Original Article

DOI: 10.5582/irdr.2018.01092

Aging-associated latent herpes viral infection in normal Japanese individuals and patients with Werner syndrome

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

[1] *Division of Anti-ageing and Longevity Sciences, Department of Medical Technology, Faculty of Medical Engineering, Toin University of Yokohama, Yokohama, Japan;*
[2] *Department of Orthopaedics & Rheumatology, East Medical Center, Tokyo Women's Medical University, Tokyo, Japan;*
[3] *Division of Rheumatology, Nerima Hikarigaoka Hospital, Tokyo, Japan;*
[4] *Department of Cancer Genomics, Cancer Institute, Japanese Foundation for Cancer Research, Tokyo, Japan;*
[5] *School of Public Health, Teikyo University, Tokyo, Japan;*
[6] *Department of Life Sciences, School of Pharmaceutical Sciences, Hokkaido University of Science, Sapporo, Japan.*

Summary **A series of our "inflammageing" study examining serum samples from a maximum of 217 healthy Japanese individuals aged between 1 and 100 years and mutation-proven 40 patients with Werner syndrome (WS) indicated normal aging-associated elevations of highly sensitive CRP (hsCRP) and matrix metalloproteinase-9 (MMP-9). To further study the contribution of environmental factors such as persistent herpes viral infection to inflammageing, IgG antibodies against varicella/zoster virus (VZV) and cytomegalovirus (CMV) were examined in the same serum samples as has been done for hsCRP and MMP-9 analyses. The mean levels of serum IgG viral antibodies were comparable between normal (mean ± SE: 31.0 ± 4.3 unit) and WS (38.6 ± 7.6) for CMV, and between normal (42.0 ± 12.2) and WS (29.8 ± 3.8) for VZV, respectively. Significant associations of aging with IgG anti-CMV antibody were in normal aging (p = 0.023) and WS (p = 0.037), but not with IgG VZV in both conditions. Aging-associated change of IgG anti-CMV antibody titer in WS increased significantly (1.32 times higher) compared with normal aging (p = 0.037). IgG anti-CMV level was significantly elevated in the male gender than female in both conditions (p = 0.006). Elevated hsCRP level was significantly associated with IgG anti-CMV (p = 0.016) and IgG anti-VZV (p = 0.008) antibodies in normal aging, but not in WS. Serum MMP-9 was significantly associated with IgG anti-CMV level (p = 0.0002) in normal aging, but not in WS. Persistent herpes viral infection may constitute a part of "inflammageing" in normal aging and WS.**

Keywords: Aging, CRP, Cytomegalovirus, Herpes virus, Inflammageing, MMP-9, Werner syndrome

1. Introduction

Human aging is probably controlled by a combination of intrinsic/genetic factors including metabolism/catabolism functions (*1*), and extrinsic/environmental factors such as infections (*2*). The possible intrinsic/genetic factors include genetically-determined progeroid syndrome genes such as *WRN* for Werner syndrome (WS) (*3*). WS has been proposed as the representative human natural ageing model. Because patients with WS, transmitted autosomal-recessively by the homozygous mutation of *WRN* (RecQ3 DNA helicase), usually manifest their premature aging phenotypes immediately after adulthood. Typical aging signs and symptoms in WS include voice change, bilateral cataracts, gray hair/alopecia, skin atrophy, skin pigmentation, type II diabetes mellitus, central obesity, atherosclerosis, hyperlipidemia, skin ulcer, renal dysfunction, osteoporosis, and cancer/sarcoma. The

**Address correspondence to:*
Dr. Makoto Goto, Division of Rheumatology, Nerima Hikarigaoka Hospital, 2-11-1 Hikarigaoka, Nerima-Ku, Tokyo 179-0072, Japan.
E-mail: werner.goto@gmail.com

average age of death either by myocardial infarction or malignancy is about 50 years old. So far, 1,300 cases have been recognized all over the world, in which roughly 75% of the patients are of Japanese origin (*3*).

Human aging is inevitably encountered by an increasing chance of environmental attack from infectious agents such as herpes viruses during daily living leading to a minor/latent inflammation that could be evaluated by highly sensitive CRP (hsCRP) and matrix metallo-proteinase-9 (MMP-9) (*1,2,4,5*).

Chronic elevation of hsCRP in the normal elderly population may probably be associated with aging-related pathological conditions including diabetes mellitus (DM), sarcopenia, osteoporosis, cancer, atherosclerosis, cognitive decline and finally death (*6,7*). Aging-related persistent, systemic, minimal and asymptomatic inflammation, termed 'inflammageing', is tightly associated with an imbalance between an increase in pro- and a decrease in anti-inflammatory substances including cytokines/chemokines (*8- 11*). Inflammation is widely recognized as a patho-physiologically fundamental metabolism to generate energy with thermogenesis, leading to wound healing and tissue destruction during healthy development and aging (*6,12,13*).

We have reported possible biomarkers for aging in a series of inflammageing studies by using the same serum samples from normal Japanese individuals aged between 1 and 100 years old and mutation-proven progeroid patients with WS (*4,5,8,9*).

The aim of this study was to clarify the associations of serum level of latent herpes viral infections including varicella/zoster virus (VZV) and cytomegalovirus (CMV) with normal aging and the WS patients, and also with the aging-associated increase of hsCRP and MMP-9 by directly comparing the same serum samples used in the series of our inflammageing study.

2. Materials and Methods

2.1. *Study population*

All the samples studied in the present experiment were the same sera as were used in the previous hsCRP and MMP-9 studies (*4,5*). A total of 72 normal serum samples from both sexes (M = 32, F = 40) aged between 18 and 100 years were used for the study (Table 1). Normal individuals, enjoying the usual daily life at home or nursing home, had neither apparent inflammatory diseases including infection, cancer, lymphoproliferative disorders, DM, 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*).

Serum samples were also obtained from 40 mutation-proven WS patients without any medication at the time of serum sampling (M = 23, F = 17; between 32 and 70 years old); a part of "Goto collection of Werner syndrome" (*http://cell.brc.riken.jp/ja/gmc.html*).

As indicated in Table 2, nine WS patients were free from skin ulcers (SU) [SU (-)], while 31 had SU [SU (+)]. 23 had DM [DM (+)], but 17 did not [DM (-)]. WS patients were sub-grouped into 1) (SU (+) DM (+) (n = 19), 2) SU (+) DM (-) (n = 12), 3) SU (-) DM (+) (n = 4) and 4) SU (-) DM (-) (n = 5).

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.

2.2. *Screening of latent herpes viral infection*

Serum levels of IgG anti- CMV antibody and IgG anti- VZV antibody were measured using a CYTOMEGALLO IgG (II)-EIA "SEIKEN" kit and a varicella-Zoster IgG EIA "SEIKEN" kit according to the manufacturer's manual, respectively (Denka Seiken Co., Ltd., Tokyo, Japan). Colorimetric measurements were taken with an automatic ELISA reader (Behringer ELISA processor III) using a 486 nm filter. Assays were carried out in duplicate and the antibody titer was read from a standard curve made with known concentrations of the respective viral antibody. Results are given in arbitrary units, as defined by the reference serum diluted 1:400 and 1:102,400.

2.3. *Determination of hsCRP and MMP9*

The data of hsCRP (ug/mL) used in this study was obtained in the previous experiment (*4*) by using a CircuLex high-sensitivity CRP ELISA kit (Cyclex Co., Nagano, Japan) according to the user's manual. The concentration of MMP-9 (ng/mL) in the sera was determined by specific sandwich ELISA using a Human MMP-9 ELISA kit (Fuji Chemical Industries, Toyama, Japan) as described before (Table 1 and Table 2) (*5*).

2.4. *Data analysis and statistics*

We examined association of the respective serum IgG anti-CMV antibody or IgG anti-VZV antibody and the respective healthy aging individuals or WS patients by using linear regression model expressed as

$$Y = \alpha + \beta_1 * \text{Group} + \beta_2 * \text{Age} + \beta_3 * (\text{Age} * \text{Group}) + \beta_4 * \text{Sex}, \quad (1)$$

where Y means IgG anti-CMV antibody titer or IgG anti-VZV antibody titer (unit/ml). And α means an estimated intercept. Furthermore, β_1 indicates estimated coefficient for Group, where Group = 0 in normal aging and Group = 1 in WS, β_2 indicates coefficient for

Table 1. Herpes viral infection in normal Japanese individuals

Age	Sex	hsCRP[a](μg/mL)	MMP9[b](ng/mL)	anti-VZV[c]	anti-CMV[d]
18	F	0.23	93.4	5.1	22.5
25	F	0.18	327	24.2	2
25	M	0.76	55.4	26.3	2
26	M	0.29	247	3.3	10.5
27	F	0.23	191	15.8	2
28	M	0.67	154	11.8	6.1
30	F	0.23	126	12.4	30.1
31	F	0.20	0.8	16.2	11.1
32	M	0.29	4.7	16.9	12.4
32	F	2.30	348	7.5	2
33	F	0.08	134	55.3	2
35	M	0.83	79.2	51.5	3.9
35	M	0.33	145	33.8	7.9
36	F	0.61	206	13.3	21.8
36	F	0.31	302	149	28.4
37	F	0.42	58.8	15.2	19.7
38	F	0.57	84.5	27.5	41.1
43	M	0.13	26.8	24.5	2
46	F	8.88	155	18.8	75.5
47	F	0.20	198	15.1	58.1
48	M	0.30	51.4	3.6	4.6
49	F	1.14	91.2	32	28.4
53	M	0.35	105	9.5	23.6
55	M	0.22	149	15.4	8.4
57	M	0.76	40.4	9.3	24.5
57	M	1.98	94.1	51.2	9.8
60	M	0.17	54.3	2.9	18.7
60	M	0.93	49.7	20.2	12
60	M	0.08	62.0	23.5	2
60	M	2.79	70.3	24.7	49.7
60	M	0.35	36.8	64.5	25.4
61	M	0.97	4.8	7.6	23.2
62	M	7.02	98.9	18.8	14.6
62	F	0.28	26.3	18.8	14.6
66	M	1.24	92.2	26.4	14.2
67	M	0.37	78.8	9.8	10.3
68	M	0.23	26.8	18.4	26.4
68	M	2.83	24.9	68.4	14.2
71	M	0.50	56.0	22.8	22.9
73	M	0.76	101	13.3	36.7
74	F	0.55	240	9.2	15.5
74	F	3.06	52.3	9.6	38.1
75	M	0.11	212	39.6	16.5
75	F	1.94	40.4	22.3	19.4
77	F	0.60	122	3.7	11.4
78	M	0.28	354	5.7	19.6
81	M	29.6	784	64.7	34.6
81	F	19.1	531	18.9	97
81	F	0.84	341	64.7	34.6
82	F	2.26	284	33	39.9
82	F	0.30	123	4.9	17.2
83	M	2.90	29.0	182	28.2
83	F	0.34	276	95	182
84	F	0.54	62.2	16.4	95
86	F	17.7	228	11.1	59.6
88	F	4.36	156	37	24.1
88	F	3.48	145	42.2	27.2
89	F	10.9	10.2	116	27.5
89	F	11.9	4.9	13.2	70.6
91	F	0.11	47.5	19	21.8
91	F	18.2	172	57.2	25.8
92	F	0.19	160	9.7	28.3
94	M	4.75	317	15.3	14
94	F	16.5	508	5.2	23.4
94	F	2.67	356	11.9	14.8
96	M	3.06	435	53.3	222
96	M	2.81	128	19.6	15.2
96	F	23.6	509	26.9	34.5
96	F	0.65	186	56.5	42.5
98	F	0.75	295	6.6	112
99	F	1.40	106	14.4	44.2
100	F	23.2	295	54.2	29

[a]hsCRP: highly sensitive CRP, [b]MMP-9: matrix metalloproteinase-9, [c]anti-VZV: anti-varicella/ zoster virus, [d]anti-CMV: anti-cytomegalovirus.

Table 2. Herpes viral infection in Werner syndrome patients

Subgroups				Age	Sex	hsCRP[a] (ug/mL)	MMP-9[b] (ng/mL)	anti-VZV[c]	anti-CMV[d]
	SU[e]	DM[f]	ID						
1	+	+	WS12901	32	F	2.61	134	20.7	35.1
1	+	+	WS57201	37	M	22.8	351	8.9	9.4
1	+	+	WS56301	39	M	0.79	284	35.9	12.3
1	+	+	WS19201	38	M	7.03	82.2	19.2	28.9
1	+	+	WS5501	40	F	11.1	10.2	4.6	43.3
1	+	+	WS57801	41	M	1.04	122	45.5	2
1	+	+	WS51301	42	M	17.4	252	11.8	70.4
1	+	+	WS4705	45	F	42.4	224.3	33.9	33.8
1	+	+	WS6301	46	M	27.2	184	4.5	7.3
1	+	+	WS53601	46	M	16.3	24	37.2	28
1	+	+	WS0101	47	M	15	49.3	9.2	25.6
1	+	+	WS58501	51	M	4.02	414	2.7	22.3
1	+	+	WS58301	53	M	3.21	357	75	53.7
1	+	+	WS4704	54	M	3.42	32.1	53.4	41.4
1	+	+	WS17201	54	F	2.26	198.5	50.2	44.1
1	+	+	WS0801	55	F	8.99	167	44.1	50.1
1	+	+	WS54801	57	M	5.88	127	38.7	22.9
1	+	+	WS56201	70	M	10.3	85.8	12.7	10.3
1	+	+	WS1801	70	M	28.3	235.5	17.5	189
2	+	-	WS6103	32	M	1.62	2	3.7	2
2	+	-	WS6104	32	M	25	867.6	260	2
2	+	-	WS14501	35	M	4.55	129	40.8	25.5
2	+	-	WS53101	38	F	22.9	294	10.7	37.1
2	+	-	WS51601	36	F	0.98	222	28.8	2
2	+	-	WS53901	43	F	1.28	38.4	18.1	30.7
2	+	-	WS53801	46	F	0.98	103	44.8	172
2	+	-	WS2101	50	F	8.66	30.8	8.4	34.3
2	+	-	WS55801	53	F	18.2	45.4	21.3	66
2	+	-	WS52901	54	F	10.8	45.8	5.4	28.6
2	+	-	WS54001	57	F	7.04	70.3	6	5.9
2	+	-	WS4701	59	F	11.8	33.6	56.2	15.1
3	-	+	WS57701	38	F	2.07	197	22.6	82.8
3	-	+	WS58701	35	M	2.93	108	15.4	2
3	-	+	WS57401	41	M	26.9	0	23	2
3	-	+	WS4401	41	M	24.9	30	13.3	16.6
4	-	-	WS5801	43	M	1.28	2.9	5.7	8.2
4	-	-	WS10501	52	F	3.86	16.7	72.7	150
4	-	-	WS0402	47	M	1.15	19.5	488	2
4	-	-	WS7501	48	M	22.3	66.7	8.3	28.2
4	-	-	WS0401	49	F	4.76	52.9	2	2

[a]hsCRP: highly sensitive CRP, [b]MMP-9: metalloproteinase-9, [c]anti-VZV: anti-varicella/ zoster virus, [d]anti-CMV: anti-cytomegalovirus, [e]SU: skin ulcer, [f]DM: diabetes mellitus.

continuous age, β_3 indicates a coefficient of interaction for Age and Group, and β_4 indicates coefficient for Sex, where Sex = 1 for male and Sex = 0 for female. In order to show a reliability of mean, we show its standard error of the mean. Furthermore, we used Pearson correlation coefficients to show a statistical relationship between two variables. *P*-values < 0.05 are considered to be statistically significant.

3. Results

3.1. *Latent herpes viral infection in normal aging and WS*

The serum levels of IgG viral antibodies were comparable between normal aging (mean ± SE: 31.0 ± 4.3 unit) and WS (38.6 ± 7.6) for CMV, and between normal aging (42.0 ± 12.2) and WS (29.8 ± 3.8) for VZV, respectively (Table 1 and Table 2).

In WS, IgG anti-VZV antibody levels in respective subgroups were 1) 27.7 ± 4.7 (mean ± SE), 2) 42.0 ± 20.4, 3) 18.6 ± 2.5, and 4) 115.3 ± 94.1. The mean ± SE of IgG anti-VZV antibody was not significantly different between any combination of subgroups. IgG anti-CMV antibody levels in respective subgroups of WS were 1) 38.4 ± 9.3, 2) 43.4 ± 17.4, 3) 25.9 ± 19.3 and 4) 38.1 ± 28.4. The mean ± SE of IgG anti-CMV antibody was not significantly different between any combination of subgroups in WS.

Furthermore, we did not observe any significant

Table 3. Association of anti-CMV antibody, age and sex in normal aging and WS

Variables	Estimated regression coefficient	SE	*p* value
Intercept	- 10.303	12.17	0.399
Group(1, 0)	- 38.812	30.648	0.208
Age	0.404	0.175	0.023
Age*Group	1.318	0.624	0.037
SEX(1, 0)	19.988	7.141	0.006

Linear regression model used in this study was expressed as $Y = \alpha + \beta_1$*Group + β_2*Age + β_3*(Age*Group) + β_4*Sex, where Y means IgG anti-CMV antibody titer (unit/mL). And α means an estimated intercept. Furthermore, β_1 indicates estimated coefficient for Group, where Group = 0 in normal aging and Group = 1 in WS, β_2 indicates coefficient for continuous age, β_3 indicates a coefficient of interaction for Age and Group, and β_4 indicates coefficient for Sex, where Sex = 1 for male and Sex = 0 for female. Estimated regression coefficients are indicated in the left column and *p* values in the right column. IgG anti-cytomegallovirus (CMV) antibody titer is expressed as an arbitrary unit/mL.

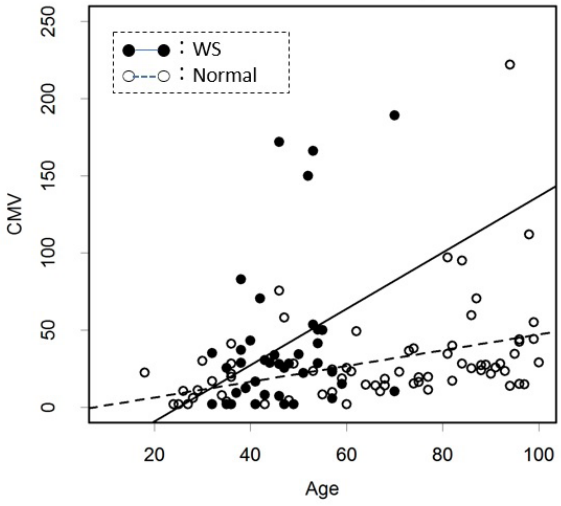

Figure 1. Age-associated increase in IgG anti-CMV antibody in normal individuals and Werner syndrome. Linear regression model for female described in Table 3 was used. WS: solid line, normal aging: dotted line. Regression lines for male (bold line) in both groups were higher than the fine line of female by a value of 20 as indicated in Table 3.

relationship of the titers of IgG anti-VZV antibody/ IgG anti-CMV antibody and patients' calendar age, sex, hsCRP, and MMP-9 in the respective subgroups of WS by linear regression analysis.

3.2. *Aging-associated changes of latent herpes viral infection*

Simple Pearson correlation of IgG anti-CMV antibody and normal aging was 0.39 ($p = 0.0008$) for normal group and 0.35 ($p = 0.027$) for WS. The results of the multiple linear regression model (1) for IgG anti-CMV antibody titer and age with covariates for group and sex are indicated in Table 3. Estimated regression lines for WS and normal aging are shown in Figure 1. Here, solid line indicates WS (male: bold line and, female: fine line) and dotted line normal aging (male: bold and female: fine).

Aging-associated change of IgG anti-CMV antibody titer in WS increased significantly (1.32 times higher) compared with that in normal aging ($p = 0.037$). IgG anti-CMV level was significantly elevated in male gender compared to female in both conditions ($p = 0.006$).

IgG anti-VZV antibody titer neither correlated with calendar aging in normal aging nor WS.

3.3. *hsCRP-associated changes of latent herpes viral infection*

Inflammation monitored by the serum level of hsCRP was significantly associated with healthy aging as shown in the previous report. No significant gender difference was observed concerning to the age-associated increase in hsCRP level (*4*).

IgG anti-CMV antibody was significantly correlated with hsCRP ($p = 0.016$) in normal aging, if age and sex were adjusted.

IgG anti-VZV antibody was significantly ($p = 0.008$) correlated with serum level of hsCRP in normal aging, if sex was adjusted, but not, if age was adjusted.

In WS, neither IgG anti-CMV antibody nor IgG anti-VZV antibody was correlated with hsCRP.

3.4. *MMP-9-associated changes of latent herpes viral infection*

IgG anti-CMV antibody significantly ($p = 0.0002$) correlated with serum level of MMP-9 in normal aging, if age and sex were adjusted, but not in WS.

IgG anti-VZV antibody significantly correlated with sex ($p = 0.019$) in normal aging, if MMP-9, age and sex were adjusted, but not in WS.

4. Discussion

We have for the first time reported in the present study that calendar aging-associated anti-CMV antibody titer in WS significantly increased compared with that in the healthy aging Japanese population living under a similar environment. As aging-associated increase in IgG anti-CMV antibody in healthy aging has been well documented (*15-19*), the persistent viral infections during aging may attribute to a mild but significant decline in immune function with normal aging and WS followed by tissue-destructive chronic inflammation (inflammageing) after maturation stage (*12,13*).

We have already reported the increasing level of serum hsCRP and MMP-9 in accordance with healthy calendar aging and also significantly more elevation of hsCRP compared with healthy aging controls in patients with a genetically-determined progeroid syndrome such as WS (*4,5*).

The pathogenesis of aging-associated inflammation,

probably driven by a combination of environmental factors including viral infections and genetic factors, is not well studied (*20*).

Possible environmental factors, which can produce inflammatory cytokines may include beta-herpes virus such as CMV (*21,22*). Human CMV causes many infection-related birth defects, and may trigger a variety of age-associated inflammatory diseases such as vascular diseases, autoimmune diseases, hepatitis, interstitial pneumonitis, gastrointestinal diseases and atherosclerosis (*23,24*). The CMV-infected mononuclear cells produce a variety of cytokines such as IL-2, IL-6 and TNF-α through the MAPK/ERK pathway (*21*). Human herpes virus 6, the same subfamily of CMV, has recently been shown to have ATPase, helicase, exonuclease and DNA-binding activities and can integrate into telomeres of the human chromosome (*22*).

CRP, induced by IL-6 can act as pro-inflammatory by inducing the expression of TNF-α and IL-1β, and is the prototypical acute-phase reactant in man (*25*). Serum hsCRP has been proposed as a marker of atherosclerosis-associated diseases including coronary heart disease and cerebro-vascular accidents, and also inflammageing (*7,26*).

CRP can also function as a component of the innate immune system by activating the classical pathway of the complement system (*27*), enhancing phagocytosis (*28*). 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, transforming growth factor-β and IL-12 (*25-29*). So, CRP has an antagonistically pleiotropic activity and the elevates inflammation associated with healthy aging and WS may not be the direct result of one-way traffic destruction of tissues, but the sum result of ongoing tissue degradation and repair by MMPs and cytokine/chemokine circuit-driven inflammation and regeneration (*6*).

Inflammation has been believed to be an energy supply mechanism to proceed to normal repair mechanisms during a whole life and normal development before maturation followed by tissue destruction after a senescent stage, though the precise mechanism of inflammageing is still uncovered (*20*).

The possible pathogenetical contribution of VZV and CMV to SU and DM either in natural aging and WS has never been reported and we did not find a relationship between SU/DM and VZV/CMV in WS, although the incidence of VZV and CMV infection and the frequency of SU and DM are generally age related.

The reason why there is more inflammation with an elevated level of anti-CMV antibody in WS than healthy counterparts is a complete mystery.

As the recent WS gene (WRN) knock-out mice study suggested an induction of immune dysfunction followed by chronic inflammation (*30*), we would like to speculate that the loss-of-function mutation of WRN may lead to some immune dysfunction leading to more susceptibility to CMV infection in WS than normal aging populations. Because natural killer cell function declined significantly in WS and WS patients produced more auto-antibodies compared with the normal aging population as we already reported (*4,5,8,9,31-35*). Obviously, this is a highly speculative idea and further study may be needed to clarify the pathogenesis of mild inflammation: inflammageing in healthy aging and also in WS.

Acknowledgements

This study was supported by JSPS KAKENHI Grant Number 24590902. We would like to thank Drs. S. Hayashi at Fukui General Hospital, T. Ogino at Kyoritsu Ogino Hospital and Ms. T. Watanabe at Wayoen Nursing Home for collecting serum samples from healthy elderly individuals.

References

1. Finkel T. The metabolic regulator of aging. Nat Med. 2015; 21:1416-1422.
2. Paschos K, Allday MJ. Epigenetic reprogramming of host genes in viral and microbial pathogeneis. Cell. 2010; 18:439-447.
3. Goto M. Cinical characteristics of Werner syndrome and other premature aging syndromes; Pattern of aging in progeroid syndromes. In: From premature gray hair to helicase: Werner syndrome: Implications for aging and cancer. Monograph on Cancer Research No.49 (Goto M, Miller RW, eds.). Karger, Tokyo, Japan, 2001; pp.27-39.
4. Goto M, Sugimoto K, Hayashi S, Ogino T, Sugimoto M, Furuichi Y, Matsuura M, Ishikawa Y, Iwaki-Egawa S, Watanabe Y. Aging-associated inflammation in healthy Japanese individuals and patients with Werner syndrome. Exp Gerontol. 2012; 47: 936-939.
5. Goto M, Chiba J, Matsuura M, Iwaki-Egawa S, Watanabe Y. Inflammageing assessed by MMP9 in normal Japanese individuals and the patients with Werner syndrome. Intract Rare Dis Res. 2016; 5:103-108.
6. Balkwill F, Coussens LM. Cancer: An inflammatory link. Nature. 2004; 431:405-406.
7. Pai JK, Pischon T, Ma J, Manson JE, Hankinson SE, Joshipura K, Curhan GC, Rifai N, Cannuscio CC, Stampfer MJ, Rimm EB. Inflammatory markers and the risk of coronary heart disease in men and women, N Engl J Med. 2004; 351:2599-2610.
8. Goto M, Hayata K, Chiba J, Matsuura M, Iwaki-Egawa S, Watanabe Y. Ageing-associated inflammation (Inflammageing): Multiplex cytokine analysis in healthy Japanese individuals. Jacobs J Gerontol. 2015; 1:1-7.
9. Goto M, Hayata K, Chiba J, Matsuura M, Iwaki-Egawa S, Watanabe Y. Multiplex cytokine analysis of Werner syndrome. Intract Rare Dis Res. 2015; 4:190-197.
10. Goto M. Inflammaging (inflammation+aging): A driving force for human aging based on an evolutionarily antagonistic pleiotropy theory? BioSci Trends. 2008; 2:218-230.
11. Goto M. Emerging concept of ageing: Inflammageing. Inflamm Regen. 2009; 29:249-257.

12. Shaw AC, Goldstein DR, Montgomery RR. Age-dependent dysregulation of innate immunity. Nat Rev Immunol. 2013; 13: 875-887.
13. Kolattukudy PE, Niu J. Inflammation, endoplasmic reticulum stress, autophagy, and the monocyte chemoattractant protein-1/CCR2 pathway. Circ Res. 2012; 6:174-189.
14. Ligthart GJ, Corberand JX, Fournier C, Galanaud P, Hijmans W, Kennes B, Muller-Hermelink HK, Steinmann GG. Admission criteria for immunogerontological studies in man: The SENIEUR protocol. Mech Age Dev. 1984; 28:47-55.
15. Bennette JM, Glaser R, Malarkey WB, Beversdorf DQ, Peng J, Kiecolt-Glaser JK. Inflammation and reactivation of latent herpesviruses in older adults. Brain Behav Immun. 2012; 26:739-746.
16. Loke P, Gallagher I, Nair MG, Zang X, Brombacher F, Mohrs M, Allison JP, Allen JE. Alternative activation is an innate response to injury that requires CD4+T cells to be sustained during chronic infection. J. Immunol. 2007; 179:3926-3936.
17. Bosnjak L, Sahlström P Paquin-Proulx D, Leeansyah E, Moll M, Sandberg JK. Contact-dependent interference with invariant NKT cell activation by HSV-infected cells. J Immunol. 2012; 188:6216-6224.
18. Furui Y, Satake M, Hoshi Y, Uchida S, Suzuki K, Tadokoro K. Cytomegalovirus (CMV) seroprevalence in Japanese blood donors and high frequency of CMV DNA in elderly donors. Transfusion. 2013; 53:2190-2197.
19. Doi Y, Ninomiya T, Hata J, Yonemoto K, Tanizaki Y, Arima H, Liu Y, Rahman M, Iida M, Kiyohara Y. Seroprevalence of herpes simplex virus 1 and 2 in a population-based cohort in Japan. J Epidemiol. 2009; 19:56-62.
20. Gerli R, Monti D, Bistoni O, Mazzone AM, Peri G, Cossarizza A, Di Gioacchino M, Cesarotti ME, Doni A, Mantovani A, Franceschi C, Paqanelli R. Chemokines, sTNF-Rs and sCD30 serum levels in healthy aged people and centenarians. Mech Ageing Dev. 2000; 121:37-46.
21. Zheng Q, Tao R, Gao H, Xu J, Shang S, Zhao N. HCMV-encoded UL128 enhances TNF-α and IL-6 expression and promotes PBMC proliferation through the MAPK/ERK pathway in vitro. Viral Immunol. 2012; 25:98-105.
22. Trempe F, Gravel A, Dubuc I, Wallaschek N, Collin V, Gilbert-Girard S, Morissette G, Kaufer BB, Flamand L. Characterzation of human herpesvirus 6A/B U94 as ATPase, helicase, exonuclease and DNA-binding proteins. Nucleic Acids Res. 2015; 43:6084-6098.
23. Scalzo AA, Corbett AJ, Rawlinson WD, Scott GM, Degli-Esposi MA. The interplay between host and viral factors in shaping the outcome of cytomegalovirus infection. Immunol Cell Biol. 2007; 85:46-54.
24. Söderberg-Nauclér C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? J Intern Med. 2006; 259:219-246.
25. Du Clos TW. C-reactive protein as a regulator of autoimmunity and inflammation. Arthritis Rheum. 2003; 48:1475-1477.
26. Bharadwaj D, Stein MP, Volzer M, Mold C, Du Clos TW. The major receptor for C-reactive protein on leukocytes is fcgamma receptor ll. J Exp Med. 1999; 190: 585-590.
27. Kaplan MH, Volanakis JE. Interaction of C-reactive protein complexes with the complement system. I. Consumption of human complement associated with the reaction of C-reactive protein with pneumococcal C-polysaccharide and with the choline phosphatides, lecithin and sphingomyelin. J Immunol. 1974; 112:2135-2147.
28. 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 antiinflammatory innate immune response: Implications for systemic autoimmunity. J Exp Med. 2000; 192:1353-1364.
29. Kim S, Elkon KB, Ma X. Transcriptional suppression of interleukin-12 gene expression following phagocytosis of apoptotic cells. Immunity. 2004; 21:643-653.
30. Hui CW, St-Pierre MK, Detuneq J, Aumailley L, Dubois MJ, Couture V, Skuk D, Marretta A, Tremblay JP, Lebel M, Tremblay MÈ. Nonfunctional mutant Wrn protein leads to neurological deficits, neuronal stress, microglial alteration, and immune imbalance in a mouse model of Werner syndrome. Brain Behav Immun. 2018; 73:450-469.
31. Goto M, Kawata M, Ohmori K, Okumura K, Tada T. Immunological abnormalities of aging: An analysis of T lymphocyte subpopulations of Werner's syndrome. J Clin Invest. 1979; 64:695-699.
32. Goto M, Tanimoto K, Aotsuka S, Okawa M, Yokohari,R. Age-related changes in auto- and natural antibody in the Werner's syndrome. Am J Med. 1982; 72:607-614.
33. Goto M, Tanimoto K ,Kuwata T. Reduced NK activity of lymphocytes from patients with Werner's syndrome and recovery of its activity by human leukocyte interferon. Scand J Immunol. 1982; 15:389-397.
34. Goto M. Immunosenescent features of segmental progeroid syndrome: Werner's syndrome. AGING Immunol Infect Dis. 1992; 3:203-213.
35. Goto M. Elevation of soluble Fas(APO-1,CD95) ligand in natural ageing and Werner syndrome. BioSci Trends. 2008; 2:124-127.

(Received August 27, 2018; Revised November 12, 2018; Accepted November 20, 2018)