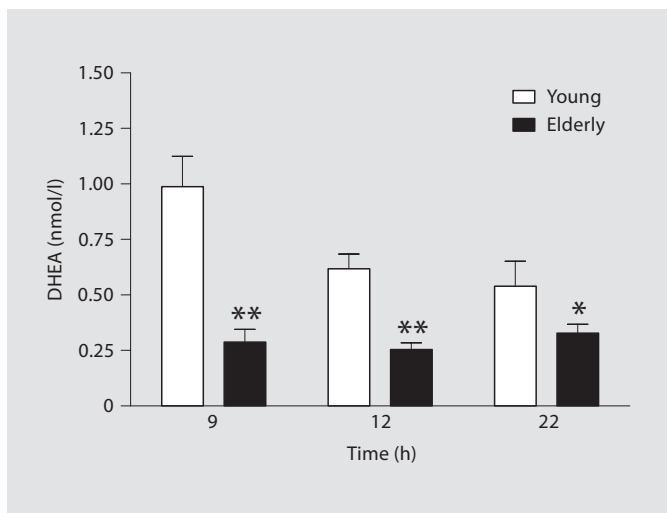
DHEA levels vs. group [ $F(2,128) = 12.02, p < 0.0001$ ]. Post hoc analyses revealed that only the young adults displayed a regular circadian rhythm, with peak DHEA levels in the morning and lower levels in the evening ( $p < 0.05$ ). In contrast, the elderly subjects presented a flat circadian pattern. Furthermore, morning serum (total) DHEA levels were found significantly reduced in the elderly ( $8.40 \pm 0.69$  nM) compared to young subjects ( $19.90 \pm 1.92$  nM;  $t = 5.63, d.f. = 70, p < 0.0001$ ).

#### Serum versus Immunoreactive GH

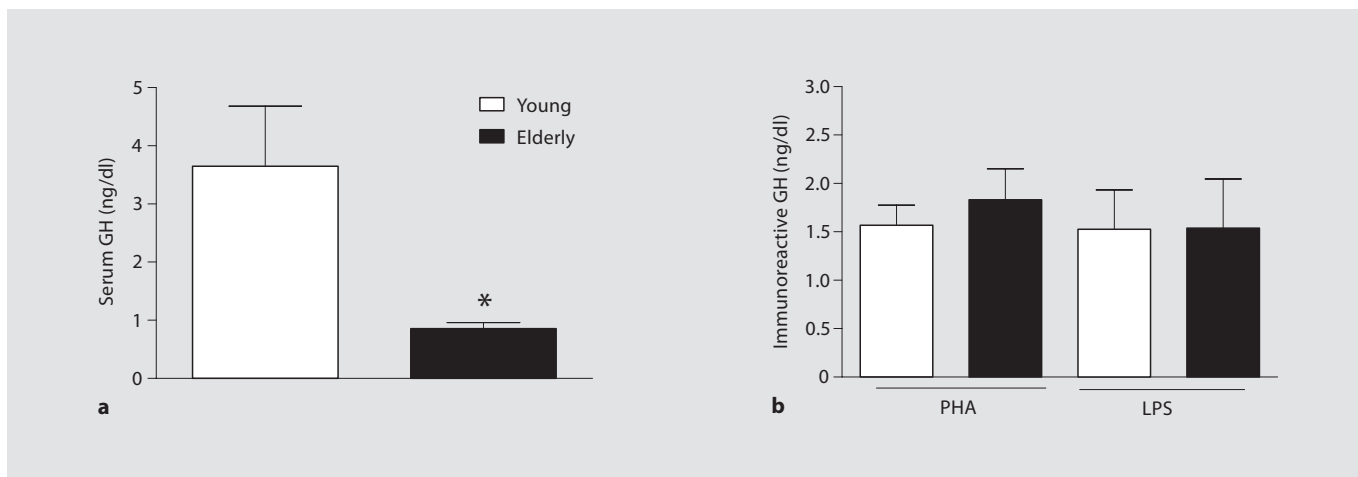
We investigated somatosenescence by evaluation of serum GH levels. We found that elders had significantly lower ( $-77\%, p = 0.003$ ) serum GH levels compared to young adults. Peripheral immunoreactive GH levels were also measured to investigate whether this specific function could parallel somatosenescence. However, immunoreactive GH production did not differ between elders and young adults (fig. 2). This applied for GH production by both stimulated monocytes or lymphocytes. Unstimulated cells did not produce immunoreactive GH.

#### Lymphocyte Sensitivity to Steroids

The measurement of peripheral hormones may not be sufficient to finally determine the functional hormonal action in target tissues. Here, we investigated the peripheral lymphoid sensitivity to steroids by analyzing the ability of GCs or DHEA in suppressing T-cell prolifera-



**Fig. 1.** Circadian pattern of salivary DHEA. Salivary DHEA levels were detected by RIA. Mean ( $\pm$  SE) levels of DHEA at each time point are shown. \*  $p < 0.05$ , \*\*  $p < 0.001$ .



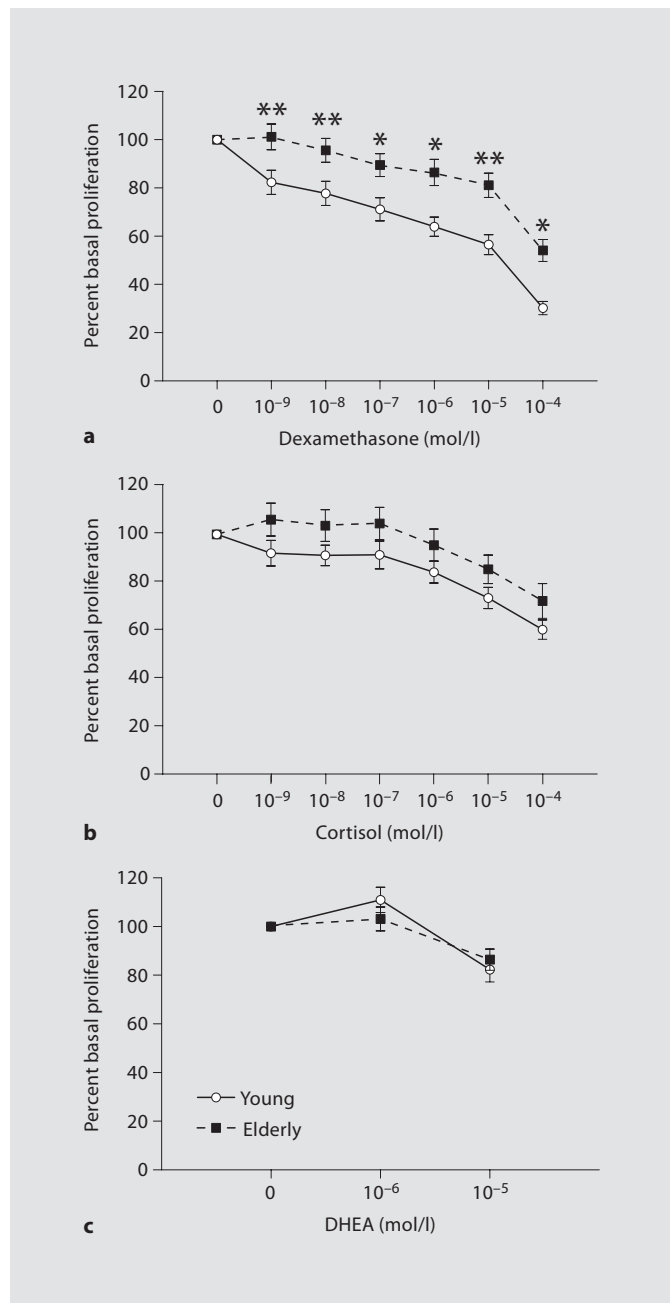
**Fig. 2.** Serum (a) versus immunoreactive GH (b). PBMCs were isolated and cells were stimulated in vitro with PHA or lipopolysaccharide (LPS) for 96 and 24 h, respectively. GH levels were checked by RIA. Means  $\pm$  SE. \*  $p < 0.003$ .

tion in vitro. Dexamethasone, cortisol and DHEA produced significant dose-dependent suppression of T-cell proliferation (all  $p < 0.0001$ ). Interestingly, we observed that lymphocytes from healthy elders were less sensitive to in vitro GC treatment compared with young subjects

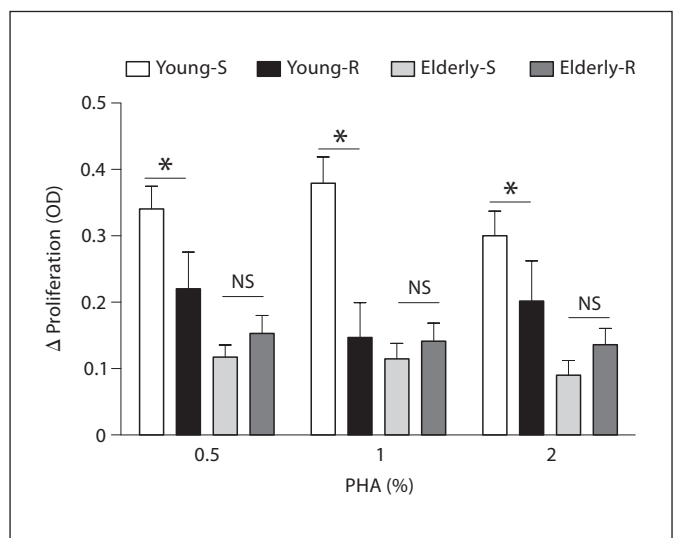
(fig. 3a). In particular, it was observed that cells from elders required higher dexamethasone concentrations to suppress PHA proliferation to the same extent as cells from young subjects [ $F(1,72) = 8.19, p = 0.006$ ]. In particular, cells from elders required 3.14 times more dexamethasone to suppress proliferation compared to cells of young subjects, as estimated by the pharmacological index  $IC_{50}$  ( $2.17 \times 10^{-5}$  vs.  $6.90 \times 10^{-6}$ , respectively). Although cortisol treatment produced a similar effect (fig. 3b), it did not reach statistical significance [ $F(1,66) = 1.64, p = 0.20$ ]. Lymphocytes from young adults and elders presented similar sensitivities to DHEA treatment in vitro [fig. 3c;  $F(1,72) = 0.11, p = 0.74$ ].

We then investigated the proportion of individuals who were more sensitive or resistant to dexamethasone effects. Subjects were classified as 'resistant' when AUC dexamethasone was higher than its median values (elderly:  $> 400.4$  nM/h; young adults:  $> 352.1$  nM/h). There was an increased proportion of dexamethasone-resistant subjects in the elderly group (48.9%) compared to young adults (28.6%), although this only approached statistical significance ( $\chi^2 = 2.94, d.f. = 1, p = 0.08$ ).

We then sought to investigate to what extent the peripheral cellular sensitivity to dexamethasone is associated to mitogen-induced T-cell proliferation. An interesting picture emerged when these sensitive/resistant subgroups were re-analyzed for PHA proliferation (fig. 4). T-cell proliferation of 'dexamethasone-resistant' and



**Fig. 3.** Lymphocyte sensitivity to GCs in vitro. Data are presented as percent of basal proliferation (0 = PHA 1% without steroids). \*\*  $p < 0.01$  and \*  $p < 0.05$  (Tukey).



**Fig. 4.** T-cell proliferation and peripheral sensitivity to GCs. Subjects were classified into sensitive (S) and resistant (R) to in vitro dexamethasone treatment. \*  $p < 0.05$ .

'dexamethasone-sensitive' subjects differed significantly [ $F(1,63) = 13.60, p < 0.0001$ ]. Post hoc analyses revealed that cells of young 'resistant' subjects had significantly lower proliferative responses than 'sensitive' individuals ( $p < 0.05$ ). In fact, proliferation of 'resistant' young adults was as low as proliferation of elders (both 'sensitive' or 'resistant'). However, this steroid-related changes were not observed in the elderly.

## Discussion

Aging is associated with several immune-related diseases including neoplasias, and autoimmune and infectious diseases. However, this altered morbidity is not evenly distributed and should thus be influenced by other immune-modulating factors. It has repeatedly been shown that there is a bidirectional communication between the nervous, endocrine and immune systems [41]. In this study, we investigated to what extent adrenal and somatosenescence are associated reciprocal changes in the immune system during strictly healthy aging. To control for age-related diseases that would interfere with our immunological analyses, strictly healthy individuals were recruited by means of the SENIEUR protocol [1] from 1,118 community-dwelling elders. We observed that SENIEUR elders had elevated BMI compared to young adults. BMI changes, associated to both obesity and lack of regular exercise, have been associated to immunological changes in adults [42, 43]. However, our elderly cohort did not seem to have nutritional changes, since there were no changes in serum vitamin B<sub>12</sub> levels, and ferritin and folic acid levels were significantly increased compared to young adults. Nutritional analyses should be part of future aging studies to further improve the SENIEUR protocol in recruiting strictly healthy individuals.

We observed that healthy elderly subjects had reduced salivary DHEA levels compared to young adults. These data are in agreement with previous studies [11, 44, 45]. However, it should be noted that in most previous studies serum levels of peripheral hormones have been assessed at only one time point. In this study, multiple daily salivary sampling enabled us to identify that elders presented a flat circadian pattern for DHEA. The impaired DHEAS secretion, together with the increase in cortisol [31], results in an enhanced exposure of various tissues (including the brain and immune system) to the cytotoxic/immunomodulatory effects of GCs. The net physiological effects would be associated with exacerbated senescent features, including cognitive impairments, osteoporosis,

cardiovascular morbidities and immunosenescence. In accordance with previous work [20], we reported here that healthy aging is also associated with a significant drop in serum GH levels (i.e. somatosenescence). The lack of peripheral GH immune signaling may be also detrimental for immunosenescence. In particular, in GH-deficient rodents, there is significant immune dysfunction, which is reversed after GH replacement [46]. In addition, treatment with recombinant human GH (rhGH) or rhIGF-I enhances immune function in monkeys [47]. However, GH is not exclusively produced by the pituitary gland, human immune cells are also able to secrete several neuropeptides including GH [21, 22]. Immunoreactive GH has immunoenhancing properties, including increased IFN- $\gamma$  production, and may thus be important in modulating both humoral and cellular immune function [21, 23]. However, lower peripheral GH levels may not be paralleled by a reciprocal decline in immunoreactive GH during aging. It was found that secretion of immunoreactive GH by both stimulated lymphocytes and monocytes was not affected in the elderly. Therapies designed to enhance immunoreactive GH production may be of clinical value for the elderly.

Recent work suggests that cytokines and hormones could be considered as possible links between endocrinosenescence and immunosenescence [48]. The age-related decline in DHEA production has been associated with increased serum levels of IL-6 [5, 49]. In addition, increased plasma TNF- $\alpha$  levels were correlated to major depression in the elderly [50]. DHEA and its metabolites have been reported to have immunomodulatory properties, including diminished TNF- $\alpha$  or IL-6 production [5, 14]. Therefore, DHEA supplementation would attenuate chronic low-grade inflammation and age-related frailty by inhibiting production of pro-inflammatory cytokines.

The measurement of peripheral hormones may not be sufficient to finally determine the functional hormonal action in target tissues. Therefore, to further examine the cross talk between peripheral hormones and the immune system, we also investigated lymphocyte sensitivity to both synthetic (dexamethasone) and naturally occurring steroids (cortisol and DHEA). We reported that strictly healthy (SENIEUR) aging is associated with reduced lymphocyte sensitivity to dexamethasone. These data indicated that healthy aging is associated with alterations in neuroendocrine-immunoregulation. This phenomenon has previously been observed during major depression [25, 26] and chronic stress [27–29], and it seems to be GR-specific since cells from elders and young adults

showed a comparable lymphocyte sensitivity to cortisol (which binds to both GR and MR). There is considerable evidence for a shift in lymphocyte sensitivity to GCs during the ontogeny. For instance, peripheral T cells of infants younger than 12 months have been reported to be highly sensitive to dexamethasone treatment in vitro [51]. After this period, lymphocyte sensitivity to steroids is gradually decreasing, reaching adult levels at 1 year of life. A reduced sensitivity to GCs can also be observed at the central level during aging. Indeed, higher cortisol levels have been described in the elderly compared to young subjects during some pharmacological challenges, such as the dexamethasone suppression test, stimulation by human or ovine corticotrophin-releasing hormone or by physostigmine [52, 53].

The mechanisms underlying acquired steroid resistance are poorly understood. Based on our previous observations [31], we suggest that higher cortisol levels would render lymphocytes less sensitive to the effects of GCs in vitro. Indeed, there is some evidence in the literature suggesting that changes in GC sensitivity could be the result of chronic GC treatment [54, 55]. Reduced intracellular GC receptors may account for the putative underlying mechanisms of age-related GC resistance [15, 56, 57], but changes in GR affinity cannot be ruled out. Cellular sensitivity to GCs can also be modulated by changes in peripheral cytokines. Franchimont et al. [58] have shown that TNF- $\alpha$  decreases and IL-10 increases the sensitivity of human monocytes to dexamethasone via down- or upregulation of GRs, respectively. GC-induced acquired resistance may have an important physiological role in the protection of cells from the dangerous effects of prolonged GC-related immunosuppression. Additionally, altered steroid immunoregulation may have important therapeutic implications in clinical situations where GCs are administered, including treatment of autoimmune diseases, organ transplantation, and allergies.

Our data also illustrate a functional cross talk between cellular sensitivity to GCs and T-cell proliferation. In particular, we showed that T cells of young adults who were resistant to dexamethasone had the lowest T-cell proliferation (fig. 4). These data indicate the physiological significance of GC sensitivity in the regulation of cell-mediated immunity. The functional cross talk at the molecular level between immune signals and GCs is essential to determine the biological response to both mediators and constitutes the ultimate level of interaction between immune and neuroendocrine mediators [59]. The communication between immune and GC signals could also be interpreted from an evolutionary perspective as being

of adaptive significance. In a 'low cortisol milieu' (young subjects), the net cortisol effects on the immune system may be stimulatory in subjects who are sensitive to steroids. Conversely, in a 'high cortisol milieu' (stress or aging), subjects who are sensitive to steroids would be of higher risk for steroid-related immunosuppression. This should be further investigated in future studies.

These data indicate that healthy aging is associated with adrenal and somatosenescence as well as impaired neuroendocrine-immunoregulation at the level of the lymphocyte. In particular, we demonstrated that lymphocyte responses were associated with reduced lymphocyte sensitivity to a synthetic GC. However, the underlying mechanisms of acquired steroid resistance require further investigation. We do not yet know whether this altered GC sensitivity is associated with resistance to other medications and we are currently investigating this possibility. In addition, somatosenescence may not be associated with a reciprocal decline in immunoreactive GH.

#### Acknowledgments

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 thank Dr. Sidia Marques (Department of Genetics, Universidade Federal do Rio Grande do Sul, Porto Alegre, Brazil) for statistical assistance. This study was supported by grants from FAPERGS (00/0168.9, M.E.B.) and CNPq (551180/01-3, M.E.B.).

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