Age-associated alterations of inflammatory and immune response, and oxidative stress in healthy and non-obese Koreans

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무엇보다 많이 지지해주시고 응원해주신 사랑하는 가족들, 믿어주시고 지켜봐주신 부모님과 형제자매들에게 감사드립니다. 마지막으로 항상 곁에서 힘이 되어 주는 남편과 챙겨주지 못해서 많이 미안하지만, 잘 자라고 있는 두 자녀 지원이 지우에게 고마운 마음과 사랑을 전합니다.

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이 동 희

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BMI	Body mass index
BP	Blood pressure
DC	Dendritic cell
HDL	High density lipoprotein
hs-CRP	High-sensitive C-reactive protein
IFN	Interferon
IL	Interleukin
LDL	Low density lipoprotein
NK	Natural killer
NKT	Natural killer thymus
PBMC	Peripheral blood mononuclear cell
T	Thymus
TC	Total cholesterol
TG	Triacylglycerol
Th	Thymus helper
TLR	Toll-like receptor
TNF	Tumor necrosis factor
8-epi-$PGF_{2\alpha}$	8-epi-prostaglandin $F_{2\alpha}$

ABSTRACT

Age-associated alterations of inflammatory and immune response, and oxidative stress in healthy and non-obese Koreans

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The objective of this study was to determine age-dependent alterations in serum cytokines, peripheral blood mononuclear cell (PBMC) cytokine production, natural killer (NK) cell activity and urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$). In total, 987 healthy and non-obese subjects was divided into five age groups: 20-34 (group 1, *n*=128), 35-44 (group 2, *n*=135), 45-54 (group 3, *n*=276), 55-64 (group 4, *n*=301), and 65-80 (group 5, *n*=147) years of age. After adjusted for body mass index, gender distribution, and smoking and drinking status, serum interferon (IFN)-γ levels decreased in group 3, 4 and 5 compared

with those in group 1, and 2. Production of IFN-γ by the unstimulated PBMCs was lower in the older groups (group 4 and 5) than in the younger groups (group 1 and 3). Serum interleukin (IL)-12 was lower in group 5 than in groups 1 and 2. In contrast, both serum and PBMC IL-6 were higher in group 5 than in group 1, 2, and 3. Urinary 8-epi-$PGF_{2\alpha}$ increased in group 3 compared with that in group 1 and further increased in group 5. Multiple linear regression analysis revealed that serum IFN-γ levels were negatively affected by age, and NK cell activity at a ratio of effector cell: target cell=5:1 was positively affected by PBMC IFN-γ. Our study shows the age-related reductions in serum and PBMC IFN-γ and serum IL-12, and age-related increase in serum and PBMC IL-6 and oxidative stress in healthy non-obese subjects. Additionally, circulating IL-6 levels may be a better marker of a chronic low-grade inflammatory activity associated with aging than systemic levels of high-sensitivity C-reactive protein, tumor necrosis factor-α, and IL-1β.

Keywords: Aging; Serum cytokine; PBMC cytokine; NK cell activity; Oxidative stress; Inflammation

1. INTRODUCTION

Aging is often associated with a dysregulation of the immune system [1] and oxidative stress [2]. Regulatory mechanisms involve a complex network of cytokines that are involved in differentiation, proliferation, and survival of lymphoid cells [7]. It has been suggested that the dysregulation of cytokines may be partly responsible for the increased morbidity and mortality and the subtle presence of infection in the elderly [3].

Previous studies have shown that several immune parameters, including the number of circulating thymus (T) lymphocytes and natural killer (NK) cell cytotoxicity, do not differ between generally healthy and well-nourished old and young women [4]. Additionally, a shift toward increased production of proinflammatory cytokines, such as tumor necrosis factor (TNF)-α and interleukin (IL)-6, has been reported with aging in the healthy older individuals compared to the younger control individuals [5, 6]. However, some reports are not consistent with increased production of proinflammatory cytokines, such as IL-1β and IL-6, and reduced production of IL-2 with aging when health and nutritional status are maintained [7]. These discrepancies may be a consequence of the selection criteria used for healthy elderly individuals, although other factors, such as experimental differences among the research groups and small sample size, must be considered. Since altered production of certain cytokines has been postulated to explain some of the age-related functional changes in the immune response, our interest was to examine the age-dependent alterations in serum cytokines, peripheral blood mononuclear

cell (PBMC) cytokine production, NK cell activity, and urinary 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$) in healthy and non-obese Koreans.

2. BACKGROUND

2.1 Immune system in aging

Aging leads to the deterioration of not only a defective T cell response but also the number and function of other cells of the innate immune system [11] and to the accumulation of cells with signal transduction defects.

Forming the first line of defense against virally infected and malignant cells, NK cells are critical effector cells of the innate immune system. With age, significant impairments have been reported in the two main mechanisms by which NK cells confer host protection: direct cytotoxicity and the secretion of immunoregulatory cytokines and chemokines. In elderly subjects, decreased NK cell activity has been shown to be associated with an increased incidence and severity of infection, highlighting the clinical implications that age-associated changes in NK cell biology have on the health of older adults.

IL-12 is known as a T cell-stimulating factor, which can stimulate the growth and function of T cells. It plays an important role in the activities of NK cells and T cell. IL-12 mediates enhancement of NK cells activity. Enhanced functional response is demonstrated by interferon (IFN)-γ production and killing of target cells. It stimulates the production of IFN-γ and TNF-α from T cells and NK cells.

2.1.1 Aging-associated adaptive immunity : T cells

The thymus is the key organ that directs T cell differentiation, maturation, and selection early in life, and T cells that are selected subsequently migrate to secondary lymphoid tissues where antigens can be surveyed when presented by antigen-presenting cells [12, 13, 14]. Once encountering a specific antigen that T cells recognize, the T cells can become activated, proliferate, and differentiate into effector T cells, a process that is facilitated by co-stimulatory molecules and cytokines. These T cells can rapidly proliferate and respond to antigen if it is again encountered.

T cells produce restricted cytokines and provide B cells, interacting with the innate immune system, and can mediate autoimmune responses. T cells have been classified as thymus helper (Th) 1, Th2 [12, 13] that is dependent on the milieu of cytokines which regulate Th1 differentiation [15]. These subset of T cell have different roles in defending against pathogens, Th1 cells inducing control of intracellular organisms via production of IFN-γ that activates macrophages. T cells also differentiate into other key immunoregulatory effector that can suppress or regulate inflammatory immune responses and acquire cytotoxic functions. When it is stimulated with antigen plus appropriate co-stimulation, such acquisition of cytotoxic functions with the expression of IFN-γ can be driven by exposure to cytokines such as IL-12 and is also regulated by transcription factors [16].

Aging-associated adaptive immunity means decrease of T cells. That means all of T cell receptor signaling, cell proliferation, cytokine production, and vaccine responses

declines with advancing age. One explanation for a decline in the maximal proliferation response of T cells in elderly individuals is age-associated telomere shortening [17, 18].

Lowering T-cell cytotoxicity responses has been linked to a warning ability to respond adequately to viral infections [19], and production of IL-2 and cell proliferation by virus-specific T cells. T cells were found to have a decline in their ability to suppress target cell proliferation in the elderly.

2.1.2 Aging-associated innate immunity: NK cells

Innate immune system includes neutrophils, monocytes, macrophages, dendritic cells (DC), basophils, mast cells, eosinophils, and NK cells. These cells are responsible for phagocytosis; the production of various inflammatory mediators; the activation of and interaction with adaptive immune responses and cell destruction and clearance. Although the innate immune system can lead to a strong inflammatory response independent of adaptive immunity, the recruitment of both systems provides a more robust defense, and DCs play a key role in the interactions between the innate and adaptive immune systems. Following endocytosis of foreign antigens in the periphery, DCs act as antigen-presenting cells which can travel to lymph nodes where they activate the T cell of the adaptive system [20].

Individual components of the innate immune system may undergo significant change with aging, though the innate system is generally understood to be better preserved in the aging adult, and these changes vary significantly between individuals. In one study of

adults older than age 85, it was shown that older adults who had an impaired innate immune response characterized by decreased production of inflammatory cytokines, and it had an associated twofold increase in overall mortality, even controlling for chronic illness [21]. Age-related decline in the innate system includes decreasing phagocytic capacity and oxidative burst in neutrophils and macrophages, decreased cytotoxicity of NK cells, and a decreased ability of DCs to find lymph nodes and stimulate T cells in vivo studies [22]. Other investigators have reported decreased NK cell cytotoxicity [23] and it reduced number and function of plasmacytoid DCs in blood [24] with advancing age. However, components of the innate system may increase with aging, such as the number of NK cells and serum levels of inflammatory mediators, cytokines [25].

Studies of NK cell numbers and functionality in the very elderly illustrate the virus-infected as well as tumor cells in a major histocompatibility complex-unrestricted manner regarding compensation with age. It might also indirectly modulate adaptive immunity via altered cytokine production affecting the activation of DCs and monocytes, and promoting inflammation [26].

2.1.3 Aging-associated immune dysregulation

Aging is accompanied by immune, hormonal, and adipose changes leading to a chronic inflammatory state. Immune dysregulation is reflected not only in diminished immunity with decreased response against pathogens, but also in increased inappropriate immune activation, leading to autoimmunity and a subsequent increase in the incidence of autoimmune diseases as well as possibly other detrimental manifestations.

Age-related changes with regard to lymphocytes, leads to a kind of dysregulation of cytokine production primarily from T cells, which results in chronic activating of the immune system and ‘inflammaging’ and can be explained as a compensation mechanism in the attempt to overcome alterations on cytokine receptor signaling [27].

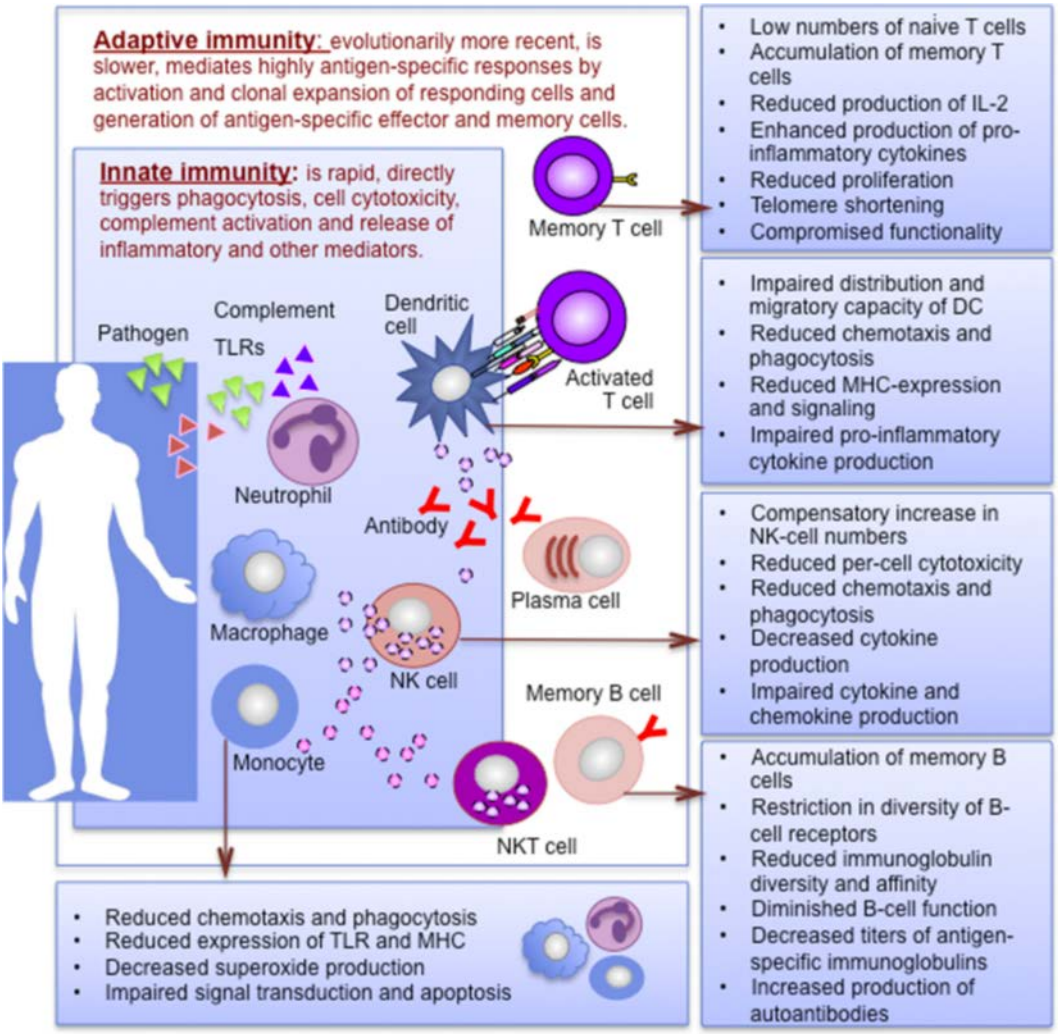

Figure 1. Impact of aging on innate and adaptive immunity [43]

2.2 Cytokines

Cytokines are small, nonstructural proteins with molecular weights ranging from 8 to 40,000 d. Originally called lymphokines and monokines to indicate their cellular sources, it became clear that the term “cytokine” is the best description, since nearly all nucleated cells are capable of synthesizing these proteins and in turn, of responding to them. There are presently 18 cytokines with the name IL [28]. Other cytokines have retained their original biological description, such as TNF-α.

Some cytokines clearly promote inflammation and are called proinflammatory cytokines, whereas other cytokines suppress the activity of proinflammatory cytokines and are called antiinflammatory cytokines. IFN-γ is another example of the pleiotropic nature of cytokines. Like IFN-α and IFN-β, IFN- γ possesses antiviral activity. IFN- γ is also an activator of the pathway that leads to cytotoxic T cells.

For the most part, cytokines are primarily involved in host responses to disease or infection, and any involvement with homeostatic mechanisms has been less than dramatic.

2.2.1 Cytokines in acute and chronic inflammation

Inflammation is mediated by a variety of soluble factors, including a group of secreted polypeptides known as cytokines. Several cytokines play key roles in mediating acute inflammatory reactions, namely IL-1, TNF-a, IL-6, IL-11.

Chronic inflammation may develop following acute inflammation and may last for weeks or months, and in some instances for years. During this phase of inflammation, cytokine interactions result in monocyte chemotaxis to the site of inflammation where macrophage activating factors, such as IFN-ɣ and other molecules then activate the macrophages while migration inhibition factors retain them at the inflammatory site. The macrophages contribute to the inflammatory process by chronically elaborating low levels of IL-1 and TNF which are responsible for some of the resulting clinical symptoms such as anorexia, cachexia, fever, sleepiness, and leukocytosis.

2.2.2 Proinflammatory cytokines

Cytokines represent the major factors involved in the communication between T cells, macrophages and other immune cells in the course of an immune response to antigens and infectious agents. Also cytokines are regulators of host responses to infection, immune responses, inflammation, and trauma. IL 12 is produced mainly by macrophages and its production can be upregulated by IFN-ɣ. These cytokines, originally described as simple antiviral substances, are now taken to be important regulators of the immune response. All this emphasizes the importance of macrophage-cytokine interactions in determining the type of immune response.

IL-1 and TNF-α are proinflammatory cytokines, and when they get into humans, they produce fever, inflammation, tissue destruction, and in some cases, even shock and death.

2.2.3 Interukin-6

IL-6 is a glycoprotein ranging from 21 to 28 kDa depending on the degree of post-translational modification. The IL-6 cDNA was cloned in 1986 and the gene encoding IL-6 was mapped to chromosome 7 in humans [29]. IL-6 is produced by a variety of cells including mononuclear phagocytes, T cells, B cells, neutrophils, adipocytes, glial cells, endothelial cells, chondrocytes, vascular smooth muscle cells and fibroblasts [29, 30, 31]. In addition to the stimulation of acute phase protein synthesis by the liver, IL-6 acts as a growth factor for mature B cells and induces their final maturation. It is involved in T cell activation and differentiation, and participates in the induction of IL-2 and IL-2 receptor expression. Some of the regulatory effects of IL-6 involve inhibition of TNF production, providing negative feedback for limiting the acute inflammatory response. Upregulation of IL-6 production has been observed in a variety of chronic inflammatory and autoimmune disorders such as thyroiditis, type I diabetes, rheumatoid arthritis [32, 33], systemic sclerosis [34], mesangial proliferative glomerulonephritis and psoriasis, and neoplasms such as cardiac myxoma, renal cell carcinoma, multiple myeloma, lymphoma, and leukemia [32].

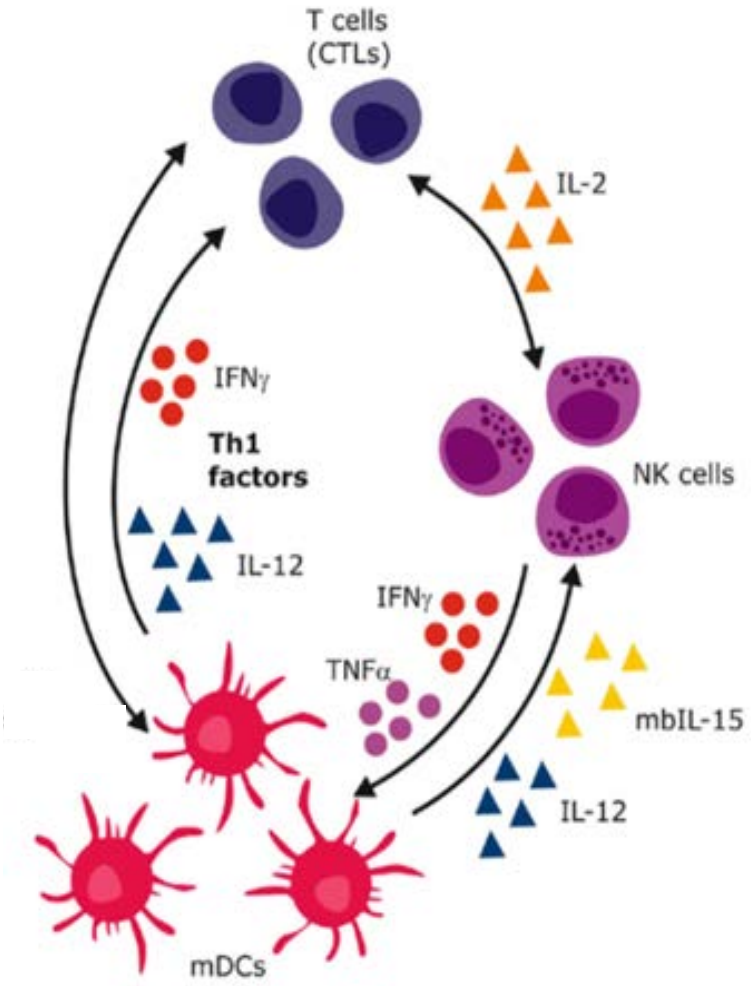

Figure 2. Cytokines in immune cells such as T cells, NK cells and DCs

NK cell engender dominant pro-inflammatory signaling through secretion of IFN-γ, Th1 cytokines, and activation of both innate and adaptive immune cells [74].

2.3 Inflammation in aging

Inflammation is the response of tissue to injury in the acute phase by increased blood flow and vascular permeability along with the accumulation of fluid, leukocytes, and inflammatory mediators such as cytokines. Age-associated inflammation may not only play a role in functional decline but may also increase susceptibility of the elderly. Increased susceptibility to infection caused by warning immune responses. Inflammatory responses to infection in the elderly may be greater in magnitude than those of younger individuals. These immune dysregulation in the elder individual associated with immunosenescence, as reflected by significantly higher level of high sensitive C reactive protein (hs-CRP) and soluble TNF receptors in elderly [35]. In additions, inflammatory process induces oxidative stress and reduces cellular antioxidant capacity.

2.3.1 Aging-associated oxidative stress with urinary 8-epi-$PGF_{2\alpha}$

Aging is often associated with oxidative stress. Cells and tissues are constantly exposed to reactive oxygen intermediates, and an imbalance between oxy-radicals and endogenous antioxidant defenses that characterized oxidative stress is one aspect of the aging process that can affect immune cell function. Urinary 8-epi-$PGF_{2\alpha}$ is produced in vivo by free radical-dependent peroxidation of lipid-esterified arachidonic acid. It is an accurate marker of basal oxidative stress [2].

2.3.2 Chronic low-grade inflammation

Aging is characterized by quantitative and qualitative changes of the immune system. This phenomenon is accompanied by cytokine dysregulation, which is an increase of pro-inflammatory cytokines and reduction of anti-inflammatory cytokines, leading to a chronic low-grade inflammatory state.

High levels of such IL as IL-6, IL-1, TNF-α, and hs-CRP are associated in elderly individuals with increased risk of morbidity and mortality [36]. In particular, cohort studies have indicated TNF-α and IL-6 levels as markers of frailty [37]. High plasma levels of the pro-inflammatory cytokine IL-6, correlated to a peculiar lipid profile, such as high plasma levels of lipoprotein-a [38]. In old people, high plasma levels of IL-6 are considered a marker of increased risk of frailty [39] and are associated with reduced muscle strength [40].

Age-related changes in the immune system and low-grade inflammation were shown to contribute to the risk of cardiovascular diseases, which play a major role in morbidity and mortality of old individuals. Age-associated dysregulation of inflammatory pathways also appears to affect the central nervous system and be involved in the pathophysiological mechanisms of neurodegenerative disorders, such as Alzheimer's disease [37].

The effects of cytokine dysregulation are independent of the other usual mortality risk factors (tobacco use, diabetes, arterial hypertension, hypercholesterolemia) and comorbid conditions.

3. MATERIALS AND METHODS

3.1 Study participants

Study participants were recruited from the Health Promotion Center of the Ilsan Hospital during routine health examination visits between February 2014 and June 2016. Based on data obtained from the health examination, subjects who met the study criteria and agreed to participate in the study were referred to the Department of Family Medicine. Participants completed a personal health and medical history questionnaire which served as a screening tool. The health of potential subjects was reassessed, and subjects who met the study criteria were ultimately enrolled. The exclusion criteria consisted of current and/or a history of hypertension, cardiovascular disease, diabetes mellitus, dyslipidemia, liver disease, renal disease, pancreatitis, or cancer; pregnancy or lactation; and the use of any medications or supplements. The aim of the study was carefully explained to all participants who provided their written informed consent. The Institutional Review Boards of Yonsei University and Ilsan Hospital approved the study protocol which complied with the Declaration of Helsinki.

In total, 987 healthy and non-obese subjects were enrolled in our study. The participants were divided into five age groups: 20-34 (group 1, n=128), 35-44 (group 2, n=135), 45-54 (group 3, n=276), 55-64 (group 4, n=301), and 65-80 (group 5, n=147) years of age.

3.2 Anthropometric parameters and blood pressures

Body weight (in lightweight clothes and without shoes) (UM0703581; Tanita, Tokyo, Japan) and height (GL-150; G-tech International, Uijeongbu, Korea) were measured in the morning, and BMI was calculated in units of kilograms per square meter (kg/m^2). Systolic and diastolic blood pressure (BP) levels were assessed in the supine position after a resting period (20 min). BP was measured twice on the left arm using an automatic BP monitor (FT-200S; Jawon Medical, Gyeongsan, Korea); the two measurements were averaged.

3.3 Blood and urine collection

Venous blood samples were collected following an overnight fast of at least 12 hours. The blood specimens for assessment of clinical characteristics were collected in ethylenediaminetetraacetic acid-treated tubes and plain tubes. The blood samples were centrifuged to obtain plasma and serum. The collected samples were stored at -80 °C until analysis.

Urine samples were collected in a polyethylene bottle containing 1% butylated hydroxytoluene following an overnight fast at least 12 hours. The bottles were immediately covered with aluminum foil and stored at -20 °C until further analysis.

3.4 Serum fasting lipid profiles

Fasting triacylglycerol (TG) and total-cholesterol (TC) levels were measured via enzymatic assay using TG and CHOL kits (Roche, Mannheim, Germany), respectively. High-density lipoprotein (HDL) cholesterol was measured via selective inhibition using a HDL-C plus kits (Roche, Mannheim, Germany) and the resulting color reaction was monitored with a Hitachi 7600 autoanlyzer (Hitachi, Tokyo, Japan). Low-density lipoprotein (LDL) cholesterol was indirectly calculated using the Friedewald formula, wherein LDL-cholesterol = TC – [HDL-cholesterol + (TG/5)].

3.5 Serum fasting glucose and insulin levels

Serum fasting glucose levels were measured via the hexokinase method using GLU kits (Roche, Mannheim, Germany). Serum fasting insulin levels were measured via an immunoradiometric assay using an Insulin IRMA kits (Diasource, Louvain, Belgium). The resulting color reactions were monitored with a Hitachi 7600 (Hitachi, Tokyo, Japan) for fasting glucose and with the SR-300 radioimmunoassay (Stratec, Birkenfeld, Germany) for insulin.

3.6 Serum albumin, hs-CRP, and urinary 8-epi-$PGF_{2\alpha}$ levels

Serum albumin concentrations were analyzed through the BCG method using an ALB kit (Roche, Mannheim, Germany) with a Hitachi 7600 autoanalyzer (Hitachi, Tokyo, Japan). Serum hs-CRP levels were measured using CRP kits (Roche, Mannheim, Germany) and the resulting colorimetric reaction was monitored with a Hitachi 7600 autoanalyzer (Hitachi, Tokyo, Japan). The urinary 8-epi-$PGF_{2\alpha}$ levels were measured using a Urinary Isoprostane ELISA kit (Oxford Biomedical Research Inc., Rochester Hills, MI, USA), and urinary creatinine levels were determined with the Jaffe method (alkaline picrate reaction).

3.7 Isolation of PBMCs

To measure cytokine levels in PBMC supernatants and assess of NK cell activity, PBMCs were isolated from whole blood samples. Whole blood samples were mixed with the same volume of RPMI 1640 (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), gently overlaid onto histopaque (Sigma-Aldrich, Irvine, UK) and then centrifuged (20 min, 1800 rpm, 15°C). After separation, a thin layer of buffy coat was isolated and washed twice with RPMI 1640. The pellet was resuspended in RPMI 1640 supplemented with penicillin/streptomycin (Gibco, Thermo Fisher Scientific, Waltham, MA, USA). The isolated PBMCs were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (Gibco, Thermo Fisher Scientific, Waltham, MA, USA), seeded into 12-well plates (1.0×10^6 cells/ml), and incubated at 37°C under 5% CO_2 for no more than 46 h ± 30 min. After

incubation, the supernatants were collected and stored at -80°C.

3.8 Cytokine assay in serum and PBMC supernatants

IFN-γ levels in serum and PBMC supernatants were measured with an IFN-γ High Sensitivity Human ELISA Kit (Covalab, Villeurbanne, France) and IL-12 levels in serum and PBMC supernatants were analyzed by a High-Sensitivity Human IL-12 (P70) ELISA kit (Genway Biotech Inc., San Diego, CA, USA) according to the manufacturer's instructions. The color reaction were read at 450 nm using a Victor x5 2030 multilabel plate reader (PerkinElmer, Norwalk, CT, USA). IL-6, IL-1β, and TNF-α levels in serum and PBMC supernatants were measured using the Bio-Plex™ Reagent Kit (Bio-Rad Laboratories, Hercules, CA, USA).

3.9 NK cell activity

The isolated PBMCs from the whole blood samples were incubated with K562 cells to analyze NK cell cytotoxic activity. As mentioned in the isolated PBMCs section, the same steps were conducted to isolate PBMCs from the whole blood sample, except that an isolated buffy coat layer was washed once with serum free media and then resuspended in 1 mL of serum free media. The isolated PBMCs (effector cell, E) were seeded into 96-well plates at ratios of 10:1, 5:1, and 1.25:1 with the K562 cells (2.0×10^4 cells/well) (target cell, T) and then incubated at 37°C under 5% CO_2 for more than 4 hours. The cytolytic activities

of NK cells were analyzed via the CytoTox 96® Non-Radioactive Cytotoxicity Assay Kit (Promega Co., Fitchburg, WI, USA) according to the manufacturer's instructions. The color reactions were read at 490 nm using a Victor x5 2030 multilabel plate reader (PerkinElmer, Norwalk, CT, USA), and the results were calculated by the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{Experimental-Effector Spontaneous-Target Spontaneous}}{\text{Target Maximum-Target Spontaneous}} \times 100$$

3.10 Statistical analysis

Statistical analysis was performed using SPSS version 23.0 (IBM, Chicago, IL, USA). Logarithmic transformation was performed on skewed variables. For descriptive purposes, the mean values are presented as untransformed values. The results are expressed as the means ± standard error. A two-tailed *P*-value <0.05 was considered statistically significant. One-way analysis of variance (ANOVA) with the Bonferroni correction was used to test for age-group effects. A general linear model analysis was also performed with adjustment for BMI, gender distribution, and smoking and drinking status. Multiple linear regression analyses were performed to identify major independent predictors of serum IFN-γ levels and NK cell activity. Pearson's correlation coefficient was used to examine relationships between variables. A heat map was created to visualize and evaluate relationships among variables in the study population.

4. RESULTS

4.1 Clinical and biochemical characteristics according to age group

We examined samples from 987 healthy non-obese subjects from 20 to 80 years of age to elucidate the impact of age on serum cytokines, PBMC cytokine production, NK cell activity, and oxidative stress. The study subjects were divided into five age groups: 20-34 (group 1, *n*=128), 35-44 (group 2, *n*=135), 45-54 (group 3, *n*=276), 55-64 (group 4, *n*=301), and 65-80 (group 5, *n*=147). BMI (group 1: 25.1 ± 0.27, group 2: 24.2 ± 0.26, group 3: 23.8 ± 0.16, group 4: 24.0 ± 0.14, group 5: 23.5 ± 0.17 kg/m^2; *P* <0.001), gender distribution, and smoking and drinking status were significantly different across the age groups. Table 1 and Table 2 shows the clinical and biochemical characteristics in the 5 age groups. Systolic BP was higher in group 4 than in group 1, 2 and 3 and was even higher in group 5. Diastolic BP was higher in group 4 and 5 than in groups 1 and 2. Serum TG was higher in group 5 than in groups 1 and 2. TC and LDL cholesterol were higher in groups 3 and 4 than in groups 1 and 2, but decreased in group 5. HDL cholesterol was lower in group 5 than in group 1, 2, 3, and 4. Serum glucose was higher in groups 4 and 5 than in groups 1 and 2. Serum albumin was higher in group 1 than in groups 2,3,4 and 5. Serum hs-CRP did not show any significant difference among the age groups, however, after adjusting BMI, gender distribution, and smoking status, and significant difference was observed among the 5 groups. Serum levels of IL-1β and TNF-α did not significantly differ among the age

groups (Table 2).

Table 1. Clinical and biomedical characteristics in healthy non-obese subjects according to the age group

	Group 1	Group 2	Group 3	Group 4	Group 5	*P*-value
	20-34 (*n*=128)	35-44 (*n*=135)	45-54 (*n*=276)	55-64 (*n*=301)	Over 65 (*n*=147)	
Age (year)	27.8±0.32^{e}	40.6±0.29^{d}	49.9±0.20^{c}	59.3±0.19^{b}	68.8±0.28^{a}	<0.001
Systolic BP (mmHg)	112.8±1.27^{d}	114.4±1.15^{d}	118.6±0.80^{c}	122.0±0.76^{b}	126.2±1.10^{a}	<0.001
Diastolic BP (mmHg)	68.5±0.90^{c}	73.2±0.81^{b}	76.1±0.57^{a}	77.2±0.54^{a}	77.5±0.78^{a}	<0.001
Triglyceride (mg/dL)$^{\phi}$	98.3±7.26^{b}	113.5±6.15^{a,b}	126.5±4.22^{a}	121.6±3.96^{a}	129.3±5.71^{a}	<0.001
Total-cholesterol (mg/dL)$^{\phi}$	194.1±3.58^{b}	201.7±3.03^{b}	218.4±2.08^{a}	220.4±1.95^{a}	202.7±2.82^{b}	<0.001
LDL-cholesterol (mg/dL)$^{\phi}$	60.6±1.38^{a}	56.6±1.17^{a,b}	57.5±0.80^{a,b}	57.5±0.75^{a}	53.5±1.09^{b}	0.001
HDL-cholesterol (mg/dL)$^{\phi}$	114.7±3.28^{b}	122.4±2.77^{b}	136.0±1.90^{a}	138.5±1.78^{a}	123.4±2.56^{b}	<0.001
Glucose (mg/dL)$^{\phi}$	82.3±1.03^{c}	85.5±0.87^{b,c}	88.3±0.60^{a,b}	90.0±0.56^{a}	90.4±0.81^{a}	<0.001
Insulin (μIU/dL)$^{\phi}$	13.0±0.55^{a}	11.1±0.47^{a,b}	9.94±0.32^{c}	9.36±0.30^{c}	10.3±0.43^{b,c}	<0.001
hs-CRP (mg/L)$^{\phi}$	1.00±0.18	0.92±0.15	0.96±0.10	1.09±0.10	0.96±0.14	0.026
Serum albumin (mg/dL)$^{\phi}$	4.73±0.04^{a}	4.61±0.03^{a,b}	4.59±0.02^{b}	4.63±0.02^{a,b}	4.55±0.03^{b}	0.002
IL-1β (pg/mL)$^{\phi}$	0.91±0.13	0.74±0.11	0.92±0.07	0.88±0.07	0.88±0.12	0.681
TNF-α (pg/mL)$^{\phi}$	6.19±1.78	8.28±1.51	11.7±0.99	9.84±0.99	8.69±1.56	0.164

Mean ± SE. $^{\phi}$ were tested by logarithmic transformation. One-way ANOVA was used to calculate P^{a}-values. P^{b}-values adjusted for BMI, gender distribution, and smoking and drinking status. All alphabetical letters $P<0.05$ were derived from Bonferroni post hoc tests; no significant differences are marked with the same letter, and significant differences are marked with a different letter.

4.2 Serum IFN-γ, IL-12, IL-6, and urinary 8-epi-$PGF_{2\alpha}$ according to age group

Figure 3 shows the mean value of serum IFN-γ, IL-12, IL-6, and urinary 8-epi-$PGF_{2\alpha}$ according to age group. Serum IFN-γ significantly was significant lower in groups 3, 4 and 5 than in groups 1 and 2 (Figure 3). Serum IL-12 was significantly lower in group 5 than in group 1 and 2. In contrast, serum IL-6 was significantly higher in group 5 than in groups 1, 2, and 3. Urinary 8-epi-$PGF_{2\alpha}$ was significantly higher than in group 3 than in group 1 and was even higher in group 5 (Figure 3).

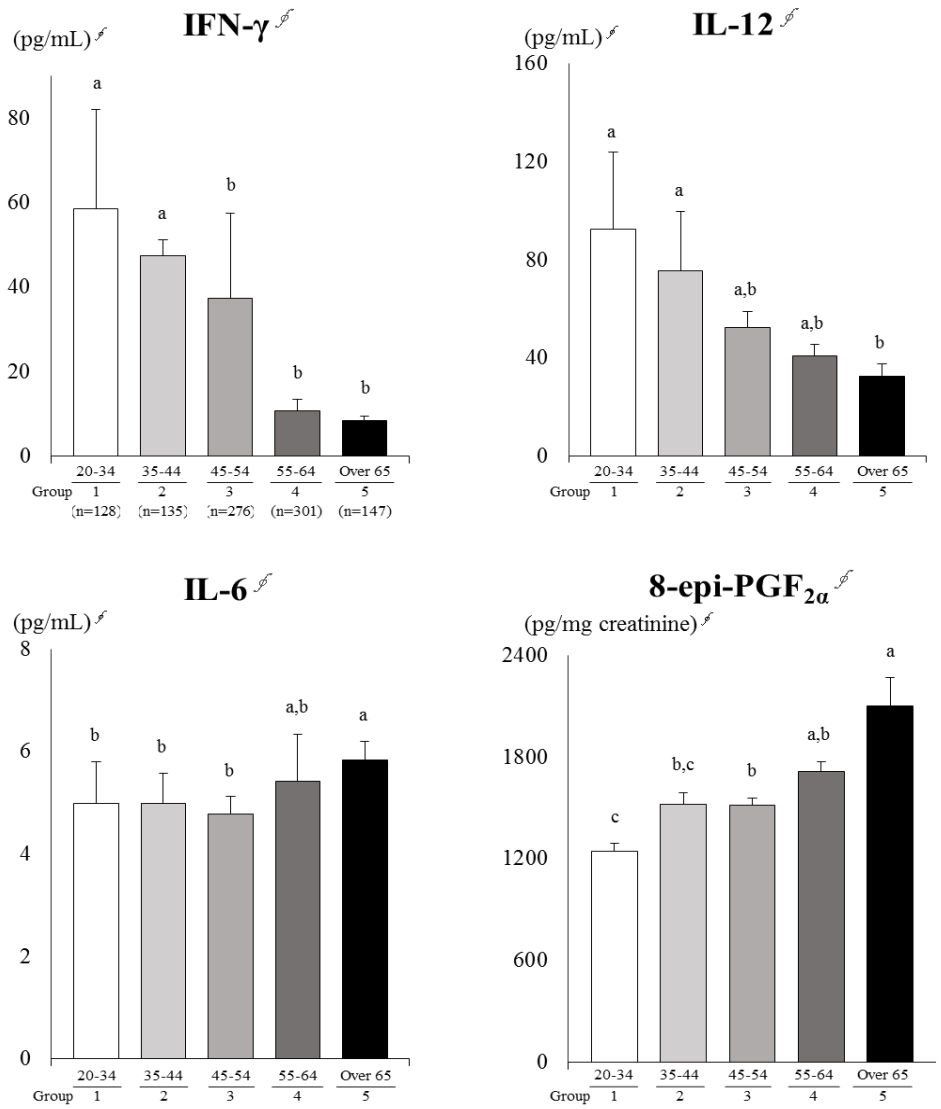

Figure 3. Serum IFN-γ, IL-12, IL-6, and urinary 8-epi-PGF$_{2\alpha}$ levels in healthy non-obese subjects according to age group

Mean ± SE.[§] were tested using logarithmic transformation. One-way ANOVA was used to calculate *P*-values. *P*-values adjusted for BMI, gender distribution, and smoking and drinking status. All alphabetical letters $P<0.05$ were derived from Bonfferoni post hoc tests; no significant differences are marked with the same letter, and significant differences are marked with a different letter.

4.3 PBMC cytokine production and NK cell activity according to age group

Table 2 also provides the mean value of the cytokines produced by cultured PBMCs and NK cell activity according to age group. Non-stimulated PBMC from subjects in the oldest group (group 5; age 65-80 years) secreted significantly higher amounts of IL-6 into the culture media than those from the younger groups (group 1, 2, and 3; age 20-54 years). Production of IFN-γ by the unstimulated PBMCs was significantly lower in the older groups (group 4 and 5; age 55-80 years) than in the younger groups (group 1 and 2; age 20-44 years). Secretion of IL-12 from the unstimulated PBMCs was significantly differ between group3 and 4. However, the production of IL-1β, TNF-α by the unstimulated PBMCs did not statistically differ among the age groups (Table2). NK cell activity (%) was measured based on the Effector cell:Target cell ratios of 10:1, 5:1, or 1.25:1. There were no significant differences in NK cell activity among the age groups (Table 2).

Table 2. Cytokine production by non-stimulated PBMCs, and NK cell activity in healthy non-obese subjects according to the age group

	Group 1	Group 2	Group 3	Group 4	Group 5	*P*-value
	20-34 (*n*=128)	35-44 (*n*=135)	45-54 (*n*=276)	55-64 (*n*=301)	Over 65 (*n*=147)	
Nonstimulated PBMCs						
IL-1β (pg/mL)[ϕ]	1.12±0.66	0.69±0.54	1.24±0.36	2.03±0.35	2.06±0.59	0.860
IL-6 (pg/mL)[ϕ]	19.5±21.5^{b}	13.0±17.0^{b}	20.6±11.4^{b}	46.9±11.1^{b}	68.4±18.7^{a}	<0.001
TNF-α (pg/mL)[ϕ]	8.76±8.97	3.27±7.09	3.75±4.85	6.55±4.67	27.9±7.96	0.821
IFN-γ (pg/mL)[ϕ]	2.02±0.93^{a,b}	1.80±0.48^{a}	1.69±0.32^{b}	1.52±0.34^{a,b}	0.86±0.56^{a,b}	0.036
IL-12 (pg/mL)[ϕ]	6.00±2.33^{a,b}	4.70±1.20^{b}	4.94±0.81^{b}	5.09±0.86^{a}	4.26±1.40^{a}	<0.001
NK cell activity						
E:T=10:1 (%)[ϕ]	28.2±4.37	22.9±3.03	24.5±2.42	26.8±1.93	23.2±2.35	0.214
E:T=5:1 (%)[ϕ]	17.9±3.23	19.2±2.24	15.8±1.79	18.7±1.43	14.6±1.74	0.089
E:T=1.25:1 (%)[ϕ]	15.6±3.50	15.8±2.43	15.6±1.94	16.5±1.54	14.2±1.88	0.709

Mean ± SE. [ϕ] were tested by logarithmic transformation. One-way ANOVA was used to calculate P^{a}-values. P^{b}-values adjusted for BMI, gender distribution, and smoking and drinking status. All alphabetical letters $P<0.05$ were derived from Bonferroni post hoc tests; no significant differences are marked with the same letter, and significant differences are marked with a different letter.

4.4 Correlations among age, serum albumin and serum cytokines, PBMC cytokines, NK cell activity and urinary 8-epi-$PGF_{2\alpha}$

Figure 4 shows correlation matrix among age, serum albumin, serum cytokines, PBMC cytokines, NK cell activity at a ratio of E:T=5:1, and urinary 8-epi-$PGF_{2\alpha}$. Age positively correlated with serum IL-6, serum TNF-α, urinary 8-epi-$PGF_{2\alpha}$, and PBMC IL-6 and negatively with serum albumin, serum IFN-γ, serum IL-12, PBMC IFN-γ and NK cell activity. Serum albumin positively correlated with NK cell activity and negatively with urinary 8-epi-$PGF_{2\alpha}$, PBMC IL-6, and PBMC TNF-α. Serum IL-1β positively correlated with serum IL-6, serum TNF-α, and serum IFN-γ. Serum IL-6 positively correlated with serum TNF-α, urinary 8-epi-$PGF_{2\alpha}$, PBMC IL-1β, and PBMC IL-6. Serum TNF-α positively correlated with urinary 8-epi-$PGF_{2\alpha}$ and PBMC TNF-α and negatively with PBMC IFN-γ. Serum IL-12 positively correlated with PBMC IFN-γ. Urinary 8-epi-$PGF_{2\alpha}$ negatively correlated with NK cell activity. PBMC IL-1β positively correlated with PBMC IL-6, and PBMC TNF-α. PBMC IL-6 positively correlated with PBMC TNF-α and PBMC IFN-γ. PBMC IFN-γ positively correlated with NK cell activity (Figure 4).

Multiple linear regression analysis was performed to identify the major clinical factors associated with the serum IFN-γ levels (dependent variable) that had a key role in both adaptive and innate immunity. Age, BMI, gender, smoking, drinking, serum albumin, serum IL-1β, serum IL-6, serum TNF-α, serum IL-12, PBMC IL-1β, PBMC IL-6, PBMC TNF-α, PBMC IFN-γ, PBMC IL-12, and NK cell activity were

defined as independent variables. In all subjects, serum IFN-γ levels were negatively affected by age (r^2=0.286, *P*=0.020). We next determined the independent effects of the following variables on NK cell activity that was a representative in innate immunity : age, BMI, gender, smoking, drinking, serum albumin, serum IL-1β, serum IL-6, serum TNF-α, serum IFN-γ, serum IL-12, PBMC IL-1β, PBMC IL-6, PBMC TNF-α, and PBMC IFN-γ. In all subjects, NK cell activity was positively affected by PBMC IFN-γ (r^2=0.211, *P*=0.030).

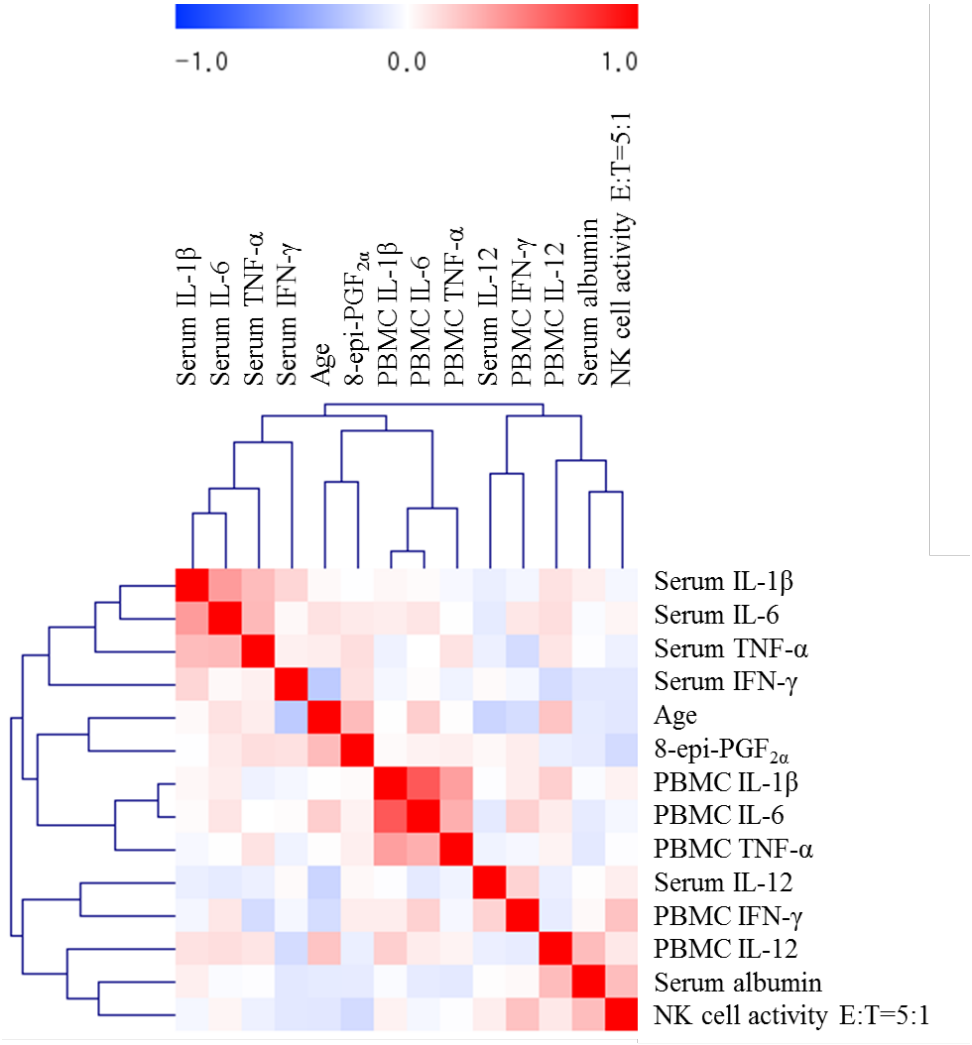

Figure 4. Correlation matrix among age, serum albumin, serum cytokines, PBMC cytokines, NK cell activity (E:T=5:1), and urinary 8-epi-$PGF_{2\alpha}$

Correlations were obtained using *Pearson*'s correlation coefficient. *Red* indicates a positive correlation and *Blue* indicates a negative correlation.

5. DISCUSSION

We examined samples from 987 healthy non-obese subjects from 20 to 80 years of age to elucidate the impact of age on serum cytokines, PBMC cytokine production, and NK cell activity. In our study, circulating level of IFN-γ, a potent immune-stimulatory cytokine [41], were lower in healthy, non-obese and older subjects (age 45-80 years), without significant changes in the circulating levels of TNF-α or NK cell activity, than in younger subjects (20-44 years). Similarly, PBMC IFN-γ was lower in older subjects (55-80 years) than in younger subjects (20-44 years). Additionally, multiple linear regression analysis revealed that circulating IFN-γ was independently and negatively correlated with age. Furthermore, NK cell activity was independently and positively correlated with PBMC IFN-γ.

PBMCs consist of monocytes and lymphocytes including T cells, B cells, and NK cells [42]. Particularly, NK cells are involved in the early defense against foreign cells and autologous cells undergoing various forms of stress, such as microbial infection or tumor transformation [44]. Upon stimulation, NK cells secrete large amounts of cytokines, including IFN-γ and TNF-α [11],[43, 44, 45]. However, a recent study underscores the complexity of the cytokine-induced functional activities of NK cells and illustrated the differential response of NK cells from the younger to the elderly under cytokine stimulation [46]. For example, an age-related decrease in IFN-γ but not TNF-α in our study supported the previous observation that IFN-γ production is lower in the elderly than in the young individuals, but TNF-α

production in the elderly was not significantly different from that in the young [11],[43, 44]. Indeed, early secretion of IFN-γ in response to IL-12 or IL-2 has been reported to be lower in the elderly than in the young people. However, aging does not significantly alter other NK cell functions, such as TNF-α production. NK cells are also cytotoxic, for example inducing apoptosis of cells recognized as targets [44]. The finding that NK cell activity was similar among the age groups in our study is consistent with the previous findings that NK cell cytotoxicity does not differ healthy and well-nourished old and young subjects [4],[7],[11].

Endogenous IL-12 contributes to NK cells-dependent IFN-γ production [11], [47]. In the present study, circulating IL-12 was positively correlated with PBMC IFN-γ production. Additionally, circulating levels of IL-12 were decreased in the oldest (65-80 years) compared to those in younger subjects (20-44 years). IL-12 which is produced by macrophages, DCs and other antigen presenting cells, is a pivotal cytokine with multiple immunoregulatory properties that stimulates Th1 cytokines [7],[47]. Th cells are divided into two functional subclasses, Th1 and Th2 cells, based upon the cytokines that they produce and their effects on cell-mediated and humoral immunity [48]. Th1 cells produce IL-2, IFN-γ, TNF-α, and IL-12 and enhance cell-mediated immunity. On the other hand, Th2 cells produce IL-4, IL-5, IL-6, and IL-10 and up-regulate humoral immunity.

Proinflammatory cytokines such as IL-1β, IL-6 and TNF-α are increased in the elderly and secreted by macrophages, lymphocytes, NK cells, and vascular smooth muscle cells [6],[49]. Our study also showed an age-related increases in serum IL-6

and PBMC IL-6 levels. The age-related increase in circulating IL-6 levels, but not in IL-1β or TNF-α, or hs-CRP levels, observed in our study is consistent with the findings from previous studies for healthy population [6]; in terms of hs-CRP, statistical significance was likely to be showed after adjusting BMI, gender distribution, and smoking and drinking status, however, any significant differences were not observed between each ae group when we conducted Bonferroni post hoc test. Therefore, circulating IL-6 levels may be a better marker of chronic low-grade inflammatory activity in aging individuals, particularly in the oldest group (65-80 years), than systemic levels of IL-1β, TNF-α and hs-CRP. The improved correlation between age and IL-6 could explain that locally produced IL-1β and TNF-α do not escape into the circulation, even though they have a strong systemic IL-6 response [6]. Moreover, IL-1β and TNF-α are known to stimulate IL-6 production, while IL-6 influences the synthesis of IL-1β and TNF-α [50]. Thus, the levels of these cytokines may be directly associated with one another. Indeed, our present findings confirm the previous report of a strong intercorrelation among IL-6, IL-1β, and TNF-α in serum or PBMC [6]. These results supports the activation of a myriad of interconnected proinflammatory cytokine networks.

A role of inflammation and oxidative stress has been suggested in the pathogenesis of aging and several age-associated disorders, including atherosclerosis, osteoporosis, and dementia [51, 52, 53]. Oxidative stress occurs when free radical generation exceeds the system's ability to neutralize and eliminate the free radicals [22, 23]. Free radicals may activate nuclear factor-kB and trigger an inflammatory

cascade, which induces more free radical production; thus, a vicious cycle is created [54]. As in a previous study [5], the urinary 8-epi-$PGF_{2\alpha}$ levels, a biomarker of oxidative stress [55], was higher in individuals aged 45-54 years than in individual aged 20-34 years and even higher in individuals aged of 65-80 years. Additionally, the urinary 8-epi-$PGF_{2\alpha}$ level was positively correlated with serum IL-6 and TNF-α and negatively with serum albumin and NK cell activity.

In summary, our study reported age-related reductions in serum and PBMC IFN-γ and serum IL-12 and age-related increases in serum and PBMC IL-6 and oxidative stress in healthy nonobese subjects. Specially, circulating IFN-γ was independently and negatively correlated with age and NK cell activity was independently and positively correlated with PBMC IFN-γ. Additionally, circulating IL-6 levels could be a better marker of a chronic low-grade inflammatory activity associated with aging than systemic levels of hs-CRP, TNF-α and IL-1β.

6. CONCLUSIONS

Our study shows the age-related reductions in serum and PBMC IFN-γ and serum IL-12 and age-related increases in serum and PBMC IL-6 and oxidative stress in healthy nonobese subjects. Additionally, circulating IL-6 levels may be a better marker of the chronic low-grade inflammatory activity associated with aging than systemic levels of hs-CRP, TNF-α and IL-1β.

REFERENCES

1. Welzl K, Kern G, Mayer G, Weinberger B, Säemann MD, Sturm G, Grubeck-Loebenstein B, Koppelstaetter C. Effect of different immunosuppressive drugs on immune cells from young and old healthy persons. Gerontology. 2014; 60:229-238.
2. Lessiani G, Santilli F, Boccatonda A, Iodice P, Liani R, Tripaldi R, Saggini R, Davì G. Arterial stiffness and sedentary lifestyle: Role of oxidative stress. Vascular Pharmacology. 2016; 79:1-5.
3. Alvarez-Rodríguez L, López-Hoyos M, Muñoz-Cacho P, Martínez-Taboada VM. Aging is associated with circulating cytokine dysregulation. Cellular Immunology. 2012; 273:124-132.
4. Deanna K, Andrea MM, Gordon H, Helen SW, Mary P, Namanjeet A. Immune function did not decline with aging in apparently healthy, well-nourished women. Mechanisms of Ageing Development. 1999; 112:43-57.
5. Paik JK, Chae JS, Kang R, Kwon N, Lee SH, Lee JH. Effect of age on atherogenecity of LDL and inflammatory markers in healthy women. Nutrition, Metabolism & Cardiovascular Disease. 2013; 23:967-972.
6. Kim OY, Chae JS, Paik JK, Seo HS, Jang Y, Cavaillon JM, Lee JH. Effects of aging and menopause on serum interleukin-6 levels and peripheral blood mononuclear cell cytokine production in healthy nonobese women. Age. 2012; 34:415-425.

7. Namanjeet A, Andrea MM, Rick B, Mary PM, Roshni R, Gordon H. Cytokine production by stimulated mononuclear cells did not change with aging in apparently healthy, well-nourished women. Mechanisms of Ageing and Development. 2001; 122:1269-1279.
8. Heremans H, Dillen C, van Damme J, Billiau A. Essential role for natural killer cells in the lethal lipopolysaccharide-induced Shwartzman-like reaction in mice. Journal of Immunology. 1994; 24:1155-1160.
9. Roy S, Pavitrakar D, Gunjikar R, Ayachit VM, Bondre VP, Sapkal GN. Monocytes and B cells support active replication of Chandipura virus. BMC Infectious Diseases. 2016; 16:487.
10. Solana R, Alonso MC, Pen J. Natural killer cells in healthy aging. Experimental Gerontology. 1999; 34:435-443.
11. Rafael S, Erminia M. NK and NK/T cells in human senescence. Vaccine. 2000; 18:1613-1620.
12. Lee N, Shin MS, Kang I. T-cell biology in aging, with a focus on lung disease. Journals of Gerontology Series A: Biological Sciences and Medical Sciences. 2012; 67:254-263.
13. Goronzy JJ, Li G, Yu M, Weyand CM. Signaling pathways in aged T cells : A reflection of T cell differentiation, cell senescence and host environment. Seminars in Immunology. 2012; 24:365-372.

14. Fulop T, Larbi A, Wikby A, Mocchegiani E, Hirokawa K, Pawelec G. Dysregulation of T-cell function in the elderly: scientific basis and clinical implications. Drugs Aging. 2005; 22:589-603.
15. Oestreich KJ, Weinmann AS. Transcriptional mechanisms that regulate T helper 1 cell differentiation. Current Opinion in Immunology. 2012; 24:191-195.
16. Arens R, Schoenber SP. Plasticity in programming of effector and memory CD8 T-cell formation. Immunological Review. 2010; 235:190-205.
17. Weng NP, Hathcock KS, Hodes RJ. Regulation of telomere length and telomerase in T and B cells: a mechanism for maintaining replicative potential. Immunity. 1998; 9:151-157.
18. Effros RB. Telomere/telomerase dynamics within the human immune system: effect of chronic infection and stress. Experimental Gerontology. 2011; 46:135-140.
19. Treanor J, Falsey A. Respiratory viral infections in the elderly. Antiviral Research. 1999; 44:79-102.
20. Janeway Jr C, Medzhitov R. Innate immune recognition. Annual Review of Immunology. 2002; 20:197-216.
21. Van den Biggelaar AH, Huizinga TW, de Craen AJ, Gussekloo J, Heijmans BT, Frölich M. Impaired innate immunity predicts frailty in old age. The Leiden 85-plus study. Experimental Gerontology. 2004; 39:1407-1414.
22. Singh U, Devaraj S, Jialal I. Vitamin E, oxidative stress, and inflammation. Annual Review of Nutrition. 2005; 25:151-174.

23. Shoelson SE, Lee J, Yuan M. Inflammation and the IKK beta/I kappa B/NF-kappa B axis in obesity- and diet-induced insulin resistance. International Journal of Obesity and Related Metabolic Disorder. 2003; 27:S49-52.
24. Weiskopf D, Weinberger B, Grubeck-Loebenstein B. The aging of the immune system. Transplant International. 2009; 22:1041-1450.
25. Solana R, Pawelec G, Tarazona R. Aging and innate immunity. Immunity. 2006; 24:491-494.
26. Solana R, Tarazona R, Gayoso I, Lesur O, Dupuis G, Fulop T. Innate immunosenescence: effect of aging on cells and receptors of the innate immune system in humans. Seminar in Immunology. 2012; 24:331-341.
27. Fulop T, Larbi A, Douziech N, Levesque I, Varin A, Herbein G. Cytokine receptor signaling and aging. Mechanism of Ageing and Devlopment. 2006; 127:526-537.
28. Charles A, Dinarello MD. Proinflammatory Cytokines. CHEST Journal. 2000; 118:503-508.
29. Van Snick J. Interleukin-6: an overview. Annual Review of Immunology. 1990; 8:253-278.
30. Hirano T. The biology of interleukin-6. Chemical Immunology and Allergy. 1992; 51:153-180.
31. Hirano T, Taga T, Matsuda T, Hibi M, Suematsu S, Tang B, Murakami M, Kishimoto T. Interleukin 6 and its receptor in the immune response and hematopoiesis. The International Journal of Cell Cloning. 1990; 8:155-166.

32. Hirano T. Interleukin-6 and its relation to inflammation and disease. Clinical Immunology and Immunopathology. 1992; 62:S60-65.
33. Tan PLJ, Farmiloe S, Yeoman S, Watson JD. Expression of the interleukin 6 gene inrheumatoid synovial fibroblasts. Journal of Rheumatology. 1990; 17:1608-1612.
34. Feghali CA, Bost KL, Boulware DW, Levy LS. Mechanisms of pathogenesis in scleroderma. I. Overproduction of IL-6 by fibroblasts cultured from affected skin sites of patients with scleroderma. Journal of Rheumatology. 1992; 19:1207-1211.
35. Bruunsgaard H, Skinhoj P, Qvist J, Pedersen BK. Elderly humans show prolonged in vivo inflammatory activity during pneumococcal infections. Journal of Infectious Diseases. 1999; 180:551-554.
36. Jenny. Inflammation in aging: cause, effect, or both? Discovery Medicine. 2012; 13:451-460.
37. Michaud M, Balardy L, Moulis G, Gaudin C, Peyrot C, Vellas B. Proinflammatory cytokines, aging, and age-related diseases. Journal of American Medical Directors Association. 2013; 14:877-882.
38. Baggio G, Donazzan S, Monti D, Mari D, Martini S, Gabelli C, Dalla VM, Previato L, Guido M, Pigozzo S, Cortella I, Crepaldi G, Franceschi C. Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors. The FASEB Journal. 1998; 12:433-437.

39. Ershler WB, Keller ET. Age-associated increased interleukin-6 gene expression, late-life diseases, and frailty. Annual Review of Medicine. 2000; 51:245-270.
40. Barbieri M, Ferrucci L, Ragno E, Corsi A, Bandinelli S, Bonafe M, Olivieri F, Giovagnetti S, Franceschi C, Guralnik JM, Paolisso G. Chronic inflammation and the effect of IGF-I on muscle strength and power in older persons. American Journal of Physiology. Endocrinology and Metabolism. 2003a; 284: E481-E487.
41. Heremans H, Dillen C, van Damme J, Billiau A. Essential role for natural killer cells in the lethal lipopolysaccharide-induced Shwartzman-like reaction in mice. Journal of Immunology. 1994; 24:1155-1160.
42. Roy S, Pavitrakar D, Gunjikar R, Ayachit VM, Bondre VP, Sapkal GN. Monocytes and B cells support active replication of Chandipura virus. BMC Infectious Diseases. 2016; 16:487.
43. Matt K, George M. 2015. Handbook of the Biology of Aging. Edition No.8. Handbooks of Aging : Academic Press
44. Thierry W, Marc D, Scott HR, Laurence Z, Eric V. Natural-killer cells and dendritic cells: "l'union fait la force". Blood. 2005; 106:2252-2258.
45. Panda A, Arjona A, Sapey E, Bai F, Fikrig E, Montgomery RR, Lord JM, Shaw AC. Human innate immunosenescence: causes and consequences for immunity in old age. Trends Immunology. 2009; 30:325-333.
46. Nogusa S, Murasko DM, Gardner EM. Differential effects of stimulatory factors on natural killer cell activities of young and aged mice. Journal of Gerontology. 2012; 67:947-954.

47. Zhang Y1, Wang Y, Gilmore X, Xu K, Mbawuike IN. Independent and synergistic effects of interleukin-18 and interleukin-12 in augmenting cytotoxic T lymphocyte responses and IFN-gamma production in aging. Journal of Interferon and Cytokine Research. 2001; 21:843-850.
48. Naira MP, Kandaswami C, Mahajan S, Chadhac KC, Chawda R, Naira H, Kumard N, Naira RE, Schwartza SA. The flavonoid, quercetin, differentially regulates Th-1 (IFNɣ) and Th-2 (IL4) cytokine gene expression by normal peripheral blood mononuclear cells. Biochimica et Biophysica Acta. 2002; 1593:29-36.
49. Tousoulis D, Oikonomou E, Economou EK, Crea F, Kaski JC. Inflammatory cytokines in atherosclerosis: current therapeutic approaches. European Heart Journal. 2016; 37:1723-1735.
50. Bruunsgaard H, Pedersen M, Pedersen BK. Aging and proinflammatory cytokines. Current Opinion in Hematology. 2001; 8:131-136.
51. Martocchia A, Stefanelli M, Falaschi GM, Toussan L, Ferri C, Falaschi P. Recent advances in the role of cortisol and metabolic syndrome in age-related degenerative diseases. Aging clinical and Experimental Research. 2016; 28:17-23.
52. Lahousse L, Moyse E, Krantic S, Brusselle GG. Eur RJ. Understanding age-related diseases: report of the 2015 Ageing Summit. European Respiratory Journal. 2016; 47:5-9.

53. Barnes PJ. Mechanisms of development of multimorbidity in the elderly. European Respiratory Journal. 2015; 45:790-806.
54. Federico A, Morgillo F, Tuccillo C, Ciardiello F, Loguercio C. Chronic inflammation and oxidative stress in human carcinogenesis. International Journal of Cancer. 2007; 121:2381-2386.
55. Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. Free Radical Biology and Medicine. 2000; 28:505-513.
56. Passeri G, Pini G, Troiano L, Vescovili R, Sansoni P, Passeri M, Gueresi P, Del signore R, Pedrazzoni M, Franceschi C. Low vitamin D status, high bone turnover, and bone fractures in centenarians. The Journal of Clinical Endocrinology and Metabolism. 2003; 88:5109-5115.
57. Claudio F, Miriam C, Daniela M, Sergio G, Fabiola O, Federica S, Maria PP, Laura I, Laura C, Maria S, Elisa C, Gastone C, Castellani, Stefano S. Inflammaging and anti-inflammaging: A systemic perspective on aging and longevity emerged from studies in humans. Mechanisms of Ageing and Development. 2007; 128:92-105.
58. Hansen S, Sun L, Baptiste KE, Fjeldborg J, Horohov DW. Age-related changes in intracellular expression of IFN-c and TNF-a in equine lymphocytes measured in bronchoalveolar lavage and peripheral blood. Developmental and comparative immunology. 2013; 39:228-233.
59. Martin M, Laurent B, Guillaume M, Clement G, Caroline P, Bruno V, Matteo C, Fati N. Review. Proinflammatory Cytokines, Aging, and Age-Related

Diseases. Journal of the Americal Medical Directors Association. 2013; 14:887-882.

60. Miller RA. The aging immune system: Primer and prospectus. Science. 1996; 273:70-74.
61. Bauer ME. Chronic stress and immunosenescence: A review. Neuroimmunomodulation. 2008; 15:241-250.
62. Fried LP, Tangen CM, Walston J. Frailty in older adults: Evidence for a phenotype. Journal of Gerontology Series A: Biological Sciences and Medical Sciences. 2001; 56:M146-M156.
63. Alley DE, Crimmins E, Bandeen-Roche K. Three-year change in inflammatory markers in elderly people and mortality: The Invecchiare in Chianti study. Journal of American Geriatrics Society. 2007; 55:1801-1807.
64. Bruunsgaard H, Andersen-Ranberg K, Hjelmborg JB. Elevated levels of tumor necrosis factor alpha and mortality in centenarians. American Journal of Medicine. 2003; 115:278-283.
65. Ferrucci L, Harris TB, Guralnik JM. Serum IL-6 level and the development of disability in older persons. Journal of American Geriatric Society. 1999; 47:639-646.
66. Harris TB, Ferrucci L, Tracy RP. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. American Journal of Medicine. 1999; 106:506-512.

67. Volpato S, Guralnik JM, Ferrucci L. Cardiovascular disease, interleukin-6, and risk of mortality in older women: The women's health and aging study. Circulation. 2001; 103:947-953.
68. Reuben DB, Cheh AI, Harris TB. Peripheral blood markers of inflammation predict mortality and functional decline in high-functioning community dwelling older persons. Journal of American Geriatric Society. 2002; 50:638-644.
69. Walston JD, Matteini AM, Nievergelt C. Inflammation and stress-related candidate genes, plasma interleukin-6 levels, and longevity in older adults. Experimental Gerontology. 2009; 44:350-355.
70. Bruunsgaard H, Ladelund S, Pedersen AN. Predicting death from tumour necrosis factor-alpha and interleukin-6 in 80-year-old people. Clinical Experimental Immunology. 2003; 132:24-31.
71. Franceschi C, Bonafè M, Valensin S, Olivieri F, De Luca M, Ottaviani E. Inflamm-aging. An evolutionary perspective on immunosenescence. Annals of the New York Academy of Sciences. 2000; 908:244-254.
72. Jing Y, Shaheen E, Drake RR, Chen N, Gravenstein S, Deng Y. Aging is associated with a numerical and functional decline in plasmacytoid dendritic cells, whereas myeloid dendritic cells are relatively unaltered in human peripheral blood. Human Immunology. 2009; 70:777-784.
73. Gomez CR, Boehmer ED, Kovacs EJ. The aging innate immune system. Curr Opin Immunol. 2005; 17:457-462.

74. Gras Navarro A, Bjorklund AT, Chekenya M. Therapeutic potential and challenges of natural killer cells in treatment of solid tumors. Frontier in Immunology. 2015; 6:202.

국문요약

건강한 한국인을 대상으로 연령에 따른 염증과 면역반응 그리고 산화스트레스 변화에 대한 연구

노화가 진행되면 면역시스템의 이상조절로 인해서 만성적인 염증이 생기고 이것이 노인성 질환을 일으킨다. 본 연구에서는 면역과 관련된 INF-γ, PBMC production, IL-6, TNF-α 그리고 NK cell 활성 등이 연령에 따라 어떻게 변화하는지 살펴보았다. 987명의 건강한 정상체중의 성인에 대하여 BMI, 성별, 음주와 흡연 여부를 보정 후 나이를 기준으로 5개의 그룹: 20-34세 (group 1, N=128), 35-44세 (group 2, n=135), 45-54세 (group 3, n=276), 55-62세 (group 4, n=301), 65-90세 (group 5, n=147)으로 구분하였다. Serum INF-γ 와 PBMC INF-γ 는 고연령군에서 저연령군보다 낮은 수준으로 확인되었고 Serum IL-12도 고연령군에서 낮게 관찰되었다. 반면에 Serum IL-6와 PBMC IL-6는 저연령군보다 고연령군에서 높게 관찰되었다. 또한 산화스트레스 수준을 반영하는

urinary 8-epi-$PGF_{2\alpha}$ 도 고연령군에서 높게 관찰되었으며 이들은 유의적인 차이를 보였다. 다중회귀분석 결과, PBMC IFN-γ 는 NK cell 활성을 증가시키는 것으로 확인되었다. 결론적으로 건강한 정상체중의 성인에게서 연령에 따라 IFN-γ 와 PBMC IFN-γ 는 감소하는 경향이 있고 IL-6와 PBMC IL-6 그리고 산화스트레스는 증가하였다. 또한 만성적인 염증에 대해서는 전체적인 수준의 hs-CRP, TNF-α, IL-1β 보다 circulating IL-6 가 더 나은 지표일 수도 있다.

핵심되는 말: 노화, Serum cytokine; PBMC cytokine, NK cell activity, Oxidative stress, Inflammation