The association between cytomegalovirus infection and aging process

Virginija Kanapecienė, Julius Kalibatas, Elyra Redaitienė, Jelena Čeremnych
Institute of Hygiene, 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 immune-fluorescence.

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 immunosupression, 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
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 particulars 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 anamnestic (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 special 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. Reynolgld, 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 immunoenzymatic 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 multiform analysis of variance (ANOVA) was used. The results were considered statistically significant at P≤0.05. Polynomial logistic regression was used in the case-control study,
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 the 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>
<thead>
<tr>
<th>Antibody titres against CMV</th>
<th>Premature aging (case) N=51</th>
<th>Physiological aging (control) N=58</th>
<th>OR* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG anti-CMV-EA &lt;8 (no antibodies) ≥8</td>
<td>9/42</td>
<td>13/45</td>
<td>1.0** 1.58 (0.59–4.22)</td>
</tr>
<tr>
<td>IgG anti-CMV-LA ≤128 256 and &gt;</td>
<td>18/33</td>
<td>18/40</td>
<td>1.0** 0.9 (0.39–2.04)</td>
</tr>
<tr>
<td>IgA anti-CMV-EA &lt;8 (no antibodies) ≥8</td>
<td>33/18</td>
<td>31/27</td>
<td>1.0** 0.68 (0.31–1.53)</td>
</tr>
<tr>
<td>IgA anti-CMV-LA ≤64 128 and &gt; P</td>
<td>10/41</td>
<td>37/21</td>
<td>1.0** 9.8 (3.67–26.0) P&lt;0.001</td>
</tr>
</tbody>
</table>

*Odds ratios (OR) adjusted for age. **Comparison index.

Medicina (Kaunas) 2007; 43(5)
Table 3. Association of slow and physiological aging with cytomegalovirus infection

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

*Odds ratio (OR) adjusted for age. **Comparison index.
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**

V. Kanapecienė, J. Kalibatė, E. Redaitienė, J. Čeremnych

**Raktas:** Citomegalovirusas, imuninis atsakas, senėjimas.

**Santrauka.** Mokslingos literatūros duomenų analizė rodo, jog citomegalovirusinė infekcija turi įtakos įžmojas senėjimo procesams, ypač imuninės sistemos silpnumą ir su tuo susijusius infekcinių ligų bei mirtingumo nuo jų padidėjimu tarp vyresnio amžiaus žmonių.

**Darbo tikslas.** Ivertinti ryšį tarp citomegalovirusinės infekcijos ir senėjimo eigąs (priešlaikinio, fiziologinio ir sulėtėjusio senėjimo).

**Medžiaga ir metodai.** Ištirtos 146 sveikos vyresnio amžiaus moterys (60–90 metų), kurios pagal specialiają biologinio amžiaus vertinimo metodiką suskirstytos į tris grupes: pirma grupę – sulėtėjusio senėjimo grupę (37 moterys, 25.4 proc.), antra – fiziologinio senėjimo grupę (58 moterys, 39.7 proc.), trečia – priešlaikinio senėjimo grupę (51 moteris, 34.9 proc.). Imuninis atsakas į citomegalovirusinę infekciją tirtas naudojant imunofermentininį metodą bei netiesioginės imunofluorescencijos metodą.

**Rezultatai.** Palyginti imuninių atsakų į citomegalovirusinę infekciją skirtingose senėjimo grupėse, didžiausia IgG antikūnų prieš ankstyvuosius antigenus (CMV-AA) titrai ir IgA antikūnų prieš vėlyvuosius struktūrinus antigenus (CMV-VA) titrai rasti priešlaikinio senėjimo moterų grupėje. Nustatyta, kad priešlaikinis senėjimas labai susijęs su padidėjusiu IgA antikūnų, būdingų simptominei citomegalovirusinėi infekcijai ir dažnoms infekcijos reaktyvacijoms, kiekv. 

**Išvada.** Citomegalovirusinė infekcija susijusi su padidėjusia priešlaikinio senėjimo (OR = 9.8; p < 0.01) rizika.

**References**


**Address:** V. Kanapecienė, Higienos institutas, Didžioji 22, 01128 Vilnius
El. paštas: virginija.kanapeciene@hi.lt


Received 4 May 2006, accepted 19 March 2007

Straipsnis gautas 2006 05 04, priimtas 2007 03 19