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TRANSFORMING GROWTH FACTOR BETA AND SOLUBLE ENDOGLIN IN THE HEALTHY SENIOR AND IN ALZHEIMER´S DISEASE PATIENTS

B. JURASKOVA[1], C. ANDRYS[2], I. HOLMEROVA[3], D. SOLICHOVA[1], D. HRNCIARIKOVA[1], H. VANKOVA[3], T. VASATKO[4], J. KREJSEK[2]

1. Department of Gerontology and Metabolism, Charles University in Prague, Medical Faculty and University Hospital, Hradec Kralové, Czech Republic; 2. Department of Clinical Immunology and Allergy, Charles University in Prague, Medical Faculty and University Hospital, Hradec Králové, Czech Republic; 3. Centre of Gerontology, Charles University in Prague, Faculty of Humanities, School of Management and Supervision in Prague, Centre of Gerontology, Simunkova 1600, Prague 8 – Kobylisy, Czech Republic; 4. Second Department of Internal Medicine, Charles University in Prague, Medical Faculty and University Hospital, Hradec Králové, Czech Republic. Address for correspondence: Dr. Bozena Juraskova, Department of Gerontology and Metabolism, Charles University in Prague, Medical Faculty and University Hospital in Hradec Kralove, Sokolska St. Hradec Kralove, Czech Republic, juraskovabozena@seznam.cz

Abstract: *Objectives:* Senescence of the immune system and of endothelial cells can contribute to age-dependent vascular and neurodegenerative disorders including Alzheimer´s disease. The aim of this study is an assessment of putative relationships of serum levels of transforming growth factor beta (TGFβ) and soluble endoglin (sCD105) and neurodegeneration, and of changes of these molecules in the course of ageing. *Design:* The subjects of the study consisted of three groups, the first one was 63 otherwise healthy middle – aged participants, 31 females, 32 males, of average age 35 years. The second group was formed by 58 healthy, self-dependent inhabitants of nursing homes, 44 females and 14 males, average age 83.5 years. The third group comprised of 129 Alzheimer´s disease patients, 86 females, 43 males, of average age 80 years, with MMSE score that ranged from 16 to 20. *Measurement:* Serum levels of TGF beta and soluble endoglin were measured by the ELISA method in samples of peripheral blood using commercial kits. *Results:* The serum level of TGFβ was 34,339 ± 6,420 pg/ml in the healthy younger group, 37,555 ± 11,944 pg/ml in the healthy seniors, and 29,057 ± 11,455 pg/ml in Alzheimer´s disease patients. Compared to healthy seniors, the serum level of TGFβ was significantly decreased in Alzheimer´s disease patients ($p<0.01$). The serum level of endoglin were 4.88 ± 0.95 μg/ml in the healthy younger group; 6.11 ± 1.38 μg/ml in healthy seniors, and 7.20 ± 1.72 μg/ml in patients with Alzheimer´s disease, respectively. The serum level of endoglin was significantly higher ($p<0.001$) in senescent healthy persons compared to the younger control group. When compared with healthy seniors, patients with Alzheimer´s disease had significantly elevated ($p<0.001$) serum level of endoglin. *Conclusions:* Decreased levels of TGFβ in Alzheimer's disease may result in impairment of cerebral circulation reflected in the increased endoglin levels. These findings may indicate involvement of the immune system in Alzheimer's disease pathogenesis.

Key words: Endoglin, TGFβ, old age, Alzheimer´s disease.

Introduction

The individual immune response changes substantially during ageing, reflecting actual physiological demands. The Th1 and Th2 subsets are the principal contributors to regulation of individual immune response. Th1 subset activity, which reaches its maximum in the early adulthood, gradually declines during ageing. Th1 subset activity is characterized by highly effective but potentially harmful cytotoxic responses and granuloma formation and must be tightly regulated by numerous mechanisms. As these regulatory mechanisms are disturbed in the elderly, there is a risk of „self-tolerance" failure and the development of immunopathological reactions. Furthermore, the risk of the development of autoimmunity in the elderly is enhanced by the formation and (or) release of autoantigens which are typical feature of ageing. Systemic proinflammatory activity which characterizes senescence is manifested in the upregulation of numerous substances, such as proinflammatory cytokines, chemokines, soluble adhesion molecules, soluble receptors for cytokines, arachidonic acid derivatives, to name only some. The associated long-term exaggerated inflammation frequently culminates in degenerative fibrotic processes.

The potentially harmful proinflammatory activities which are inseparably linked to ageing are counterbalanced by numerous regulatory circuits. The dominant role in these immune regulations is entrusted to regulatory T cells which are characterized by the production of antiinflammatory and immunosuppressive cytokines, such as TGFβ and IL-10. TGFβ is a pluripotent cytokine responsible for numerous effects at cell level. TGFβ is produced not only by T cells, but is also generated by monocyte-macrophage cells, granulocytes, chondrocytes, fibroblasts, and astrocytes, as well as by abnormal cells, such as cancer cells (1). Physiological role of TGFβ is regulation of cell differentiation, growth and tissue repair. The target cells for its biological activities are endothelial cells, smooth muscle cells and fibroblasts. The extracellular matrix remodelling is one of its principal activities. In addition, TGFβ is regulating angiogenesis and oncogenesis (2, 3). An integral part of the receptor for TGFβ is endoglin. Endoglin is a transmembrane glycoprotein which is expressed predominantly on the endothelial cells. Endoglin resembles betaglycan in its structure and contributes substantially to the process of angiogenesis and vascular remodeling via the TGFβ/FGFβR signaling pathway (4).

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Endoglin, like „yin-yang", both upregulates and downregulates the regulatory and antiinflammatory functions of TGFβ (5, 6). Endoglin in its soluble form is released into the circulation during an ongoing inflammatory process (7).

Little is known about the dynamics of TGFβ production in the healthy elderly population. It is generally accepted, and many times proven in various clinical conditions, that enhanced proinflammatory activities are the typical feature of the elderly condition.

Another threat to the elderly is the development of various forms of neurodegeneration such as Alzheimer´s disease. The pathogenesis of Alzheimer´s disease remains elusive but the participation of the immune system is hypothesized (8).

Based on these facts, we compared the serum levels of endoglin and TGFβ in the younger control group, in the healthy seniors, and seniors affected by Alzheimer´s disease.

Methods

We followed the serum levels of TGFβ and the soluble form of endoglin in three groups of subjects. The younger control group consisted of 63 healthy blood donors (31 females, 32 males, average age 35 years). The second group consisted of 58 otherwise healthy seniors recruited from residents of nursing home (44 females, 14 males, average age 83.5 years). All participants in this group were active and self-sufficient, without any signs of chronic illness, malnutrition, or neurodegenerative disorders (Mini-mental State Examination – MMSE scores were normal). The third group comprised 129 patients with diagnosed Alzheimer´s disease, also recruited from residents of nursing homes in Czech Republic (86 females, 43 males, average age 80 years). Their MMSE scores were in the range from 16 to 20. Alzheimer´s disease was diagnosed by consulting neurologist or psychiatrist.

Peripheral blood samples were allowed to clot and serum was obtained after sample centrifugation. Serum samples were stored frozen at -20˚C. Repeated thawing and freezing of samples was avoided. TGFβ1 and soluble endoglin (sCD105) were detected by ELISA using diagnostic kits Quantikine human TGFβ1 and Quantikine human endoglin/CD105 manufactured by R&D Systems (USA).

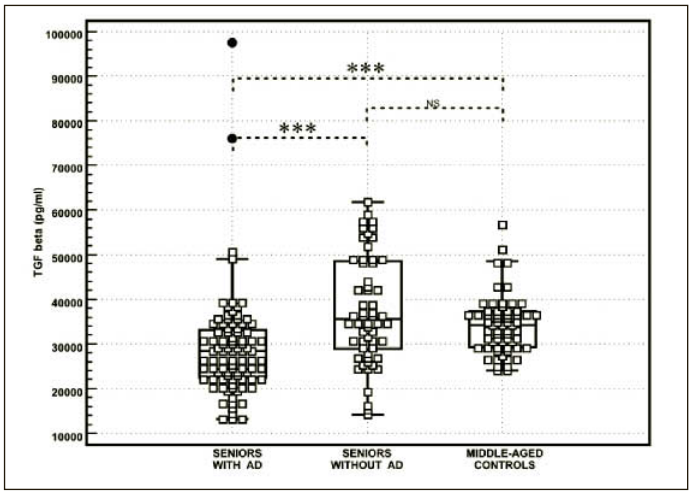
Statistical analysis of data was performed using MedCalc (Belgium) statistical software. Normal distribution of data was assessed. Student's t-test or Wilcoxon's test were used. Probability level less than 0.05 was considered as significant. All results are expressed as an average ± ISD.

Results

The serum level of TGFβ in younger control group of blood donors was 34,339 ± 6,420 pg/ml, which was not significantly different from that in healthy seniors (37,555 ± 11,944 pg/ml; p< 0.0647). When compared with healthy seniors, those with Alzheimer´s disease had significantly reduced serum levels of TGFβ (29,057 ± 11,455 pg/ml; p< 0.001) (figure 1).

Figure 1

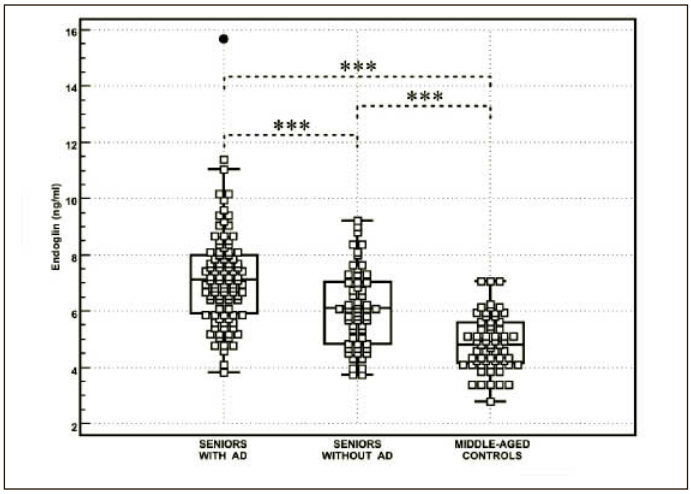
Comparisons of TGFβ levels - younger controls vs. able-bodied seniors, and able- bodied seniors vs. seniors with Alzheimer´s disease. A standard box-and-whisker plot (median, quartiles and range) was used



The serum level of soluble endoglin in the group of seniors with dementia (N = 129) was 7.20 ± 1.72 ng/ml (figure 2). Serum levels of soluble endoglin measured in the control group were 6.11 ± 1.38 ng/ml. The younger control group of healthy adult blood donors had endoglin level of 4.88 ± 0.95 ng/ml. Compared to younger control group of blood donors, both elderly groups had significantly elevated serum levels of endoglin (p<0.001). When compared to healthy seniors, seniors suffering from Alzheimer´s disease had significantly elevated serum levels of endoglin (p<0.001).

Figure 2

Comparisons of endoglin levels – younger controls vs. able-bodied seniors, and able-bodied seniors vs. seniors with Alzheimer´s disease. A standard box-and-whisker plot (median, quartiles and range) was used



Discussion

Our results indicate that aging is associated with an increase of endoglin levels in the serum, while Alzheimer´s disease is associated with decreased levels of TGFβ and further increase of endoglin levels. Ageing is accompanied by numerous changes in body physiology and individual immune responses. The immune system is skewed into immunopathological activity in association with ongoing inflammation and tissue remodelling. These processes are linked to endothelial dysfunction and vascular abnormalities which are frequently followed by the enhanced formation and shedding of markers of endothelial dysfunction in their soluble form into circulation. Soluble endoglin seems to be a prototypical example of such molecules.

TGFβ production is increased in the elderly population which is affected by atherosclerosis (8). TGFβ is also involved in CNS pathology. Here, TGFβ is suspected of involvement in the processing of amyloid precursor protein, plaques formation of both proinflammatory and antiinflammatory cytokines by microglia and astrocytes, and apoptosis of neuronal cells (2). Amyloid precursor protein processing is impaired in Alzheimer´s disease, leading to the formation of insoluble fragments of abnormal peptide β. Peptide β upregulates oxidative stress and the subsequent inflammatory reaction (2).

Alzheimer´s disease is characterized by neuronal loss, abnormal accumulation of some proteins, both extracellular and intracellular, and by activation of some immune mechanisms (10, 11). It seems plausible that sporadic form of Alzheimer´s disease is the result of combination of brain tissue damage and diminished trophic support of neurons, which leads to chronic injury to cells and their subsequent death. TGFβ is proposed by these authors as an important neuroprotective factor (10, 11).

In contrary overproduction of TGFβ and amyloid deposition were found in a transgenic mouse model (12). The brain vessels in these transgenic mice contained significantly higher levels of perlecane and fibronectin, and their basement membrane was thickened. Amyloid deposition started at 6 months and various neurodegenerative changes were identified in microvascular cells of brain from 9 months up to 18 months. Endothelial cells were thinner with numerous tiny protrusions and chromatine condensations. Pericytes were shown to cover a smaller area in the capillary lumen compared with those in normal mouse. These abnormalities resemble those found in Alzheimer´s disease brains. The authors have suggested that chronic overproduction of TGFβ can impair the basement membrane and results in „Alzheimer´s disease- like cerebrovascular amyloidosis and microvascular degeneration“(12).

The membrane glycoprotein endoglin (CD105) mediates effects of TGFβR. An overwhelming body of evidence implicating the role of endoglin in various inherited disorders, cancer, vascular pathology, and many others, has already been published (13-15). However, papers reporting its role in neurodegeneration are much less common. Endoglin seems to be a guardian for the maintenance of vascular integrity of the brain. Xu et al. (16) reported that excessive stimulation by vascular endothelial growth factor in the environment of a relative lack of endoglin, is responsible for brain blood - vessel malformation in the rat experimental model. The serum level of endoglin was significantly diminished in serum in patients suffering from amyotrophic lateral sclerosis (ALS) compared to healthy controls (17). The authors concluded that a decreased serum level of endoglin could be the reason for accelerated motoneuron degeneration in patients with ALS. Chronic ischemia caused by failed perfusion is hypothesized to be a putative cause of reduced vasculogenesis in ALS patients (17).

Whereas other neurodegenerative disorders such as ALS are associated with diminished levels of endoglin, endoglin levels are elevated in healthy seniors and this elevation is even higher in patients with Alzheimer´s disease. Elevation of endoglin levels could be related to microvascular amyloid deposits in Alzheimer´s disease that could lead to local ischemia. In agreement with that Dziewulska and Rafalowska (18) found enhanced expression of endoglin peripheral to the primary ischemic lesion together with other signs of postischemic tissue injury.

In conclusion, we propose that in patients with Alzheimer´s disease significantly decreased levels of the neuroprotective cytokine TGFβ can result in chronic impairment of brain microcirculation. This is reflected by the elevated serum level of endoglin that we have found in Alzheimer´s disease patients. The relevance of our finding to the immunopathogenesis of Alzheimer´s disease warrants further investigation.

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