JACOBS PUBLISHERS

OPEN ACCESS

Jacobs Journal of Gerontology

Research Article

Ageing-Associated Inflammation (Inflammageing) : Multiplex Cytokine Analysis In Healthy Japanese Individuals

Makoto Goto[1,2,3], Koichiro Hayata[2], Junji Chiba[2], Masaaki Matsuura[4,5], Sachiko Iwaki-Egawa[6],Yasuhiro Watanabe[6]

[1]*Department of Rheumatology, Nerima Hikarigaoka Hospital, 2-11-1 Hikarigaoka, Nerima-Ku, Tokyo 179-0072, Japan*

[2]*Department of Orthopaedics & Rheumatology, East Medical Center, Tokyo Women's Medical University, 2-1-10 Nishi-Ogu, Arakawa-Ku, Tokyo 116-0011, Japan*

[3]*Division of Anti-ageing and Longevity Sciences, Department of Medical Technology, Faculty of Medical Engineering, Toin University of Yokohama 1614 Kurogane-Cho, Aoba-Ku, Yokohama 225-8503, Japan*

[4]*Department of Cancer Genomics, Cancer Institute, Japanese Foundation for Cancer Research*

3-8-31 Ariake, Koto-Ku, Tokyo 135-8550, Japan

[5]*Graduate School of Public Health, Teikyo University 2-11-1 Kaga, Itabashi-Ku, Tokyo 173-8605, Japan*

[6]*Department of Life Sciences, School of Pharmacy, Hokkaido Pharmaceutical University 7-1 Katuraoka –Cho, Otaru 047-0264, Japan*

**Corresponding author: Dr. Makoto Goto, Department of Orthopedics & Rheumatology, East Medical Center, Tokyo Women's Medical University 2-1-10 Nishi-Ogu, Arakawa-Ku, Tokyo 116-0011, Japan, Tel: 81-03-3810-2900 ; Fax: +81-03- 3979-3868; E-mail: werner.goto@gmail.com*

Received: 08-092015

Accepted: 09-02-2015

Published: 09-05-2015

Abstract

Ageing is tightly associated with minor and life-long environmental insults, leading to a chronic and systemic inflammation, named "inflammageing". We reported the healthy ageing-associated elevation of highly sensitive CRP (hsCRP) in Japanese individuals aged between 1 and 100 years and the patients with Werner syndrome in the previous paper. To further study the association of hsCRP and 26 cytokines/chemokines, a multiple cytokine array system was used in the same serum samples as were examined for hsCRP from healthy Japanese adults.

The serum levels of IL-4, IL-6, IL-13, IL-15, GM-CSF, IFNγ, IP-10 (CXCL10) and TNFα were significantly elevated, though IL-2, IL-8 (CXCL8) and MIP-1α(CCL3) levels were significantly decreased with healthy ageing. Elevated hsCRP level was significantly associated with IL-6, IL-13, IL-15 and IP-10, while IL-8 and MIP-1α were negatively associated with hsCRP. Among these cytokines/chemokines, both IL-6 and IL-13 levels were significantly associated with serum level of hsCRP, if age and sex were taken into account.

Immunological shift to Th2 with healthy ageing may stimulate a pro-inflammatory cytokine/chemokine circuit, leading to a systemic chronic inflammation monitored by hsCRP. Further study may warrant the pathophysiology of Th2 shift and Th2-biased mild inflammation in healthy ageing (inflammageing).

Keywords: Ageing; chemokine; CRP; cytokine; inflammageing

Abbreviations

FGF: Fibroblast Growth Factor;

G-CSF: Granulocyte Colony-Stimulating Factor;

GM-CSF: Granulocyte-Monocyte Colony- Stimulating Factor;

hsCRP: Highly Sensitive CRP;

IFN : Interferon;

IL: Interleukin;

IL1ra : IL-1 Receptor Antagonist;

IP-10 (CXCL10): Ifnγinducible Protein 10;

MCP-1 (CCL2): Monocyte Chemoattractant Protein-1;

MIP-1 (CCL3/4): Macrophage Inhibitory Protein -1;

PDGF: Platelet Derived Growth Factor;

TNF: Tumor Necrosis Factor;

VEGF: Vascular Endothelial Growth Factor

Introduction

Human ageing is inevitably accompanied by an increasing chance of environmental attack from inside (such as mutants, endoplasmic reticulum (ER) stress and by-products associated with immune- surveillance activity) and outside (such as ultra violet light, air pollution, allergens, infectious agents, drugs and foods), leading to a minor inflammation that has been evaluated by highly sensitive CRP (hsCRP) [1, 2].

Chronic elevation of hsCRP in the healthy elderly population may tightly associated with the ageing-related conditions including diabetes mellitus, sarcopenia, osteoporosis, cancer, atherosclerosis, cognitive decline and finally death [3]. The ageing-associated chronic, low grade/asymptomatic and systemic inflammation, named 'inflammageing', is probably caused by an imbalance between an increase in pro- and a decrease in anti-inflammatory cytokines/chemokines [4-7].

Inflammation is widely recognized as a patho-physiologically fundamental metabolism to generate energy with thermogenesis, leading to wound healing or tissue destruction/repair during healthy development and ageing [8].

Ageing-associated changes of pro/anti-inflammatory cytokines/chemokines have been reported by using ELISA and multiplex technology. However, the results are conflicting. Ageing-associated elevation of pro-inflammatory cytokines/chemokines including interleukin (IL)-6, IL-8 (CXCL8), tumor necrosis factor (TNF)-α, macrophage inhibitory protein (MIP)-1-α(CCL3) and monocyte chemoattractant protein (MCP)-1 (CCL2) was reported by Mariani et al [9]. However, both Shurin et al [10] and Kim et al [11] described no ageing-associated changes of these cytokines/chemokines. Shurin et al [10] reported a significant age-associated increase of interferonγ inducible protein (IP)-10 (CXCL10) and eotaxin (CCL11). Elevation of IL-6, MCP-1 and IP-10 was described by Inadera et al [12] and Antonelli et al [13].

We have reported in the previous study a significant ageing-associated increase in the serum level of hsCRP in the healthy Japanese individuals and the patients with Werner syndrome [1].

The aim of this study was to clarify the association of 26 cytokine/chemokine levels examined by multiplex assay with healthy ageing, and also with the ageing-associated increase of hsCRP by directory comparing serum hsCRP levels and 26 cytokines/chemokines by using the same serum samples obtained from apparently healthy Japanese volunteers.

Materials and Methods

Study population

All the samples studied in the present experiment were the same sera as were used in the previous hsCRP study [1]. A total of 113 normal serum samples from both sexes (M=41, F=72) aged between 25 and 100 years were used for the study. The normal individuals, enjoying the usual daily life at home or nursing home, had neither apparent inflammatory diseases including infection, cancer, lymphoproliferative disorders, diabetes mellitus, Alzheimer's disease, autoimmune diseases and arthritis at the time of serum sampling, nor history of cardio-/cerebro- vascular accidents. Exclusion protocol for elderly individuals met the SENIEUR criteria [14].

All of the individuals provided written informed consent for this study, which was approved by the ethics committee of Toin University of Yokohama. All of the samples were stored at -80°C until use.

Multiplex cytokine array system

Serum levels of 26 cytokines/chemokines including interleukin (IL)-1β, IL-1 receptor antagonist (ILra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-12, IL-13, IL-15, IL-17, basic fibroblast growth factor (FGF), granulocyte-colony stimulating factor (G-CSF), granulocyte-macrophage- colony stimulating factor (GM-CSF), platelet derived growth factor (PDGF), vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF)α, interferon (IFN)-γ, and chemokines including IL-8 (CXCL8), IFNγ-induced protein (IP)-10 (CXCL10), monocyte chemoattractant protein (MCP)-1 (CCL2), macrophage inhibitory protein (MIP)-1α(CCL3), MIP-1β(CCL4), eotaxin (CCL11) were simultaneously measured using commercially-available bead-based immunofluorescence Bio-Plex Suspension Array System (BioRad; Hercules, CA) according to the manufacturer's instruction.

Briefly, six distinct sets of fluorescently dyed beads loaded with captured monoclonal antibodies specific for each cytokine/chemokine to be tested, were used. Serum samples (50ul/well of fourfold diluted serum) or standards (50ul/well) were incubated with 50ul of premixed bead sets into the pre-wetted 96 well microtiter plates at 4°C. After incu-

bation and washing, 25ul of fluorescent detection antibody mixture was added for 30 min and then the samples were washed and resupended in assay buffer.

High standard curves for each soluble factor were used. The low standard curves were obtained by tenfold diluted high standard. The formation of different sandwich immune-complexes was obtained by using the Bio-Plex Pro Human Cytokine multiplex Assay (Bio-Rad; Hercules, CA). A 50ul volume was sampled by each well and the fluorescent signal of a minimum of 100 beads per region (cytokine/chemokine) was evaluated and recorded. Values presenting a coefficient of variation beyond 10% were discarded before the final analysis.

Determination of hsCRP

The data of hsCRP used in this study was obtained in the previous experiment[1] by using CircuLex high-sensitivity CRP ELISA kit (MBL Woburn, MA) according to the user's manual.

Data analysis and statistics

We examined ageing-associated changes of serum levels of cytokine/chemokine using regression analyses expressed as log_e(cytokine/chemokine (*j*))= *a* + *b** Age, where *a* is an estimated intercept, *b* is an estimated regression coefficient for Age and *j* is an indicator for individual cytokine/chemokine. To examine the relationship between serum levels of hsCRP and cytokine/chemokine, we performed regression analyses expressed as log_e(hsCRP) = *a* + *b**log_e(cytokine/chemokine (*j*)), where *a* is an estimated intercept, *b* is an estimated regression coefficient and *j* is an indicator for individual cytokine/chemokine. Multiple regression models were used to further examine the relationship between hsCRP and cytokines /chemokines with adjustment of sex and age effects on the serum levels. The model was expressed as log_e (hsCRP) = $a + b_1$*Age + b_2*Sex + b_3*log_e (cytokine/ chemokine (*j*)), where *a* (intercept), b_1, b_2 and b_3 are estimated regression coefficients and *j* is an indicator for individual cytokine/chemokine. We used Akaike's Information Criterion (AIC) [15] for model selection between models with original data and models with log-transformed values (not shown). We show only results based on models with log-transformed values described above because they were better than models with original data. Statistical language R was used for the analyses. P-values < 0.05 were considered to be statistically significant [16].

Serum cytokine/chemokine data were analyzed using the Bio-Plex manager software version 5.0 (Bio-Rad; Hercules, CA). Standard levels between 70 and 130% of the expected values were considered to be accurate and were used. In general, at least six standards were accepted and used to establish standard curves following a five-parameter logistic regression model (5PL), Sample concentrations were immediately interpolated from the standard curves. Values were expressed as pg/ml and presented as mean±SE.

Subheadings: Multiplex cytokine analysis of inflammageing

Results

Ageing-associated changes of cytokine/chemokine

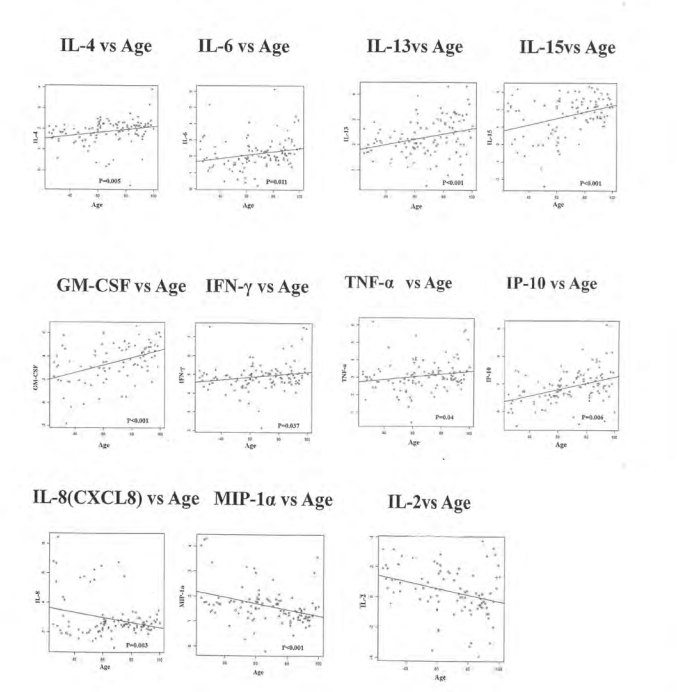
Inflammation monitored by the serum level of hsCRP was significantly associated with healthy ageing, as shown in the previous report [1]. No significant gender difference was observed concerning to the age-associated increase in hsCRP level.

Single regression analyses shown in Table 1 and supplementary Fig 1-1 indicated significant positive associations of age with IL-4, IL-6, IL-13, IL-15, GM-CSF, IFN-γ,TNF-αand IP-10, respectively. IL-8, MIP-1αand IL-2 were significantly negatively associated with ageing (supplementary Fig 1-2). The rest of the cytokine/chemokine levels examined did not change significantly with ageing. There was no significant gender difference concerning to the serum levels of 26 cytokines/chemokines examined in the present study.

Table 1. Significant ageing-associated changes of cytokine/chemokine.

Cytokines/chemokines	Estimated intercept	Estimated regression coefficient	SE	P value
IL-4	1.367	0.007	0.002	0.005**
IL-6	1.435	0.010	0.004	0.011*
IL-13	1.564	0.011	0.003	<0.001***
IL-15	0.377	0.018	0.005	<0.001***
GM-CSF	1.303	0.032	0.007	<0.001***
IFN-γ	4.431	0.007	0.003	0.037*
TNF-α	2.563	0.008	0.004	0.04*
IP-10 (CXCL10)	6.099	0.012	0.002	<0.001***
IL-8 (CXCL8)	4.010	-0.017	0.006	0.003**
MIP-1α(CCL3)	2.453	-0.012	0.003	<0.001***
IL-2	1.915	-0.022	0.007	0.003**

Regression analyses expressed as log_e(cytokine/ chemokine(*j*)) = *a* + *b**Age were indicated, where *a* is an estimated intercept, b is an estimated regression coefficient and *j* is an indicator for individual cytokine/chemokine.



Supplementary Figure 1. is inserted to better understand the data.

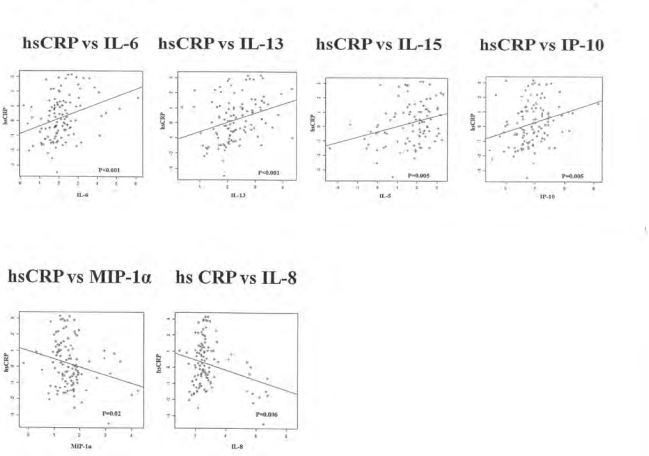
hsCRP- associated changes of cytokine/chemokine

Single regression analyses shown in Table 2 and supplementary Fig 2-1 indicated significant positive associations between serum level of hsCRP and IL-6, IL-13, IL-15, and IP-10, respectively. In contrast, the serum hsCRP level showed significantly negative associations with respective 2 chemokines: IL-8 and MIP-1α (supplementary Fig 2-2).

Table 2. hsCRP-associated significant changes of cytokine/chemokine.

Cytokines/chemokines		Estimated intercept	Estimated regression coefficient	SE	P value	N
IL-6		-0.873	0.505	0.142	<0.001***	112
IL-13		-1.210	0.615	0.177	<0.001***	112
IL-15		-0.372	0.373	0.129	0.005**	95
IP-10	(CXCL10)	-4.327	0.658	0.226	0.005**	112
IL-8	(CXCL8)	1.081	-0.31	0.112	0.006**	112
MIP-1α	(CCL3)	0.981	-0.496	0.201	0.02*	110

Regression analyses expressed as log_e(hsCRP)= a + b*log_e(cytokine/ chemokine(j)), where a is an estimated intercept, b is estimated regression coefficient and j is an indicator for individual cytokine/chemokine.



Supplementary Fig.2. is inserted to better understand the data.

Association of cytokine/chemokine with serum hsCRP and ageing

Using multiple regression models, temporal effect of age on the serum level of hsCRP was determined. Table 3 showed estimated regression coefficients with SE, p-values and variance analyses. The ANOVA indicated that all models fit the data well.

Two cytokines: IL-6 and IL-13 were significantly associated with hsCRP and healthy ageing.

The relationship between cytokine/chemokine, hsCRP and healthy ageing was ; log_e (hsCRP) = -2.596 + 0.031 x Age + 0.052 x Sex + 0.321 x log_e (IL-6), and log_e (hsCRP) = -2.756 + 0.031 x Age + 0.08 x Sex + 0.359 x log_e (IL-13), respectively. In these formulae, Sex was 1 for male and 0 in female. No sex difference was observed concerning to the ageing associated changes of 26 cytokines/chemokines examined.

Table 3. Association of serum levels of cytokine/chemokine with hsCRP and ageing.

Dependent variable	Independent variables	Estimated regression coefficient	SE	P value
hsCRP	Intercept	-2.596	0.483	<0.001***
	Age	0.031	0.006	<0.001***
	Sex	0.052	0.250	0.836
	IL-6	0.321	0.133	0.017*
hsCRP	Intercept	-2.756	0.533	<0.001***
	Age	0.031	0.006	<0.001***
	Sex	0.080	0.253	0.751
	IL-13	0.359	0.169	0.036*

Multiple regression model used to examine the relationship between hsCRP and multiple cytokines/chemokines with adjustment of sex and age effects on the serum levels was expressed as log_e(hsCRP) = $a + b_1$*Age + b_2*Sex + b_3*log_e(cytokine/chemokine(j)), where a is an estimated intercept, b_1, b_2 and b_3 are estimated regression coefficients and j is an indicator for individual cytokine/chemokine.

Discussion

CRP is the prototypical acute-phase reactant in man. Serum hsCRP has been proposed as a marker of atherosclerosis-associated diseases including coronary heart disease and cerebro-vascular accidents [17, 18]. CRP induced by IL-6 can act as pro-inflammatory by inducing the expression of TNFα and IL-1β [19].

CRP can also function as a component of the innate immune system by activating the classical pathway of complement system [20], enhancing phagocytosis [21] and binding to the Fcγreceptors on leukocytes, leading to the anti-inflammatory cytokine IL-10 production and the suppression of IL-12 secretion [22]. CRP may act as a protective machinery against a variety of inflammatory conditions and autoimmunity by interacting with many anti-inflammatory mediators such as IL-10 and IL-12 [22- 24].

We have observed significantly increasing levels of serum hsCRP [1] and cytokines/chemokines including IL-4, IL-6, IL-13, IL-15, TNF-α, GM-CSF IFN-γ and IP-10 in accordance with healthy ageing. In contrast, a significant decrease in the serum levels of IL-2, IL-8 and MIP-1α was negatively associated with healthy ageing. Among these cytokines/chemokines, both IL-6 and IL-13 levels were significantly associated with serum level of hsCRP, if age and sex were taken into account in the present experiment.

As hsCRP-associated elevation of serum levels of IL-6 and IL-13 with ageing may suggest a possible contribution of latent/persistent viral/parasitic infections such as herpes virus groups [25-27], we observed the age-associated increase in the serum level of anti-herpes viral antibodies in a separate experiment (manuscript in preparation). So, latent/persistent viral infection as a part of 'inflammageing' may induce immune imbalance during healthy ageing.

Ageing-associated increase in the serum level of IL-13 has never been reported, though ageing-associated increases in IL-4 and IL-6 have been frequently described [28-30]. IL-13 has an anti-inflammatory activity in association with other cytokines such as IL-4 and IL-6 in response to various stimuli [31,32], though IL-13 like IL-4, produced by IL-3-stimulated basophils and Th2 cells can induce an allergic inflammation against infected parasites, possibly leading to the activation of wound healing macrophages for tissue repair with fibrosis [33] and the abrogation of autophagy and autophagy-mediated killing of intracellular mycobacteria in human macrophages [8, 34]. Age-associated increase of serum level of IL-3 was already reported, though we did not studied IL-3 in the present experiment [34].

Pro-inflammatory IL-6 is produced by a variety of cell types including T cells, B cells, classically activated macrophages, adipose- tissue-associated macrophages, fibroblasts and endothelial cells [35]. Ageing-associated decrease in pro-inflammatory cytokines/chemokines including IL-2, IL-8 and MIP-1α can be produced by Th1-type T cells, macrophages, fibroblasts, neutrophils and NK cells [36].

Pro-inflammatory chemokines: IP-10 and MCP-1 have been reported to be the products from adipose-tissue-associated macrophages, classically activated macrophages, fibroblasts, endothelial cells and mast cells [35, 37]. Increase in the serum levels of IP-10 and MCP-1 with healthy ageing has already been described by Gerli et al [38] and Antonelli et al [13]. Mansfield et al [37] observed a significantly elevated production of MCP-1, IP-10 and eotaxin with healthy ageing.

These cytokine/chemokine distributions may suggest an association of MCP-1-stimulated Th2 type inflammation leading to tissue remodeling and fibrosis by wound healing macrophages with healthy ageing as already described by others [9, 30, 39]. Although the serum level of chemokine IP-10 increased significantly, other chemokines such as IL-8 and MIP-1α decreased and the levels of MCP-1, MIP-1β and eotaxin of the same class of chemokines did not change significantly with healthy ageing. As discussed above, a variety of cells can produce different types of chemokines in response to inflammation.

CRP has an antagonistic pleiotropic activity and the elevating inflammation associated with healthy ageing may not be the direct result of one-way traffic destruction of tissues, but the sum result of ongoing tissue degradation and repair by a cytokine/chemokine circuit-driven inflammation and regeneration [36, 37].

The immune system is manipulating itself by an interactive orchestration of a variety of cytokines/chemokines produced by various kind of cells including T, B, NK, dendritic cells, monocytes, neutrophils endothelial cells and even fibroblasts. Among the T cell subsets: Th1 cells mainly produce IFNγ, IL-1β, IL-2, IL-10, IL-12 and TNFα; Th2 cells mainly produce IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-13 and GM-CSF; Th17 cells mainly produce IFNγ, IL-6, IL-17, GM-CSF, TGFβ and TNFα; Treg cells mainly produce IL-10 and TGFβ. However, most cytokines/chemokines can be produced redundantly by different types of cells.

GM-CSF, IFNγ, IL-6 and TNFα were elevated, while IL-17 and IL-10 did not change significantly with ageing. Healthy ageing did not associate with the serum levels of Th17 and Treg cytokines/chemokines in the present experiment [40].

Immunological shift to Th2-type T cells with healthy ageing may stimulate a pro-inflammatory cytokine/chemokine circuit, leading to a systemic chronic inflammation (inflammageing) that can be monitored by hsCRP. The elevation of Th17 cytokines/chemokines such as GM-CSF and TNFα with ageing may suggest a contribution of Th17 cells to the ageing-associated orchestration of immune system to a certain degree, though the interaction of immune cells may not be so simple and more extended studies by using larger population from other ethnic origins may warrant the present result and inflammageing.

Conclusion

"How and what" does drive and accelerate ageing-associated minor-inflammation still a mystery. Th2 shift and Th2-biased mild inflammation:with ageing may contribute inflammageing in healthy ageing.

Conflict of interest: None.

Acknowledgements

We would like to thank Drs. S. Hayashi at Fukui General Hospital and T. Ogino at Kyoritsu Ogino Hospital and Ms. T. Watanabe at Wayoen Nursing Home for collecting serum samples from healthy elderly individuals.

Funding: This study was supported by JSPS KAKENHI Grant Number 24590902 (MG).

Contributions: Planned and designed the experiments: MG. Performed the experiments: MG, KH, JC, MM, SIE, YW. Analyzed the data: MG, MM. Contributed materials/analysis tools: MG, SIE, YW. Wrote the paper: MG.

References

1.Goto M, Sugimoto K, Hayashi S, Ogino T, Sugimoto M et al. Aging-associated inflammation in healthy Japanese individuals and patients with Werner syndrome. Exp Gerontol. 2012, 47(12): 936-939.

2. Arima H, Kubo M, Yonemoto K, Doi Y, Ninomiya T et al. High-sensitivity C-reactive protein and coronary heart disease in a general population of Japanese: the Hisayama study. Arterioscler Thromb Vasc Biol. 2008, 28(7): 1385-1391.

3. Balkwill F, Coussens LM. An inflammatory link. Nature.

2004, 431: 405-406.

4. Marucci M, Cevenini E, Pini E, Scurti M, Biondi F et al. Immune system, cell senescence, aging and longevity-inflammaging reappraised. Curr Pharm Des. 2013, 19(9): 1975-1679.

5. Goto M. Inflammaging (inflammation+aging): A driving force for human aging based on an evolutionarily antagonistic pleiotropy theory? BioScience Trend. 2008, 2(6): 218-230.

6. Shaw AC, Goldstein DR, Montgomery RR. Age-dependent dysregulation of innate immunity. Nat Rev Immunol. 2013, 13(2): 875-887.

7. Boyd SD, Liu Y, Wang C, Martin V, Dunn-Walters DK et al. Human lymphocyte repertoires in ageing. Curr Opin Immunol. 2013, 25(4): 511-515.

8. Kolattukudy PE, Niu J. Inflammation, ER stress, autophagy and MCP-1/CCR2 pathway. Circ Res. 2012, 110(1): 174-189.

9. Mariani E, Cattini L, Neri S, Malavolta M, Mocchegiani E et al. Mold C. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fc gamma R. J Immunol. 2002, 169(12): 7019-7025.

10. Shurin G, Yurkovetsky ZR, Chatta GS, Tourkowa IL, Shurin MR et al. Dynamic alteration of soluble serum biomarkers in healthy aging. Cytokine. 2007, 39(2): 123-129.

11. Kim HO, Kim H-S, Youn J-H, Shin E-C, Park S et al. Serum cytokine profiles in healthy young and elderly population assessed using multiplexed bead-based immunoassays. J Transl Med. 2011, 9: 113-119.

12. Inadera H, Egashira K, Takemoto M, Ouchi Y, Matsushima K et al. Increase in circulating levels of monocyte chemoattractant protein-1 with aging. J Inteferon Cytokine Res. 1999, 19(10): 1179-1182.

13. Antonelli A, Rotondi M, Fallahi P, Ferrari SM, Paolicchi A et al. Increase of CXC chemokine CXCL10 and cc chemokine CCL2 serum levels in normal ageing. Cytokine . 2006, 34(1-2): 32-38.

14. Ligthart GJ, Corberand JX, Fournier C, Galanaud P, Hijmans W et al. Admission criteria for immunogerontological studies in man: the SENIEUR protocol. Mech Age Dev. 1984, 28(1): 47-55.

15. Akaike H. Information theory and extension of the maximum likelihood principle. 2nd international symposium on information theory. 1973.

16. Ihaka R, Gentleman R. A language for data analysis and graphics. J Comp Grap Stat. 1996, 5(3): 299-314.

17. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE et al. Joshipura K. Inflammatory markers and the risk of coronary heart disease in men and women. N Engl J Med. 2004, 351(25): 2599-2610.

18. Ishikawa J, Tamura Y, Hoshide S, Eguchi K, Ishikawa S et al. Shimada K. low-grade inflammation is a risk factor for clinical stroke events in addition to silent cerebral infarcts in Japanese older hypertensives. Stroke. 2007, 38(3): 911-917.

19. Kaplan MH, Volanakis JE. Interactions of C-reactive protein with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal polysaccharide and with the choline phosphatides, lectin and sphingomyelin. J Immunol. 1974, 112(6): 2135-2147.

20. Stein MP, Edberg JC, Kimberly RP, Mangan EK, Bharadwaj D et al. C-reactive protein binding to FcgammaRlla on human monocytes and neutrophils is allele-specific. J Clin Invest. 2000, 105(3): 369-376.

21. Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW et al. The major receptor for C-reactive protein on leukocytes is Fcgamma receptor ll. J Exp Med. 1999, 190(4): 585-590.

22. Du Clos TW. C-reactive protein as a regulator of autoimmunity and inflammation. Arthritis Rheum. 2003, 48(6): 1475-1477.

23. Gershow D, Kim S, Brot N, Elkon KB. C-reactive protein binds to apoptotic cells, protects the cells from assembly of the terminal complement components, and sustains an anti-inflammatory innate immune response:implications for systemic autoimmunity. J Exp Med. 2000, 192(9): 1353-1364.

24. Kim S, Elkon KB, Ma X. Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. Immunity. 2004, 21(5): 643-653.

25. Bennette JM, Glaser R, Malarkey WB, Beversdorf DQ, Peng J et al. Inflammation and reactivation of latent herpesviruses in older adults. Brain Behav Immun. 2012, 26(5): 739-746.

26. Bosniak L, Sahlstrom P, Paquin-Proulx D, Leeansyah E, Moll M et al. Contact-dependent interference with invariant NKT cell activation by herpes simplex virus-infected cells. J Immunol. 2012, 188(12): 6216-6224.

27. Sparrelid E, Emanuel D, Fehniger T, Andersson U, Andersson J et al. Interstitial pneumonitis in bone marrow transplant recipients is associated with local production of TH2-type cytokines and lack of T cell-mediated cytotoxicity. Transplant. 1997, 63(12): 1782-1789.

28. Sandmand M, Bruunsgaard H, kemp K, Andersen-Ranberg K, Pedersen AN et al. Is ageing associated with a shift in the balance between Type 1 and Type 2 cytokines in humans? Clin Exp Immunol. 2002, 127(1): 107-114.

29. Wang X, Xiao X, Bao W, Shan ZL, Liu J et al. Zhang Y. Inflammatory markers and risk of type 2 diabetes: a systematic review and meta-analysis. Diab Care. 2013, 36(1): 166-175.

30. Sokol CL, Barton GM, Farr AG, Medzhitov R. A mechanism for the initiation of allergen-induced T helper type 2 responses. Nat Immunol. 2008, 9(3): 310-318.

31. Schneider E, Thieblemont N, Leite De Moraes M, Dy M. Basophils: new players in the cytokine network. Eur Cytokine Netw. 2010, 21(3): 142-153.

32. Paqanelli R, Scala E, Quinti I, Ansotequi IJ. Humoral immunity in aging. Aging. 1994, 6(3): 143-150.

33. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008, 8(12): 958-969.

34. Harris J, De Haro SA, Master SS, Keane J, Roberts EA et al. T helper 2 cytokines inhibit autophagic control of intracellular mycobacterium tuberculosis. Immunity. 2007, 27(3): 505-517.

35. Lucey DR, Clerici M, Shearer GM. Type 1 and type 2 cytokine dysregulation in human infectious, neoplastic, and inflammatory diseases. Clin Microbiol Rev. 1996, 9(4): 532-562.

36. Gerli R, Monti D, Bistoni O, Mazzone AM, Peri G et al. Chemokines, sTNF-Rs and sCD30 serum levels in healthy aged people and centenarians. Mech Ageing Dev. 2000, 121(1-3): 37-46.

37. Mansfield AS, Nevala WK, Dronca RS, Leontovich AA, Shuster L et al. Normal ageing is associated with an increase in Th2 cells, MCP-1(CCL1) and RANTES (CCL5), with differences in sCD40L and PDGF-AA between sexes. Clin Exp Immunol. 2012, 170(2): 186-193.

38. Loke P, Gallagher I, Nair MG, Zang X, Brombacher F Mohrs M et al. Alternative activation is an innate response to injury that requires CD4+T cells to be sustained during chronic infection. J Immunol. 2007, 179(6): 3926-3936.

39. Singh T, Newman AB. Inflammatory markers in population studies of aging. Age Res Rev. 2011, 10(3): 319-329.

40. Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev. 2014, 13(6): 668-677.