The association between cytomegalovirus infection and aging process

Virginija Kanapeckienė, Julius Kalibatas, Elvyra Redaitienė, Jelena Čeremnych[1]
Institute of Hygiene, [1]Institute of Experimental and Clinical Medicine, Vilnius University, Lithuania

***Key words**: cytomegalovirus; immune response; aging.*

***Summary.** Analysis of published scientific data suggests that cytomegalovirus infection has an effect on aging process in human, in particular on immunosenescence, resulting in an increased incidence of infectious diseases and consequent mortality in elderly individuals. The purpose of this study was to evaluate the association between cytomegalovirus infection and a character of aging (premature, physiological, and slow).*

Materials and methods. In accordance with special criteria of the assessment of biological age, 146 healthy elderly women aged 60–90 years were divided into three groups: Group 1 – slow aging group (37 women, 25.4%); Group 2 – physiological aging group (58 women, 39.7%); Group 3 – premature aging group (51 women, 34.9%). Immune response to cytomegalovirus was studied using methods of enzyme immunoassay and indirect immunofluorescence.

Results. Comparing immune response to cytomegalovirus in different aging groups, highest titres of both IgG antibodies against early antigens and IgA antibodies against late structural antigens were found in premature aging group. Results showed that premature aging was associated with an increased level of IgA antibodies characteristic for cytomegalovirus symptomatic infection and its frequent reactivations.

Conclusion. Cytomegalovirus infection is associated with an increased risk of premature aging (OR=9.8; P<0.01).

Introduction

Aging process is associated with changes in the immune system, which may also contribute to an increased incidence of various pathological processes such as infections and cancers and autoimmune reactions (1). Such events indicate an impairment of the protective functions of immune system. Substantial changes occur in T-lymphocyte subset, and resulting decreased functionality of T lymphocytes triggers changes in other segments of the immune system (2).

The scientific data show that cytomegalovirus (CMV) is one of the factors that affect functionality of T cells. CMV is a herpes group virus, which remains in the organism for the rest of the life after the primary infection. The cytomegalovirus is widespread in human population (50–100%). Up to 90% of the adult population in Lithuania have antibodies against CMV (3). CMV usually results in an asymptomatic infection in healthy immunocompetent individuals but may manifest as CMV mononucleosis in 10% of adults. However, primary CMV infection and endogenous (previously latent) virus reactivation can cause dangerous diseases and even a high mortality rate in population at high risk: pregnant women and newborns, recipients after organ and bone marrow transplantation, AIDS patients and other immunosupressed individuals. During the life course, CMV can reactivate periodically under the influence of endogenous and exogenous factors and cause immunosuppression, participate in the pathogenesis of autoimmune diseases, cancer, and atherosclerosis, can be the cofactor in AIDS progress, activate other organism viruses: human papillomavirus, HIV, or other herpes viruses (4–6). Immunosupressed patients with CMV infection have alterations in their immune system, which are associated with changes in $CD8^+$ T-lymphocyte subset. CMV infection induces oligoclonal proliferation of $CD8^+$ T cells (7, 8). While studying the peculiarities of aging process, it was noted that CMV seropositivity is associated with many of the same phenotypic and functional alteration to T-cell immunity (9, 10).

Morbidity and mortality due to infectious diseases is greater in the elderly than in the young, at least partly because of age-associated decreased immune competence, which renders individuals more susceptible to pathogens. The role of CMV as one of the main factors

Correspondence to V. Kanapeckienė, Institute of Hygiene, Didžioji 22, 01128 Vilnius, Lithuania
E-mail: virginija.kanapeckiene@hi.lt

in determining the oligoclonal expansion and functional properties of $CD8^+$ T cells and differences in phenotypes of these cells has been well documented (10–12). Longitudinal studies of the elderly in Sweden described a concept of "the immune risk phenotype" and demonstrated that it has some predictive input towards morbidity. The "immune risk phenotype" is closely associated with CMV seropositivity (13, 14). These data suggest that immunosenescence may be contagious (14, 15).

As it was mentioned, CMV seropositivity correlates with substantial alterations in the immune repertoire in human, *i.e.* with an increase in $CD8^+$ T-cell count and changes in phenotypes of lymphocyte subpopulations. CMV-specific cells may constitute up to 25% of all $CD8^+$ T cells in subpopulation. Such response of $CD8^+$ T cells to one virus may affect the diversity of immune responses and lead to the impairment of responses to other pathogens (16–18).

Since there are no reported data regarding particularities of immune response to CMV in the elderly by groups of different course of aging, the purpose of our study was to evaluate the association between CMV infection and character of aging (slow, physiological, and premature).

Materials and methods

Immune response to CMV was examined in 146 elderly women aged 60–90 years who were followed up at the Center of Gerontology and Rehabilitation, Institute of Experimental and Clinical Medicine (Table 1). The women were selected after clinical evaluation of their health status according to anamnesis (diseases, traumas, and medications), self-evaluation (complaints, ailments, pains, *etc.* during one year before entering the study), objective data (examination, auscultation, blood pressure measurements, ECG), laboratory analysis (general blood and urine analysis, content of hematocrit, glucose, creatinine, urea, cholesterol, beta-lipoproteins, and triglycerides in blood serum), and morbidity analysis data based on the immunological examination. Individuals with severe chronic diseases (malignant tumors, serious renal and hepatic disorders, diabetes mellitus, and autoimmune diseases) and other dangerous diseases that could distort the natural process of aging were excluded from the study.

Biological age (BA) was calculated using the standard program of multiple regression analysis based on physiological indices of the main systems of the organism and specials tests (19). Subjects with BA deviation from calendar age less than 5 years were attributed to the group of physiological aging (58 individuals). In 51 individuals, BA deviation from calendar age was more than 5 years, and they comprised the group of retarded aging.

An indirect immunofluorescence assay was used for the detection of IgG and IgM antibodies against CMV-EA (early antigens) and CMV-LA (late antigens). Preparations with viral CMV-EA and CMV-LA for indirect immunofluorescence were prepared at the Laboratory of Virology, Institute of Hygiene (according to W. Reynolgd, 1979). According to the character, frequency and intensity of luminescence, the presence of antibodies in serum dilution and serum titre were determined on luminescence microscope. A final dilution when it is still possible to detect specific luminescence is considered serum titre. Moreover, IgG and IgM antibodies were detected also using the enzyme-linked immunoenzyme assay (ELISA). Immunoenzyme systems (anti-CMV-IgG and anti-CMV-IgM) were designed in the Virology Laboratory of Institute of Hygiene (20).

Functions of peripheral blood lymphocytes were determined by their response to phytohemagglutinin. Antibodies to native DNA titres were estimated by ELISA and performed by a technique described by the manufacturer (NOVA Diagnostics, Inc., San Diego, CA 92131).

Results of CMV infection serological analysis and immunological assay and data on the women's age, character of aging were stored in Excel database. For statistical analysis Stat graphic version 5 software and GLIM statistical package were used. For comparison of quantitative variables (quantities and titers of antibodies) in particular groups, a multifactor analysis of variance (ANOVA) was used. The results were considered statistically significant at $P \leq 0.05$. Polynomial logistic regression was used in the case-control study,

Table 1. **Distribution of females by age group and character of aging**

Age group	Slow aging	Physiological aging	Premature aging	Total
60–74 years	13	39	43	96
75–90 years	24	19	8	50
Total	37	58	51	146

seeking to evaluate association between the character of aging and CMV infection.

Results

After examination of immune responses to CMV in 146 elderly women, the values of serological indices of CMV infection in women by different aging groups were compared using a multifactor variance analysis. The lowest count of IgG antibodies against CMV (in enzyme immunoassay (EIA) units) was found (in slow aging group). The mean values of IgG antibodies, in EIA units, were lower in this group of women than those in both physiological and premature aging groups, *i.e.* 39.7 (30.6–48.8), 51.2 (44.9–57.6), and 48.7 (41.8–55.7), respectively; the difference, however, was statistically insignificant (P=0.118). The levels of IgM antibodies against CMV in all groups were similar because these antibodies appear at the beginning of infection or during recurrent infection and are found in blood serum during the acute phase of infection. The levels of IgM antibodies (in EIA units) found in slow aging, physiological aging, and premature aging groups were 27.7 (22.8–31.8), 27.3 (21.2–34.2), and 30.6 (25.7–35.5), respectively.

Titres of IgG and IgA antibodies against CMV-EA and CMV-LA were detected using an indirect immunofluorescence (IF). Interestingly, titres of IgA antibodies against CMV-LA varied significantly by aging group. IgA antibodies are usually produced during active CMV infection (during reactivation and chronic infection) or during infection that manifests clinically (clinical CMV syndrome). Geometric mean titres of these antibodies in slow, physiological, and premature aging groups were 75.9 (55.7–103.5), 68.0 (54.6–83.9), and 129.9 (95.5–157.6), respectively (P=0.0004).

Differences were also significant in titres of IgG antibodies against CMV-EA (P=0.042). The mean titres of IgG antibodies in slow, physiological, and premature aging groups were 5.58 (3.19–9.88), 10.80 (7.39–15.96), and 13.87 (9.03–21.33), respectively. There were no statistically significant differences in geometric mean titres of IgG antibodies against CMV-LA by aging groups (21, 22).

Since immune responses to CMV by aging groups varied substantially, we evaluated the association between slow aging and physiological aging, premature aging and physiological aging, and a character of CMV infection (titres of IgG and IgA antibodies against CMV-EA and CMV-LA antigens, determined by indirect immunofluorescence).

A case-control study was performed, where the control group included women from physiological aging group; the case groups included women from the group of premature aging and group of slow aging. Polynomial logistic regression analysis, when age was considered, showed that premature aging of women is strongly associated with a pronounced response of IgA antibodies to CMV-LA, when titre of IgA antibodies against CMV-LA is 1:128 or higher (Table 2). The odds ratio (OR) adjusted for age, when high titres of IgA antibodies against CMV-LA were found in women, was 9.8 (3.67–26.0), P<0.001. Therefore, individuals with very high titres of IgA antibodies against CMV-LA in blood

Table 2. **Association of premature and physiological aging with cytomegalovirus infection**

Antibody titres against CMV	Premature aging (case) N=51	Physiological aging (control) N=58	OR* (95% CI)
IgG anti-CMV-EA			
<8 (no antibodies)	9	13	1.0**
≥8	42	45	1.58 (0.59–4.22)
IgG anti-CMV-LA			
≤128	18	18	1.0**
256 and >	33	40	0.9 (0.39–2.04)
IgA anti-CMV-EA			
<8 (no antibodies)	33	31	1.0**
≥8	18	27	0.68 (0.31–1.53)
IgA anti-CMV-LA			
≤64	10	37	1.0**
128 and >	41	21	9.8 (3.67–26.0)
P			P<0.001

*Odds ratios (OR) adjusted for age. **Comparison index.
CMV – cytomegalovirus, EA – early antigens, LA – late antigens.

Table 3. **Association of slow and physiological aging with cytomegalovirus infection**

Antibody titres against CMV	Slow aging (case) N=37	Physiological aging (control) N=58	OR* (95% CI)
IgG anti-CMV-EA			
<8 (no antibodies)	8	13	1.0**
≥8	29	45	1.28 (0.45–3.63)
IgG anti-CMV-LA			
≤128	16	18	1.0**
256 and >	21	40	0.51 (0.20–1.27)
IgA anti-CMV-EA			
<8 (no antibodies)	14	31	1.0**
≥8	23	27	1.97 (0.80–4.86)
IgA anti-CMV-LA			
≤64	15	37	1.0**
128 and >	22	21	1.82 (0.75–4.40)

*Odds ratio (OR) adjusted for age. **Comparison index.
CMV – cytomegalovirus, EA – early antigens, LA – late antigens.

are at an almost 10-fold higher risk of premature aging.

No association was found between premature aging and physiological aging, slow aging and physiological aging and elevated titres of IgG and IgA antibodies against CMV-EA (OR=1.58, OR=0.68 and OR=1.28, OR=1.97, respectively, P>0.05), and no relationship was observed between premature aging and physiological aging, slow aging and physiological aging and IgG antibodies against CMV-LA (OR=0.9 and OR= 0.51, P>0.05) (Table 2 and 3). The presence of these antibodies is not associated with either greater risk of premature aging or possibility of slow aging.

A reduced peripheral lymphocyte response to phytohemagglutinin was found in all groups of women by different aging course; however, in premature aging group, lymphocytes response was low in almost 50% of women, with functional activity of lymphocytes in the range of 10–17%. The percentage of women with such response in both physiological and slow aging groups was only 21%.

Antibodies to native DNA (in low titres) were found in 90% of women by all aging groups, but in premature aging group, antibodies to native DNA were mostly in high titres.

Discussion

Studies on the aging phenomenon and mechanisms associated with it have received considerable attention over the last decades. Understanding the aging etiology and age-related problems can also inform public policy so that the proper supportive environments can be planned in advance as people are interested in healthy aging. This knowledge will provide vital insights into complex and inevitable factors of aging and aid in developing measures for lifespan extension, an integral indicator of health.

Our studies revealed that CMV status might determine a character of aging in humans; namely, premature aging is associated with high immune response to CMV (particularly, IgA antibodies). And it means that after primary contact with CMV, these individuals during lifetime had recurrent reactivations of the latent virus or even chronic infection, which manifested itself by persistent viral replication in the host cells (leukocytes, cells of multiple organs such as saliva gland, kidney, *etc.*). Accordingly, all these mechanisms associated with CMV infection have impact on immunosenescence.

CMV reactivation occurs mostly in immunosuppressed persons as well as in elderly because the immune system gets weaker with age. During CMV reactivation, the virus induces additional immunosuppression because its replication takes place also in cells of the immune system, *i.e.* in polymorphonuclear leukocytes, resulting in impairment of their functions. Moreover, aging is associated with a marked accumulation of dysfunctional CMV-specific $CD8^+$ T cells together with a decrease in immediate effector function. Therefore, infection with CMV may lead to reduction of prevailing levels of immunity to other viruses. These data implicate CMV as a factor dangerous to immunosuppressed individuals, as well as to the elderly, by suppressing virus-specific immunity during aging. Furthermore, CMV infection may distort the virus-specific immune response and, as it was revealed by our findings, may induce certain pathological immune (autoimmune) reactions.

Our results also are of practical significance: on the basis of results obtained after assessing CMV status and detection high level of antibodies (especially, IgA) to CMV, it is possible to evaluate the status of individual's immune system, its ability to fight infectious pathogens, and presume the process of aging.

Conclusion

Cytomegalovirus infection is associated with an increased risk of premature aging (OR=9.8), and high levels of IgA antibodies to cytomegalovirus late antigens may be used as an informative marker of premature aging.

Citomegalovirusinės infekcijos ir senėjimo proceso ryšys

Virginija Kanapeckienė, Julius Kalibatas, Elvyra Redaitienė, Jelena Čeremnych[1]

Higienos institutas, [1]Vilniaus universiteto Eksperimentinės ir klinikinės medicinos institutas

Raktažodžiai: citomegalovirusas, imuninis atsakas, senėjimas.

Santrauka. Mokslinės literatūros duomenų analizė rodo, jog citomegalovirusinė infekcija turi įtakos žmogaus senėjimo procesams, ypač imuninės sistemos silpnėjimui ir su tuo susijusiu infekcinių ligų bei mirtingumo nuo jų padidėjimu tarp vyresnio amžiaus žmonių.

Darbo tikslas. Įvertinti ryšį tarp citomegalovirusinės infekcijos ir senėjimo eigos (priešlaikinio, fiziologinio ir sulėtėjusio senėjimo).

Medžiaga ir metodai. Ištirtos 146 sveikos vyresnio amžiaus moterys (60–90 metų), kurios pagal specialiąją biologinio amžiaus vertinimo metodiką suskirstytos į tris grupes: pirma grupė – sulėtėjusio senėjimo grupė (37 moterys, 25,4 proc.), antra – fiziologinio senėjimo grupė (58 moterys, 39,7 proc.), trečia – priešlaikinio senėjimo grupė (51 moteris, 34,9 proc.). Imuninis atsakas į citomegalovirusinę infekciją tirtas naudojant imunofermentinį metodą bei netiesioginės imunofluorescencijos metodą.

Rezultatai. Palyginus imuninį atsaką į citomegalovirusinę infekciją skirtingose senėjimo grupėse, didžiausi IgG antikūnų prieš ankstyvuosius antigenus (CMV-AA) titrai ir IgA antikūnų prieš vėlyvuosius struktūrinius antigenus (CMV-VA) titrai rasti priešlaikinio senėjimo moterų grupėje. Nustatyta, kad priešlaikinis senėjimas labai susijęs su padidėjusiu IgA antikūnų, būdingų simptominei citomegalovirusinei infekcijai ir dažnoms infekcijos reaktyvacijoms, kiekiu.

Išvada. Citomegalovirusinė infekcija susijusi su padidėjusia priešlaikinio senėjimo (OR=9,8; p<0,01) rizika.

Adresas susirašinėti: V. Kanapeckienė, Higienos institutas, Didžioji 22, 01128 Vilnius
El. paštas: virginija.kanapeckiene@hi.lt

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