Cytomegalovirus Antibodies among Elderly Women with Different Process of Ageing

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The aim of the study was to determine differences in immune response to CMV among elderly women depending on the ageing process and to evaluate association between CMV infection and the character of ageing. The study sample consisted of 146 CMV-positive healthy women of older age (60–95 years). According to a special method for evaluation of biological age they were subdivided into 3 groups: I – group of retarded ageing (37 women, 25.4%), II – group of physiological ageing (58 women, 39.7%), III – group of premature ageing (51 women, 34.9%).

Immune response to CMV in different groups of ageing was compared using multiple factor analysis of variance. Highest levels of IgG class antibodies against early antigens (CMV-AA) and IgA antibodies against late structural antigens (CMV-VA) were detected in the group of premature ageing.

Polynomial logistic regression analysis has shown that premature ageing is strongly related to an increased quantity of IgA class antibodies characteristic of symptomatic CMV infection and reactivations. Persons with high levels of those antibodies are at a risk of premature ageing almost 10 times higher than individuals with low levels of those antibodies (OR = 9.8; $p < 0.01$).

Key words: cytomegalovirus, immune response, ageing

INTRODUCTION

Cytomegalovirus (CMV) is very widely spread in human population. Around 80–90% adults in Lithuania have antibodies reflecting a former contact with this virus (1). Most often, CMV rests in the body in latent form with periodical reactivations that can be provoked by various endogenous (pregnancy) and exogenous (stress, treatment with hormonal and cytostatic medications, another pathogenic agents – HIV, etc.) factors. For people with impaired immunity (recipient after transplantation, AIDS patients), CMV infection can cause serious diseases (pneumonia, hepatitis, retinitis, ulcerous lesions of gastrointestinal tract, etc.) and even become the cause of death (2–4). In such patients, immune system changes typical of CMV infection related with the main immunocompetent cells – T lymphocytes expressing $CD8^+$ receptors. During chronic CMV infection, clonal proliferation of T lymphocytes express $CD8^+CD57^+$ receptors and their expansion instead of former $CD8^+CD57^-$ lymphocytes (5, 6).

Similar immune system changes related to the character of CMV infection take place even among healthy people: clonal proliferation of $CD8^+CD57^+$ receptor expressing lymphocytes that becomes especially evident in older age (7–10).

Studying the peculiarities of ageing and their dependence on various endogenous and exogenous factors, attention was drawn to immunological changes taking place in humans during ageing and manifested by disregulation of immune system, clonal changes of T lymphocytes expressing $CD8^+CD57^+$ receptors (11–13).

Immune response to CMV reflects the character of a long-term interaction between the virus and the human body, when the virus is latent during a long period or is frequently reactivated. In some individuals, during all their life low level of antibodies is found, and in others high levels of antibodies persist for many years (14, 15).

Studies of immune response to CMV revealed that for active CMV infection manifested by active virus reproduction or virus excretion with liquids, synthesis of antibodies against non-structural early antigens (CMV-AA) induced in cells by CMV and the former intense CMV antigen stimulation due to symptomatic infection, frequent reactivation episo-

des or due to chronic CMV infection are reflected by high quantities of antibodies against late structural CMV antigens (CMV-VA) (16–18).

It has been determined that immune response to CMV is sex- and age-dependent. Our studies confirmed the results of other authors that the number of individuals with high levels of IgG antibodies against CMV-AA increases dramatically with age (especially in women) (19–21). However, young and average age individuals (up to 60 years) were included into these studies. We have found no data on the peculiarities of immune response among older people, and especially on the relation between ageing and CMV infection. As the literature provides abundant data on immunosuppressive characteristics of CMV and age-related changes of immune system (22, 23), we consider that it is necessary to determine the serological markers of CMV infection among individuals of older age (over 60 years) as well.

The aim of this study was to determine the immune response to CMV differences among women of older age depending on the character of ageing (retarded, physiological and premature) and to evaluate the association between CMV infection and the character of ageing.

MATERIALS AND METHODS

Immune response to CMV was examined in 146 women of older age (60–90 years), inhabitants of the city of Vilnius. The women were selected after clinical evaluation of their health status according to anamnestic data, subjective evaluation of their general condition during the last years, laboratory analysis and morbidity data according to their medical records, data on immunological examination. Individuals with severe chronic diseases that could distort the natural process of ageing were excluded from the study. Biological age (BA) was calculated using the standard programme of multiple regression based on physiological indices of the main systems of the organism and special tests (24). Subjects with BA deviation from calendar age less than 5 years were attributed to the group of physiological ageing (58 individuals). For 51 individuals, BA deviation from calendar age was higher than 5 years, and they formed the group of premature ageing. For 37 individuals BA was behind the calendar age by more than 5 years, and they comprised the group of retarded ageing.

For determination of IgG and IgA class antibodies against CMV-AA and CMV-VA antigens, the method of indirect immunofluorescence (IIF) was used. IIF preparations with viral CMV-AA and CMV-VA antigens were prepared at the Virology Laboratory, Institute of Hygiene (according to W. Reynolds, 1979). According to the character, frequency and intensity of luminescence, the presence of antibodies in serum dilufson and serum titre were determined on a luminescence microscope. The final solution where specific luminescence is still visible is considered as serum titre. Moreover, IgG and IgM antibodies were determined also using the method of enzyme immunoassay (ELISA). Immunoenzyme anti-CMV-IgG and anti-CMV-IgM systems designed at the Virology Laboratory, Institute of Hygiene, and registered by the State Drug Control Service at the Ministry of Health of Lithuania in 1996 (certificates No. 8/3775/96 and No. 8/3776/96) were used (25)

Results of CMV infection serological analyses and data on the individual's age, sex and the character of ageing process were stored in *Excel* spreadsheets. For statistical analysis, *Statgraphic* programme version 5 and *Epiinfo* version 6 were used. For comparison of quantitative variables (quantities and titres of antibodies) in particular groups and determination of their relations, a multifactor analysis of variance (ANOVA) and the evaluation error p (at the confidence interval 95%) were used; $p < 0.05$ was considered as significant. Polynomial logistic regression was used in case-control study, seeking to evaluate the relation between the character of CMV infection and the cases studied (the course of ageing process: physiological and retarded ageing, physiological and premature ageing (GLIM statistical package).

RESULTS AND DISCUSSION

After examination of immune response to CMV of 146 women of older age, the values of serological CMV infection indices in the groups with different ageing process were compared using multifactor analysis. According to ELISA, the lowest quantities of IgG class antibodies against CMV were found in the group of retarded ageing (Table 1). The mean values of IgG antibodies in ELISA units in this group were lower – 39.7 (30.6–48.8) than in groups of physiological and premature ageing – 51.2 (44.9–57.6) and 48.7 (41.8–55.7), respectively, but the difference was statistically insignificant ($p = 0.118$).

In different ageing groups highest differences were found in IgA class antibodies against CMV-VA titre, determined by IIF (Table 2). IgA class antibodies usually are produced during active CMV infection, which often manifests clinically. Mean geometric titres of these antibodies in the group of retarded ageing were 75.9 (55.7–103.5), of physiological 68.0 (54.6–83.9), and in the group of premature ageing reached 129.9 (95.5–157.6). The difference

Table 1. **Quantities of IgG and IgM class antibodies to CMV in the blood of women with different processes of ageing**

Antibodies	Total n = 146	Type of ageing			p
		Retarded n = 37	Physiological n = 58	Premature n = 51	
	Mean values (95% CI) in ELISA units				
IgG	46.6 (42.2–50.9)	39.7 (30.6–48.8)	51.2 (44.9–57.6)	48.7 (41.8–55.7)	0.118
IgM	28.6 (25.5–31.7)	27.7 (22.8–31.8)	27.3 (21.2–34.2)	30.6 (25.7–35.5)	0.600

Table 2. **Titres of IgG and IgA class antibodies against CMV-AA and CMV-VA in the blood of women with different processes of ageing**

Antibodies	Total n = 146	Type of ageing			p
		Retarded n = 37	Physiological n = 58	Premature n = 51	
	Mean geometric titres (95% CI)				
IgG against CMV-AA	9.49 (7.24–12.30)	5.58 (3.19–9.88)	10.80 (7.39–15.96)	13.87 (9.03–21.33)	0.042*
IgG against CMV-VA	257.2 (212.7–314.2)	230.4 (154.5–343.8)	301.9 (208.5–399.4)	247.2 (181.3–333.6)	0.445*
IgA against CMV-AA	3.10 (2.42–3.98)	3.74 (2.22–6.36)	3.06 (2.12–4.35)	2.61 (1.77–3.90)	0.556
IgA against CMV-VA	86.49 (74.4–100.5)	75.9 (55.7–103.5)	68.0 (54.6–83.9)	129.9 (95.5–157.6)	0.000*

* The difference is statistically significant.

was statistically significant ($p < 0.05$). Differences were also significant in titres of IgG antibodies against CMV–AA.

On proving the differences of immune response to CMV in groups of different ageing, we made an attempt to assess the relations between the types of retarded and physiological, premature and physiological ageing with the character of CMV infection (titres of IgG and IgA class antibodies against CMV-AA and CMV-VA antigens, determined by IIF).

A case-control study was made, where women with physiological ageing formed the control group and the case groups consisted of women with premature and retarded ageing. Polynomial logistic regression analysis when age was considered revealed that premature ageing was closely related with pronounced response of IgA class antibodies against CMV-VA when the titre of IgA antibodies against CMV-VA was 1:128 and higher (Table 3). The adjusted chance ratio OR taking into account the age revealed that when the titres of IgA antibodies against CMV-VA were high, comparing with the group of women with low titres it was 9.8 (3.67–26.0), $p < 0.001$, implying that individuals with very high blood serum titres of IgA antibodies against CMV-VA are at an almost 10-fold higher risk of premature ageing.

We have found no association between premature and physiological ageing, retarded and physiological ageing with high levels of IgG and IgA class antibodies against CMV-AA antigens (OR = 1.58, OR = 0.68 and OR = 1.28, OR = 1.97, respectively, $p > 0.05$). We have found no relations with IgG class antibodies against CMV-VA antigens, either (OR = 0.9 and OR = 0.51, $p > 0.05$) (Tables 3 and 4). Detection of these antibodies is related neither to an increased risk of premature ageing nor to probability of retarded ageing.

These results are very interesting from the scientific point of view, as there are no data concerning the influence of CMV infection on the process of ageing. Studies of ageing become more and more actual, as people want to remain active and preserve working capacity till well-advanced age. It is also important to increase the average life expectancy, thus research for the new factors that have an impact on ageing is continuing. CMV is more often activated among elderly people, as it is well known

Table 3. **Association of premature and physiological ageing with CMV infection**

Antibody titres against CMV	Premature ageing (case) n = 51	Physiological ageing (control) n = 58	OR*
IgG anti-CMV-AA			
<8 (no antibodies)	9	13	1.0**
≥8	42	45	1.58 (0.59–4,22)
IgG anti-CMV-VA			
≤ 128	18	18	1.0**
256 and >	33	40	0.9 (0.39–2.04)
IgA anti-CMV-AA			
<8 (no antibodies)	33	31	1.0**
≥8	18	27	0.68 (0.31–1.53)
IgA anti-CMV-VA			
≤64	10	37	1.0**
128 and >	41	21	9.8 (3.67–26.0)
p			p < 0.001

*Adjusted OR, age taken into account. ** Index for comparison.

Table 4. **Association of retarded and physiological ageing with CMV infection**

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

*Adjusted OR, age taken into account. ** Index for comparison.

that the immune system gets weaker with age. During such reactivations CMV increases immunosuppression even more, as it is reproduced in cells of the human immune system (polymorphonuclear leukocytes) and can modify their functions.

On the other hand, our results are of practical significance as well: after examination of immune response to CMV and detection of high levels of anti-CMV antibodies, it is possible to evaluate the status of individual's immune system and the process of ageing.

CONCLUSION

Our study on the differences of immune response to CMV related to the process of ageing revealed that the character of CMV infection can be related with an increased risk of premature ageing, and a high titre of IgA antibodies against CMV-VA can be an informative index of premature ageing.

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VYRESNIO AMŽIAUS MOTERŲ CITOMEGALOVIRUSINĖS INFEKCIJOS SKIRTUMAI SENSTANT

S a n t r a u k a

Darbo tikslas – nustatyti imuninio atsako skirtumus į citomegalo virusą vyresnio amžiaus moterų organizme priklausomai nuo senėjimo proceso ir įvertinti CMV infekcijos ir senėjimo pobūdžio ryšį. Ištirtos 146 CMV atžvilgiu seropozityvios sveikos vyresnio amžiaus (45–95 metų) moterys pagal specialią biologinio amžiaus įvertinimo metodiką suskirstytos į 3 grupes: I – sulėtinto senėjimo grupė (37 moterys, 25,4%), II – fiziologinio senėjimo grupė (58 moterys, 39,7%), III – priešlaikinio senėjimo grupė (51 moteris, 34,9%).

Imuninis atsakas į CMV daugiafaktorinės dispersinės analizės metodu palygintas įvairiose senėjimo grupėse. Didžiausi IgG klasės antikūnų prieš ankstyvuosius antigenus (CMV-AA) titrai ir IgA antikūnų prieš vėlyvuosius struktūrinius antigenus (CMV-VA) titrai rasti priešlaikinio senėjimo moterų grupėje.

Atlikę daugybinės logistinės registracijos analizę, nustatėme, kad priešlaikinis senėjimas labai susijęs su padidėjusiu IgA klasės antikūnų, būdingų simptominei CMV infekcijai ir dažnoms reaktyvacijoms, kiekiu. Asmenų su aukštu šių antikūnų titru priešlaikinio senėjimo rizika yra beveik 10 kartų didesnė nei asmenų, kuriems šių antikūnų rasta nedaug (OR = 9,8; $p < 0,01$).

Raktažodžiai: citomegalo virusas, imuninis atsakas, moterų senėjimas