Age-dependence of angiogenic activity of human serum

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Abstract

Human serum contains various factors able to modulate neovascularisation in mouse cutaneous angiogenesis test (SIA, serum induced angiogenesis). Among them are two important growth factors: vascular endothelial growth factor (VEGF) and interleukin 18

(IL-18). In our previous study, each of them, in doses comparable to concentration in human serum, presented pro-angiogenic activity after intradermal introduction into mice skin.

The aim of the present study was to evaluate total in vivo angiogenic activity as well as concentration of VEGF and IL-18 in sera collected from 60 healthy people of various age (20-86 years old), and to analyze whether some correlations exist between studied parameters and persons age.

We have found negative correlation between age and in vivo activity of serum, negative correlation between age and VEGF concentration and positive correlation between age and IL-18 level.

Key words: *healthy people, age, sera, angiogenesis, VEGF, IL-18.*

(Centr Eur J Immunol 2009; 34 (1): 53-56)

Introduction

Physiological angiogenesis is the essential process for human development. Pathological angiogenesis occurs in many diseases, such as age-dependent macular degeneration, diabetic retinopathy, rheumatoid arthritis and tumors. Many of these pathologies are age-dependent processes directly connected with disorders of the balance pro and anti-angiogenic factors. Among them two: vascular endothelial growth factor (VEGF) and interleukin 18 (IL-18) are important modulators of neovascularisation. In our previous study, each of them, in doses comparable to concentration in human serum, presented pro-angiogenic activity after introduction into mice skin in mouse cutaneous angiogenesis test (SIA, serum induced angiogenesis) [1, 2].

The aim of the present study was to evaluate total *in vivo* angiogenic activity as well as concentration of VEGF and IL-18 in sera collected from healthy people of various age (20-86 years old), and to analyze whether some correlation exists between studied parameters and persons age. Our results will be discuss on the field of interdisciplinary research.

Material and Methods

Sera were collected from 60 people (30 men and 30 women), 20-86 years old, without immunological, inflammatory and neoplastic diseases. Informed consent was obtained from each person and the study was approved by local Ethics Committee.

Serum-(SIA)-induced cutaneous angiogenesis assay

Cutaneous angiogenesis assay was performed according to Sidky and Auerbach method [3] with own modifications [4, 5]. Studies have been performed in 2-month old, female inbred Balb/c mice. Mice have been of local laboratory breed, weighing ca 20 g each. The sera of healthy subjects were injected intradermally (0.05 ml per one injection, 3-6 injections per mouse) into regionally shaved, anaesthetized with

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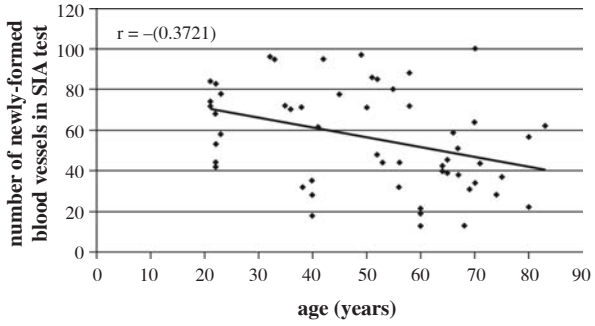

Fig. 1. Negative correlation between the age of healthy people and angiogenic activity of their sera

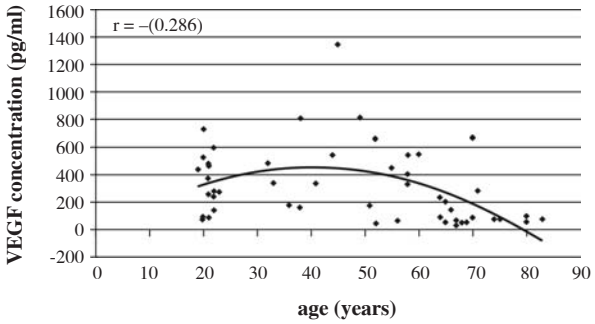

Fig. 2. Negative correlation between the age of healthy people and VEGF concentration of their sera

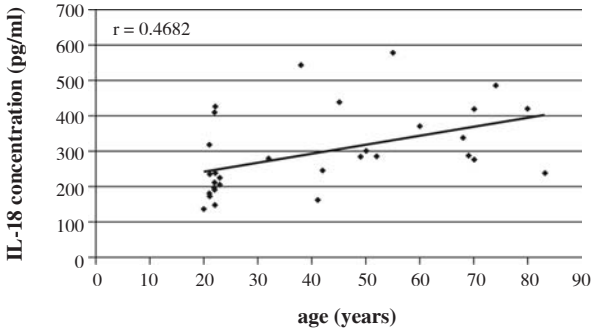

Fig. 3. Positive correlation between the age of healthy people and IL-18 content of their sera

chloral hydrate (POCH, Poland) groups of 3 or more mice. In order to facilitate the localization of injection sites later on, all injected samples were coloured with 0.1% of trypan blue. After 72 hours mice were killed with lethal dose of Morbital (Biowet, Poland). All newly formed blood vessels were identified and counted in dissection microscope in 1/3 central area of microscopic field, at 6 × magnification. Identification was based on the fact that newly-formed blood vessels differ from background vasculature by their small size, tortuosity and divarications. Mean number of newly-formed blood vessels was calculated from a dozen or so separate readings and designated as "angiogenic activity" of tested sample.

Experiments were approved and supervised by the Local Ethics Committee.

Measurement of VEGF and IL-18 concentration

Cytokine levels were determined in examined sera using sandwich ELISA kits (R&D Systems, USA) for human VEGF and IL-18, according to the producer instructions. Optical density was measured at 450 nm using spectrophotometric reader Elx800 (Biotek Instruments, Inc., USA). Cytokines concentration was expressed as pg/ml.

Statistical evaluation of the results was done by Pearson's correlation test.

Results

We have found negative correlation (r = -0.3721) between the age of healthy people and angiogenic activity of their sera (Fig. 1). We also observed negative correlation (r =-0.286) between the age of healthy people and VEGF concentration (Fig. 2) and positive correlation (r=0.4682) between the age of healthy people and IL-18 content of their sera (Fig. 3).

Discussion

On the basis of the results obtained in this study we have found, that our examined population of healthy people (persons 20 – 86 years old), presented the negative correlation between the age and angiogenic activity of their sera. This observation is in agreement with the results reported by other authors [6], considering that age – impairment of angiogenesis is a complicated process, involving lower basal NO release, decreased vasodilatation in response to acetylcholine and lower expression of VEGF. According to Hoenig et al. [7], ageing is associated with endothelial dysfunction, as well as decreased progenitor cells (EPC) function and mobilization. They suggest that lower angiogenic potential is related to depressed signaling by hypoxia inducible factor-1 (HIF-1). This factor is the main regulator of the expression of VEGF, stromal cell derived factor-1 (SDF-1) and CXC chemokine receptor-4 (CXC4). (SDF-1) and CXC are crucial regulators of progenitor cell function and homing.

Above facts have very important inter-disciplinary clinical meanings. There are interesting investigations on the field of orthopedics, focusing on influence of age on vascularisation during fracture repair [8], on the mouse model (4-weeks, 6-months and 18-months old mice). Data shows age affected vascularisation during fracture repair, and altered expression of biochemical factors involved in this process

(HIF-1, transcripts of VEGF and metalloproteinases 9 and 13). Authors concluded that enhancing vascularisation during fracture repair in the elderly may provide therapeutic opportunities.

In experimental *in vitro* model of angiogenesis in intervertebral discs tissues, Japan researchers [9] show that this process is regulated by VEGF and the NF-kappaB pathway. Both of them are induced by TNF-alpha and the level of angiogenic activity was closely related to aging. These facts are important in prognosis and management of resorption of discs in patients of different age.

Exercise-induced skeletal muscle angiogenesis experiments [10, 11] show that skeletal muscle capillarisation and VEGF expression are lower in aged compared with young men. However, exercise training increased muscle VEGF mRNA and protein and KDR mRNA independently of human age, thus the angiogenic response to aerobic exercise training is not altered during the ageing, and muscle exercise may play fundamental role in the maintenance of skeletal muscle capillarisation along the human life.

Interesting paper [12] described the effect of aging on tumor growth and angiogenesis. In mice experimental model of tumor growth two malignant cell lines (TRUMP-C2, prostate cancer and B16/F10 melanoma) were tested. VEGF levels (prostate cancer experimental group) were similar in the young and aged mice and no differences was observed in the level of angiogenesis, but tumors grew fast in aged as well as in young mice. The fastest growth of prostate tumors in this case was connected with higher levels of metalloproteineases MMP2/9 activity in intratumor matrix. In contrast, melanoma tumors grew minimally in the aged mice. These data show, that the effect of aging on tumor growth and angiogenesis depends on tumor-cell specific features and can by independent on VEGF regulation of angiogenesis. Another authors confirmed these observations [13].

Age-associated mechanisms of hearing loss are still unknown and not completely characterized. On the murine model of age-dependent hearing loss Piceotti [14] demonstrated that cochlear VEGF expression is significantly reduced as a function of age. It suggests that vascular abnormalities might play a role in this age-dependent process.

Human serum contains various factors able to induce or to suppress formation of new blood vessels. Among these angiomodulatory factors, interleukin-18 (IL-18), a recently described member of the IL-1 cytokine superfamily, is recognized as an important regulator of innate and acquired immune responses. IL-18 is expressed at sites of chronic inflammation, in autoimmune diseases, in cancers, and in the context of numerous infectious diseases. Formerly called interferon (IFN) gamma inducing factor (IGIF), IL-18 is the name of cytokine that plays an important role in the T-cell-helper type 1 (Th1) response, primarily by its ability to induce IFN gamma production in T cells and natural killer (NK) cells [15-17].

Hyperglycaemia increases inflammatory cytokines concentration in blood. Elevated levels of IL-18 were recently reported in patients with type 2 diabetes mellitus (DM2) and nephropathy.

In the present paper we observed positive correlation between the age of healthy people and IL-18 content of their sera. In our previous experiments [1], we examined IL-18 concentration in sera of elderly DM2 patients with non-proliferative retinopathy and age-matched control people, and estimated whether this cytokine plays pro- or anti-angiogenic role in *in vivo* angiogenic activity of their sera in mice cutaneous angiogenesis test. Recombinant human IL-18 injected intradermally to murine skin induced significant neovascular reaction. Our results showed that DM2 patients sera contained higher concentration of IL-18 and induced stronger neovascular reaction in mice skin than sera of corresponding control people. Sera from both groups of people after neutralization with anti-human IL-18 antibodies lost substantial part of their angiogenic activity.

These results agree with observations of authors who investigate the associations between serum IL-18 concentration and indices of lipid and carbohydrate metabolism in healthy adults. Olusi [18] showed that serum IL-18 concentration was positively correlated with serum triglyceride and glucose concentrations in both obese and diabetic subjects after controlling for the confounding effects of age, sex, and body mass index. IL-18 may be associated with obesity and glucose intolerance. Endogenous IL-18 signaling modulates food intake, metabolism, and adiposity during adulthood and might be a central or peripheral pharmacological target for controlling energy homeostasis [19]. There are, however, conflicting reports about IL-18 association in obesity, some authors [20] showed the lack of correlation of IL-18 with anthropometric, body composition variables and leptin in healthy population, what argues against a role of this cytokine in obesity.

Suchanek et al. [21] analyzed the serum level of interleukin 18 in coronary artery disease (CAD) patients with type 2 diabetes mellitus (DM), and related results to clinical findings. In the field of results they concluded: Type 2 DM predisposes patients, especially those with multi-vessel CAD who were smokers, to a higher serum level of IL-18, which may help explain their vulnerability to fatal, secondary cardiovascular events. These patients should be in the first line for stringent, secondary cardiovascular prevention.

Frayling [22] examined the role of common variation in the IL-18 gene on its serum concentrations and functioning in old age. IL-18 concentrations are associated with physical function in 65- to 80-year-olds. A polymorphism in the IL-18 gene alters IL-18 concentrations and is associated with an improvement in walk speed. IL-18 may play an active role in age-related functional impairment.

Osteoarthritis (OA) is closely related to the function of several inflammatory cytokines. Japan investigators [23] results suggest that high levels of serum IL-18 promote the

over-expression of endogenous IL-18 in articular chondrocytes, resulting in cartilage loss through suppression of aggrecan synthesis. Authors concluded, that higher serum levels IL-18 in older age, may play an important role in the pathogenesis of articular cartilage loss in osteoarthritis.

In wide context of pathology, our results showed that VEGF and IL-18 are very important cytokines for maintain homeostasis along whole human life.

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