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RESEARCH ARTICLE

Cytomegalovirus (CMV), oxidative stress, and inflammation: implications for immunosenescence and age-related diseases in the MARK-AGE population

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Abstract Cytomegalovirus (CMV) drives immunosenescence, while its reactivation is associated with inflammation and oxidative stress. This study investigates the interplay between CMV, oxidative stress and inflammation in a cohort of 2065 age-stratified individuals randomly recruited from the general population (RASIG), as part of the MARK-AGE study, to better understand the role of CMV in immunosenescence and its potential impact on age-related diseases. CMV IgG titers were associated with oxidative stress, antioxidant, and inflammatory biomarkers. Stepwise-linear regression identified positive associations with age, BMI, apolipoprotein J (ApoJ/Clu), ceruloplasmin, α-2-macroglobulin, proteasome peptidase activity, and malondialdehyde,

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and negative associations with α-tocopherol, selenium, and vitamin D. Notably, the associations with ApoJ/Clu and proteasome peptidase activity represent novel findings that point to a potential involvement of proteostasis dysregulation and cellular stress responses in CMV-related immune alterations. Quartile-based analyses revealed significantly lower antioxidant levels (α-tocopherol, selenium, ascorbic acid and vitamin D) and higher oxidative stress markers (plasma 8-isoprostanes, malondialdehyde) in the highest quartile (Q4) compared to lower quartiles. Inflammatory markers (homocysteine, ceruloplasmin and α-2-macroglobulin) ApoJ/Clu and proteasome peptidase activity were elevated in Q4. This group also exhibited a higher prevalence of cardiovascular diseases, hypertension, and diabetes. This study highlights a link between CMV IgG titers, oxidative stress, inflammation, and the prevalence of cardiovascular and metabolic diseases. Our findings suggest that CMV may contribute to immunosenescence through mechanisms involving redox imbalance and dysregulation of protein degradation pathways. Further research is needed to explore the role of CMV reactivation in aging, and its impact on age-related metabolic and cardiovascular diseases.

Keywords CMV · Aging · Redox balance · Oxidative stress · Inflammation · ApoJ/Clu · Proteasome

Introduction

Cytomegalovirus (CMV), a member of the beta-herpesvirus family, establishes a persistent latent infection with periodic episodes of reactivation. CMV infects approximately 60% of the global population, with prevalence increasing significantly with age, ranging from 40% in young adults (18–24 years old) to over 90% in older adults (75–80 years old) (Badami et al. 2009). While CMV infections are typically asymptomatic in healthy individuals, reactivation in immunocompromised individuals or neonates can cause severe diseases (Sissons and Wills 2015). Although the precise molecular mechanisms behind CMV reactivation remain unclear, oxidative stress has been suggested as a contributing factor (Scholz et al. 1996). Recent studies have further demonstrated that lipid peroxidation byproducts, such as malondialdehyde (MDA), may enhance CMV promoter activity (Jaganjac et al. 2010).

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Aging is a multifaceted process influenced by genetic, environmental, and social factors, and is often accompanied by a progressive decline in physiological function and an increased risk of age-related diseases, including cardiovascular disease, cancer, and neurodegenerative conditions (Sen et al. 2016). One of the hallmark changes of aging involves the immune system, which experiences a decrease in thymic output of naïve lymphocytes and an accumulation of chronic antigenic stress, particularly from infections such as CMV (Saavedra et al. 2023). This leads to immune cell senescence and the development of the senescence-associated secretory phenotype (SASP), characterized by the secretion of pro-inflammatory cytokines, chemokines, and proteases (Chandrasekaran et al. 2017; Pole et al. 2016; Saavedra et al. 2023). The accumulation of these senescent cells, both in immune and non-immune tissues, contributes to chronic low-grade inflammation, a phenomenon known as"inflammaging"(Franceschi et al. 2000; Fulop et al. 2017).

CMV infection has been identified as a significant contributor to immunosenescence, further exacerbating inflammaging through persistent immune activation (Aiello et al. 2019; Pawelec 2012). Immunosenescence, affects both innate and adaptive immunity, and plays a critical role in the increased susceptibility to chronic age-related diseases among the elderly (Barbe-Tuana et al. 2020; De Martinis et al. 2005; Franceschi et al. 1995).

Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is closely linked to both inflammation and aging. Although ROS play a crucial role in cellular homeostasis, signal transduction, and gene expression at low levels (Kumar and Pandey 2015), their excessive accumulation can lead to oxidative damage in cellular components including DNA, proteins, and lipids (Zhang et al. 2016), thereby promoting chronic inflammation. This process contributes to the development of numerous age-related diseases and the progressive deterioration of health that accompanies aging.

Despite increasing evidence of the involvement of CMV in promoting inflammation and immune dysregulation, its potential role in driving redox imbalance and oxidative stress in the general population remains underexplored. Prior studies have often focused on specific subgroups (e.g., elderly, immunocompromised patients), with limited investigation of large-scale, community-based cohorts. Moreover, few studies have simultaneously assessed both oxidative stress and inflammatory biomarkers in relation to CMV IgG titers, and none, to our knowledge, have evaluated the associations with emerging biomarkers of proteostasis and redox-sensitive pathways such as ApoJ/Clu and proteasome activity.

This study aims to fill these gaps by investigating the association between CMV IgG titers and a broad panel of redox, antioxidant, and inflammatory biomarkers in a large, well-characterized, population-based cohort from the MARK-AGE study. Specifically, we sought to identify biological signatures of CMV exposure potentially relevant to immunosenescence, and to explore their relationship with age-related clinical phenotypes such as cardiovascular and metabolic diseases.

Methods

Study population, recruitment, data, and blood collection

MARK-AGE is a European-wide cross-sectional population study aimed at the identification of biomarkers of aging (Burkle et al. 2015). In the present study we used peripheral blood from 2065 randomly recruited age-stratified individuals from the general population (RASIG sample) covering the age range 35–75 years. The study population represented various geographical regions across Europe including Italy, Greece, Austria, Finland, Germany, Belgium and Poland (Table 1S supplementary materials). Details of the recruitment procedures and the collection of anthropometric clinical and demographic data, as well as of laboratory parameters assays have already been reported (Capri et al. 2015; JanSen et al. 2015; Moreno-Villanueva et al. 2015a; Moreno-Villanueva et al. 2015b). Briefly, anti-coagulated whole blood, obtained by phlebotomy after overnight fasting, was collected and stored at − 80 °C. Samples were then shipped from the various recruitment centers to the MARK-AGE Biobank located at the University of Hohenheim, Stuttgart, Germany. From the Biobank, coded samples were sent to the IRCCS INRCA on dry ice, where they were stored at − 80 °C until use.

Table 1 Characteristics of study MARK-AGE population

	CMV IgG Q1 n. 571	CMV IgG Q2 n. 521	CMV IgG Q3 n. 522	CMV IgG Q4 n. 451	*p* value
Age (years)	53.54 ± 0.48**	55.70 ± 0.48**	56.52 ± 0.49^{+}	57.94 ± 0.52	< 0.01
Females (n %)	273 (47.8%)	258 (49.5%)	292 (55.9%)*	266 (52.7%)*	< 0.01
WBC ($\times 10^3/\mu L$)[a]	5.85 ± 0.06°	6.21 ± 0.14	6.31 ± 0.31	6.32 ± 0.08	NS
Neutrophils ($\times 10^3/\mu L$)[a]	3.39 ± 0.05	3.45 ± 0.09	3.49 ± 0.17	3.58 ± 0.07	NS
Lymphocytes ($\times 10^3/\mu L$)[a]	1.8 ± 0.04°	2.01 ± 0.05	2.06 ± 0.11	2.00 ± 0.03	0.030
Monocytes ($\times 10^3/\mu L$)[a]	0.47 ± 0.01	0.50 ± 0.01	0.47 ± 0.01	0.49 ± 0.01	NS
Platelets ($\times 10^3/\mu L$)[a]	238.0 ± 2.5	231.5 ± 2.7	235.6 ± 2.7	236.3 ± 2.9	NS
NLR[a]	2.03 ± 0.05^{+}	1.84 ± 0.05	1.80 ± 0.06	1.79 ± 0.06	< 0.05
PLR[a]	149.0 ± 4.4**	137.3 ± 4.7	133.2 ± 4.7	126.6 ± 5.0	< 0.01
TC (mmol/L)[a]	5.56 ± 0.04	5.546 ± 0.04	5.58 ± 0.04	5.55 ± 0.03	NS
LDL (mmol/L)[a]	3.31 ± 0.04	3.32 ± 0.04	3.34 ± 0.04	3.25 ± 0.04	NS
TG (mmol/L)[a]	1.28 ± 0.04	1.39 ± 0.04	1.37 ± 0.04	1.34 ± 0.04	NS
FG (mmol/L)[a]	5.2 ± 0.05	5.3 ± 0.05	5.2 ± 0.05	5.1 ± 0.06	NS
BMI	25.7 ± 0.2**	25.9 ± 0.2**	25.8 ± 0.2**	27.2 ± 0.2	< 0.001
Current smokers (n, %)	80 (14.0%)$^{+}$	99 (19.0%)	95 (18.2%)	101 (22.4%)	< 0.01
CVD patients (n, %)	67 (11.7%)$^{+}$	77 (14.8%)	90 (17.2%)	88 (19.5%)	< 0.01
Hypertension (n, %)	95 (16.7%)$^{+}$	120 (23.0%)	124 (23.8%)	129 (28.7%)	< 0.001
Diabetes (n, %)	14 (2.5%)$^{+}$	29 (5.6%)	21 (4.0%)	28 (6.2%)	< 0.05

ANCOVA: Analysis of covariance; Q: quartiles of CMV IgG titers; WBC: white blood cells, NLR: Neutrophil-to-Lymphocyte Ratio, PLR: Platelet-to-Lymphocyte Ratio, TC: total cholesterol, HDL: high-density lipoprotein cholesterol, LDL: Low- density lipoprotein cholesterol, TG: triglycerides, FG: fasting glucose, BMI: Body mass index, CVD: cardiovascular diseases

[a]ANCOVA analysis correcting for age, sex, country, smoking and BMI

*$p < 0.05$ compared to Q1 and Q2; °$p < 0.05$ compared to Q1, Q2, Q3; **$p < 0.01$ compared to Q4; $^{+}p < 0.05$ compared to Q4

Sero-positivity for HIV, hepatitis B virus (except seropositivity by vaccination) and HCV represented exclusion criteria. All data adhered to the principles outlined in the Declaration of Helsinki. Ethical approval for the MARK-AGE project was granted by the Local Research Ethics Committees of the respective recruiting centers and the study was retrospectively registered with the German Clinical Trials Register (DRKS00007713; Ethics Committee No.: 2008-075-f, Ethik-Kommission bei der Landesärztekammer Baden-Württemberg). Informed consent was obtained from all participants in the study.

Determination of total glutathione in whole blood

Total glutathione levels in whole blood were measured by RP-HPLC with UV–Vis detection after reduction and modifications as previously reported (Weber et al. 2017).

Determination of ascorbic acid, nitric oxide, and uric acid in plasma

Plasma ascorbic acid and uric acid were analyzed by RP-HPLC and UV detection after reduction with tris-(2-carboxyethyl)-phosphine. The details of the method have been previously reported (Weber et al. 2017). The concentration of the oxidized metabolites of NO was determined with Nitric Oxide Assay Kit (Abcam, ab65328), which provides an accurate measure of total nitrate/nitrite reflecting nitric oxide amount in samples. The detection limit of the assay is approximately 1 nmol nitrite/well.

Determination of carotenoids, tocopherols, and retinol in plasma

The carotenoids (α-carotene, β-carotene, β-cryptoxanthin, lutein, zeaxanthin, and lycopene), α-tocopherol, γ-tocopherol, and retinol were analyzed in plasma and determined simultaneously by RP-HPLC with UV and fluorescence detection as previously described (Stuetz et al. 2016).

Determination of Se in plasma

Plasma Se levels were determined by a Thermo XII Series ICP–MS device (Thermo Electron, Waltham, MA, USA) as previously reported (Giacconi et al. 2023). Plasma samples were centrifuged at 20,000×*g* for 10 min, and the supernatants were diluted 1:20 (total volume 1 ml) with a diluent containing 0.1% Triton X-100 (BDH Chemicals), 0.1% Trace Select Ultra HNO_3 (Sigma–Aldrich), and 10 ppb Rh (Merck) as an internal standard. External multielement calibration solutions containing Se (blank and 0.5–500 ppb) were prepared by serial dilution of a parent multielement solution (Inorganic Ven-tures) using the same diluent used for the samples. Data (three repeats per sample) were acquired for ^{80}Se.

Malondialdehyde, protein carbonyl analysis, 3-nitrotyrosine and isoprostanes in plasma

Plasma malondialdehyde was determined by RP-HPLC coupled with fluorescence detection after derivatization with thiobarbituric acid as previously described (Weber et al. 2017). The determination of plasma protein carbonyls and 3-nitrotyrosine was performed by a sensitive ELISA method as previously reported (RampelLi et al. 2013; Weber et al. 2014). The levels of plasma isoprostanes were detected by a Human 8-epi-prostaglandin F2 alpha (8-iso-PGF2α) time-resolved fluorescence immunoassay on an AutoDELFIA® system (PerkinElmer).

CMV IgG antibody titer

CMV-specific IgG levels in serum were measured using the DRG CMV IgG ELISA Kit (EIA-3468), in accordance with the manufacturer's specifications (DRG International Inc., United States). Each assay was performed using positive and negative controls and standard curves to ensure accuracy and reliability. The specificity and sensitivity of the kit were greater than 98%. The detection range of this assay is between 1.18 and 80 U/mL. According to guidelines CMV IgG levels greater than 11 U/mL were considered positive.

ApoJ/Clu

Quantitative measurement of serum ApoJ/CLU levels was conducted using an ELISA, following the established protocol described previously (Katsiki et al. 2004).

Proteasome peptidase activity

The chymotrypsin-like peptidase activity of the proteasome was measured using a spectrofluorometric assay with the fluorogenic peptide N-succinyl-Leu-Leu-Val-Tyr-aminomethyl coumarin (LLVY-AMC) as a substrate (Carrard et al. 2003).

Statistical analysis

Subject characteristics were reported as mean ± standard error of the mean (SEM) or percentages for continuous and categorical variables, respectively. All variables were examined for normality using the Kolmogorov–Smirnov test and visually inspected using histograms and Q–Q plots. Variables showing significant skewness were log-transformed prior to analysis. A linear regression analysis using stepwise methods was carried out to explore the main predictors of CMV IgG titers. The variables inserted were: CMV IgG levels, sex, country, age, smoking habit, BMI, hemoglobin, red blood cells, leucocyte count, platelets, C-reactive protein (CRP), ceruloplasmin, fibrinogen, homocysteine, albumin, lipid profile, plasma and urinary creatinine, fasting glucose, ferritin, γ-glutamyl-transferase, alanine aminotransferase, plasma cysteine, adiponectin, lycopene, protein carbonyls, plasmatic and urinary 8-isoprostane, carotenoids, glutathione, plasma selenium (Se), nitric oxide, 3-nitrotyrosine, Apolipoprotein J (ApoJ/Clu), α−2-macroglobulin, proteasome peptidase activity, malondialdehyde (MDA), uric acid, ascorbic acid, 25-Hydroxy-Vitamin D. In the linear regression analysis, we used a stepwise method based on biologically plausible predictors selected a priori. Therefore,

no additional correction for multiple testing (e.g., FDR) was applied. To assess multicollinearity in the regression model, Variance Inflation Factor (VIF) was calculated for all predictors. All VIF values were below the standard threshold of 5, indicating no significant multicollinearity. ANCOVA, adjusted for age, sex, and country, was used to evaluate differences in redox and inflammatory parameters across CMV IgG quartiles. Bonferroni correction was applied for multiple comparisons between quartiles.

All analyses were performed using the SPSS/Win program (Version 29.0.1.0 (171); SPSS Inc., Chicago, IL).

Results

Characteristics of the MARK-AGE population

Quartile stratification was chosen to explore potential non-linear associations and to better capture population heterogeneity in CMV IgG distributions, as no universally accepted clinical thresholds are currently available for the IgG titer interpretation in this context. Table 1 summarizes the main characteristics of the MARK-AGE population, categorized into CMV IgG quartiles (Q1, Q2, Q3, and Q4). Participants in Q4 were older and had a higher BMI compared to those in the other quartiles. The proportion of females was higher in Q4 and Q3 than in Q1 and Q2. Notably, Q1 showed a lower lymphocyte count, while Q4 exhibited a higher prevalence of smoking, cardiovascular diseases, hypertension, and diabetes. These patterns suggest increasing immune and metabolic dysregulation across CMV IgG quartiles.

Biomarkers associated with CMV IgG titer

Linear regression analysis using the stepwise method was performed to identify independent biomarkers associated with CMV IgG titers (Table 2). CMV IgG levels were positively associated with age, BMI, ApoJ/Clu, ceruloplasmin, α−2-macroglobulin, proteasome peptidase activity, and MDA. Conversely, a negative association was observed with α-tocopherol, plasma Se levels and vitamin D. These associations highlight the multifactorial nature of CMV-related changes, involving both inflammatory and oxidative stress pathways.

Table 2 Stepwise linear regression between CMV IgG titer and different parameters in the MARK-AGE population

	Unstandardized coefficients		Standardized coefficients	
	B	SEM	Beta	*p* value
Age	0.351	0.109	0.085	0.001
Countries	3.290	0.551	0.158	< 0.001
BMI	1.123	0.256	0.115	< 0.001
ApoJ/Clu	0.460	0.063	0.192	< 0.001
α−2-Macroglobulin	0.182	0.036	0.132	< 0.001
Se	−0.250	0.050	−0.138	< 0.001
α-tocopherol	−0.497	0.156	−0.081	0.002
Proteasome peptidase activity	0.029	0.007	0.116	< 0.001
Ceruloplasmin	0.517	0.157	0.085	0.001
Vitamin D	−0.133	0.061	−0.056	0.031
MDA	10.511	5.090	0.054	0.039

BMI: Body mass index, Se: Selenium, SEM: standard error of the mean, MDA: malondialdehyde, B (Unstandardized Coefficients): change in the dependent variable for a one-unit increase in the independent variable, holding other variables constant, Beta (Standardized Coefficients): effect of the independent variable in standard deviation units, allowing comparisons across variables with different scales

Quartiles of CMV IgG levels according to oxidative stress and inflammatory parameters.

To evaluate the relationship between CMV exposure and biological aging markers, we analyzed antioxidant and oxidative stress biomarkers across CMV IgG quartiles. Individuals in the highest quartile (Q4) had lower levels of antioxidants, including ascorbic acid, α-tocopherol, Se, and vitamin D (Fig. 1a–d; $p < 0.01$), and higher levels of oxidative stress markers, such as malondialdehyde (MDA) and plasma 8-isoprostanes (Fig. 1e–f; $p < 0.05$ and $p < 0.01$, respectively), as well as increased uric acid (Fig. 1g; $p < 0.05$).

Inflammatory markers such as homocysteine (Fig. 2a; $p < 0.05$), α−2-macroglobulin (Fig. 2b; $p < 0.05$) ceruloplasmin (Fig. 2c; $p < 0.01$), were also increased in Q4 compared to lower quartiles, supporting a link between CMV IgG titers and systemic inflammation. All differences reported were statistically significant after Bonferroni correction unless otherwise noted.

No significant associations were found for any other oxidative and antioxidant parameters analyzed

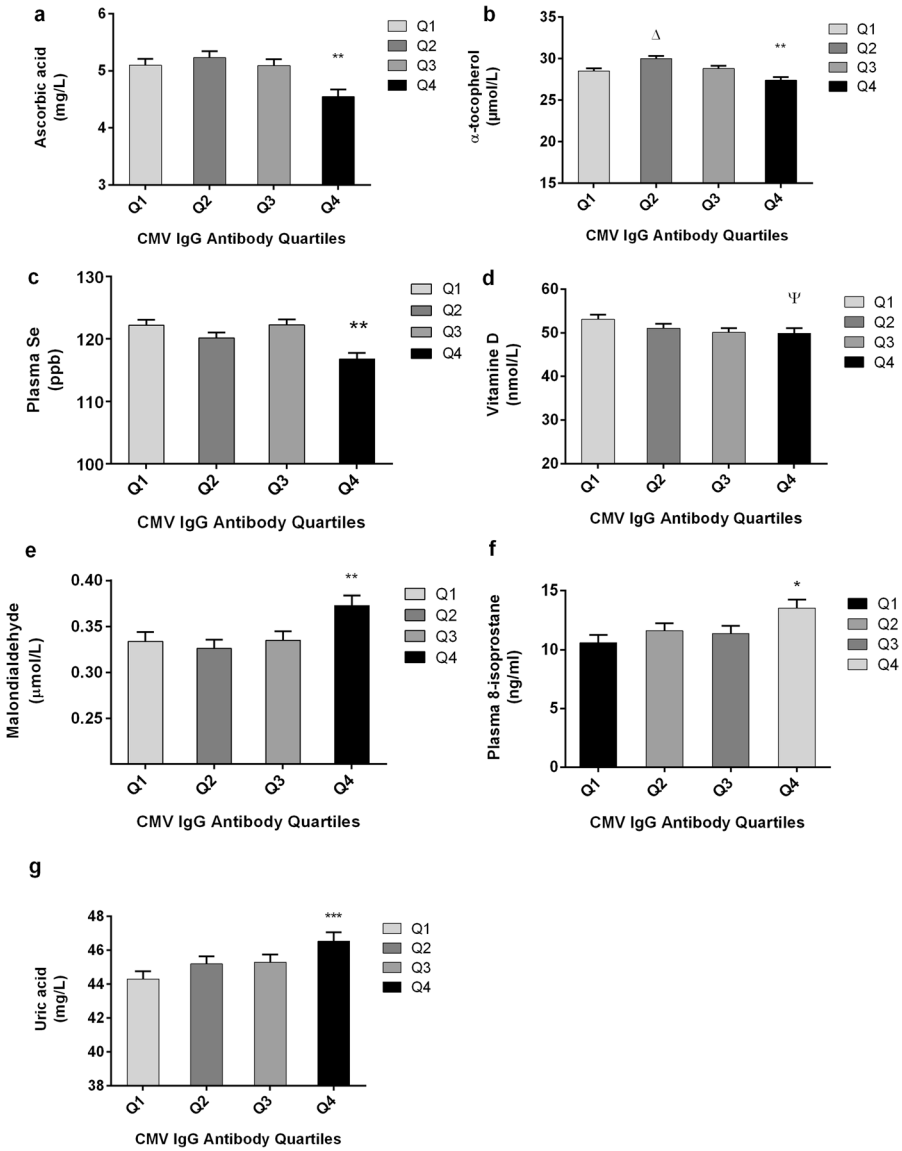

Fig. 1 Relationship between CMV IgG quartiles and oxidative and antioxidant parameters in the RASIG population. The fourth quartile (Q4) of CMV IgG showed the lowest plasma levels α-tocopherol ascorbic acid and plasma Se compared to the other quartiles. In addition, α-tocopherol were significantly increased in the second quartile compared to Q1, Q3 and Q4 quartiles. Vitamin D was diminished in Q4 compared to Q1 quartile. Q4 showed higher levels of malondialdehyde compared to the other quartiles as well as increased uric acid levels when compared specifically to Q1 and Q2. ANCOVA correcting for age, sex, country, smoking and BMI was applied. * $p < 0.05$; **$p < 0.01$. ***$p < 0.05$ Q4 compared to Q1 and Q2. Ψ $p<0.05$ Q4 compared to Q1; Δ $p<0.01$ Q2 versus Q1, Q3 and Q4 quartiles. ANCOVA: Analysis of covariance; Q: quartiles of CMV IgG titers; Se: Selenium

(plasma cysteine, adiponectin, lycopene, protein carbonyls, urinary 8-isoprostane, carotenoids, glutathione, nitric oxide, 3-nitrotyrosine; Table 2S, supplementary material).

Association between CMV IgG antibody levels and biochemical markers

Building on the significant results obtained from stepwise linear regression, we further investigated ApoJ/Clu and proteasome peptidase activity. Both ApoJ/Clu and proteasome peptidase activity were significantly elevated in Q4 compared to the other quartiles (Fig. 3; $p<0.01$). Intermediate quartiles (Q2–Q3) showed graded responses, indicating a possible dose–response relationship (Fig. 3; $p<0.05$).

These findings suggest a role for proteostasis dysregulation in CMV-related immune changes.

Discussion

Cytomegalovirus (CMV), a member of the Herpesviridae family, has a notable ability to persist for life within the human host, often remaining asymptomatic. Despite this long-term coexistence, CMV

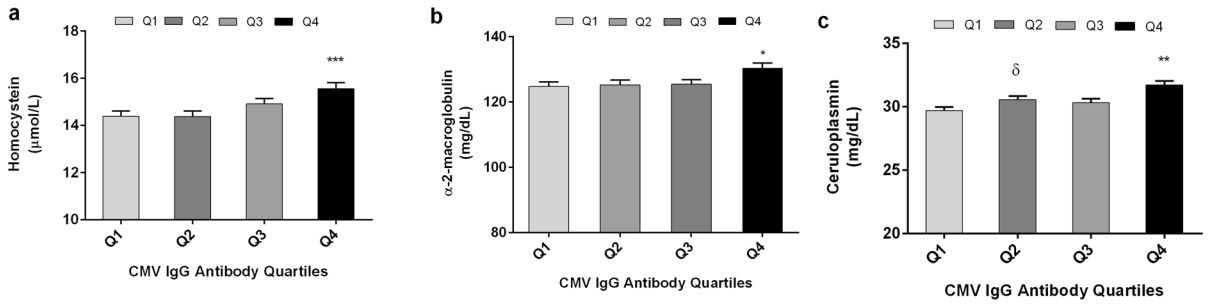

Fig. 2 Relationship between CMV IgG quartiles and inflammatory parameters in RASIG population. The fourth quartile (Q4) of CMV IgG antibodies was associated with elevated levels of homocysteine, α−2-macroglobulin, and ceruloplasmin compared to the other quartiles. Additionally, ceruloplasmin levels showed a significant difference between the second quartile (Q2) and the first quartile (Q1). ANCOVA correcting for age, sex, country, smoking and BMI was applied. * $p < 0.05$ and ** $p < 0.01$ as compared Q4 with other quartiles. *** $p < 0.05$ when compared Q4 versus Q1 and Q2. δ $p < 0.05$ Q2 versus Q1. ANCOVA: Analysis of covariance; Q: quartiles of CMV IgG titers

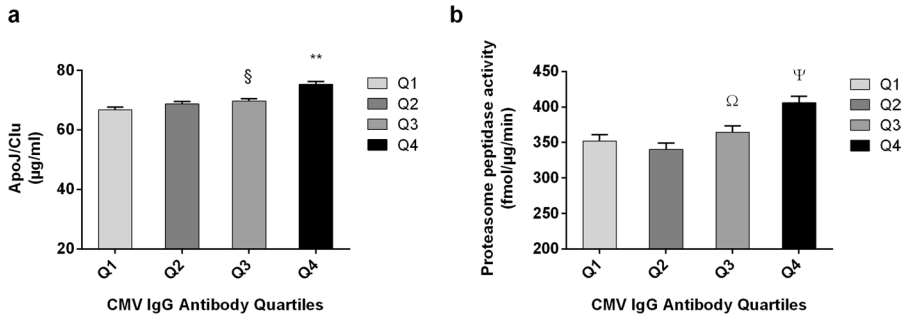

Fig. 3 Relationship between CMV IgG quartiles and biochemical markers in RASIG population. The fourth quartile (Q4) of CMV IgG antibodies was associated with elevated levels of plasma ApoJ/Clu and Proteasome peptidase activity compared to the other quartiles. In addition, ApoJ/Clu levels were significantly higher in Q3 compared to Q1, while proteasome peptidase activity was significantly greater in Q3 than in Q2. ANCOVA correcting for age, sex and country was applied. ** $p < 0.01$ and Ψ $p < 0.001$ as compared Q4 with other quartiles. § $p < 0.05$ when compared Q3 vs Q1. Ω $p < 0.05$ Q3 vs Q2. ANCOVA: Analysis of covariance; Q: quartiles of CMV IgG titers

has been associated with significant alterations in the immune system during aging. The progressive accumulation of senescent, dysfunctional T-cells, driven by CMV, can lead to chronic inflammation and age-related diseases (Moro-Garcia et al. 2018; Savva et al. 2013; Soderberg-Naucler 2006; Spyridopoulos et al. 2016). The presence of CMV IgG antibodies in peripheral blood is an indicator of past CMV infection. Viral reactivation is more frequent in older adults than in younger individuals (Stowe et al. 2007; Thomasini et al. 2017), and this reactivation is thought to be a marker of immunosenescence, although immunosenescence itself may also contribute to latent virus reactivation (Forte et al. 2020; Tu and Rao 2016). CMV latency results from a balance between minimal viral replication, the activity of specific CD8 + T cells, and stable circulating levels of antibodies against the virus (Griffiths et al. 2013; Koch et al. 2006; Sansoni et al. 2014). While CMV reactivation is often asymptomatic, especially when viral loads are low, it can trigger inflammation by promoting the secretion of pro-inflammatory cytokines (Bennett et al. 2012). This, in turn, leads to increased production of ROS, which not only induce further inflammatory responses (Vida et al. 2014), but also, when persistently generated by immune cells, cause cellular damage, recruit additional inflammatory cells, and further amplify oxidative and inflammatory stress (Federico et al. 2007; Khansari et al. 2009).

In this context, we investigated the association between CMV IgG levels, inflammatory, and oxidative markers in 2,065 individuals aged 35–75 years, as part of the MARK-AGE study. Stepwise linear regression analysis revealed that CMV IgG levels were positively associated with age, BMI, ApoJ/Clu, ceruloplasmin, α−2- macroglobulin, proteasome peptidase activity, and MDA. In contrast, negative associations were found with α-tocopherol, Se, and vitamin D. We also observed no significant associations for certain redox biomarkers, such as protein carbonyls, nitric oxide, urinary 8-isoprostane, glutathione, lycopene, and some carotenoids, which may reflect weaker biological links or measurement variability.

Further analysis based on CMV IgG quartiles highlighted a more pronounced inflammatory response in individuals with higher CMV IgG levels. Specifically, elevated inflammatory markers such as homocysteine, and acute-phase proteins like ceruloplasmin, and α−2 macroglobulin, were observed in those with the highest CMV IgG levels. These findings suggest that increased levels of inflammatory markers, associated with high CMV IgG titers, reflect a stronger immune response to CMV reactivation, aimed at controlling viral replication (Gianella et al. 2014).

Given the strong interrelation between inflammation and oxidative stress, we also examined key oxidative markers, including plasma 8-isoprostanes, malondialdehyde (MDA), and uric acid.

MDA and 8-isoprostanes, the main biomarkers of polyunsaturated fatty acid peroxidation (Mas-Bargues et al. 2021), are elevated in diabetes, cardiovascular diseases, and conditions involving vascular damage (Ahmed et al. 2006; Cherneva et al. 2017; Li et al. 2024b). Uric acid, produced from purine nucleotide degradation, displays both antioxidant and pro-oxidant effects in vitro, although its exact role in modulating oxidative stress remains uncertain (Packer 2020). Elevated uric acid levels, however, have been linked to a higher risk of severe coronary artery disease and adverse cardiovascular outcomes (Li et al. 2024a; Wang et al. 2024).

To evaluate redox balance in MARK-AGE subjects, we also assessed antioxidant compounds, including α-tocopherol, α-carotene, Se, ascorbic acid, and vitamin D, given their involvement in preventing lipid peroxidation. α-tocopherol and ascorbic acid, in particular, work synergistically to inhibit lipid peroxidation by regenerating the tocopherol radical (Sies and Stahl 1995). Furthermore, Se is essential for the activity of Glutathione Peroxidase 4 (GPx4), a key seleno-peroxidase that serves as a cornerstone of the cellular anti-peroxidant defense system (Ursini and Maiorino 2020). In subjects with the highest CMV IgG levels, the increase in oxidative markers and decrease in antioxidants suggest a shift toward redox imbalance and enhanced lipid peroxidation. This is further evidenced by the elevated plasma levels of MDA and 8-isoprostanes in the highest quartiles of CMV IgG.

Elevated CMV IgG titers have been associated with an increased risk of subclinical CMV reactivation (Kawamura et al. 2021). We hypothesize that oxidative stress plays a role in this reactivation, as supported by several studies. It has been demonstrated that pretreatment with ascorbic acid significantly reduced CMV antigen expression and viral load in vitro (Cinatl et al. 1995). Gutiérrez-Salinas et al. (Gutiérrez-SaLinas and Cruz-Tovar 2009) observed elevated MDA levels and altered antioxidant enzyme activity in children with CMV infections, further suggesting that oxidative stress contributes to CMV pathophysiology. Similarly, other researchers (Scholz et al. 1996; Speir 2000) have reported that oxidative stress enhances CMV replication and promoter activation in endothelial cells.

The exact mechanism by which oxidative stress induces CMV reactivation remains unclear. However, it may involve the activation of redox-sensitive transcription factors such as nuclear factor kappa B (NF-κB) and activator protein 1 (AP-1) (Karin and ShauLian 2001; Morgan and Liu 2011). The human CMV replication cycle is characterized by the sequential expression of immediate early (IE), early (E), and late (L) genes. The IE proteins, particularly the 72- and 86-kDa isoforms (IE1 and IE2), play a key role in initiating the lytic cycle and are essential for CMV pathogenesis (Scholz et al. 2001). During latency, IE gene expression is suppressed, resulting in reduced viral gene activity and the absence of productive replication (Cheng et al. 2017; Goodrum 2016). The CMV's IE promoter contains a high density of transcription factor binding sites, including motifs for NF-κB and AP-1 (Gutiérrez-SaLinas and Cruz-Tovar 2009; Heald-Sargent et al. 2020). Elevated levels of reactive oxygen species (ROS) activate NF-κB, which in turn promotes transactivation of the viral IE promoter. Conversely, in vitro treatment

with antioxidants has been shown to inhibit CMV IE gene expression and viral replication (Speir et al. 1996). Interestingly, during latency, CMV actively suppresses ROS production through the expression of the b2.7 long non-coding RNA, which scavenges ROS and limits NF-κB activation (Perera and Sinclair 2022). Moreover, AP-1 directly binds to the IE enhancer region, activating multiple promoters within the IE locus and thereby facilitating CMV reactivation from latency (Speir et al. 1996). In addition, lipid peroxidation products such as 4-hydroxy-2-nonenal (HNE), malondialdehyde (MDA), and other α,β-unsaturated aldehydes have also been implicated in the reactivation of CMV (Jaganjac et al. 2010).

CMV has evolved a complex relationship with the host immune system, not only by exploiting redox-sensitive transcriptional pathways to facilitate reactivation, but also by targeting protein homeostasis and immune surveillance mechanisms to ensure persistence (Forte et al. 2020).

Notably, we observed a positive association among ApoJ/Clu and proteasome peptidase activity with CMV IgG levels. ApoJ, also known as clusterin, is a multifunctional glycoprotein involved in lipid recycling, immune regulation, and cellular protection against stress (Trougakos 2013). Although its direct role in CMV infections has not been studied well, some evidence suggests that elevated ApoJ may influence viral infections by modulating pro-inflammatory processes, potentially through the formation of the membrane attack complex (MAC) involving complement components C5b, C6, and C7 (Frost et al. 2022). Alternatively, ApoJ might facilitate viral replication by stabilizing viral proteins, as observed in other viral infections (Lin et al. 2014). Proteasome activity, essential for intracellular protein degradation and antigen processing (Demartino and Gillette 2007; EhLinger and Walters 2013; Finley 2009; GOPEN_TAG_NAME_YEAR_TAGLickman and Ciechanover 2002), is a critical target in CMV's immune evasion strategy. CMV encodes immunoevasins such as US2 and US11, which reroute MHC class I molecules to the cytosol for proteasome-mediated degradation, thereby impairing antigen presentation and CD8 + T-cell recognition (Tortorella et al. 2000; Tran et al. 2010).

Beyond this, CMV extensively reprograms the ubiquitin–proteasome system to degrade host restriction factors, facilitate viral protein turnover, and suppress host antiviral responses (Le-TrilLing and Trilling 2020). This targeted manipulation of the proteasome not only promotes viral replication, but also contributes to the establishment of latency and immune evasion. Notably, pharmacological inhibition of the proteasome has been shown to impair CMV replication (Kaspari et al. 2008), underscoring its functional relevance.

Taken together, these findings support a model in which CMV reactivation and immune evasion are coordinated through redox-sensitive transcriptional regulation and manipulation of proteostasis pathways. Our observed associations with ApoJ/Clu and proteasome activity may reflect host responses to latent viral reactivation or, conversely, viral strategies to subvert host defenses.

Conclusions

Our findings indicate that high CMV IgG concentrations are associated with increased inflammation and redox imbalance, which may contribute to CMV reactivation and the progression of age-related diseases. These results support the role of CMV in immunosenescence and highlight its potential impact on cardiovascular and metabolic health. Further research is needed to clarify the relationship between CMV IgG levels and plasma viremia, as well as to improve our understanding of the clinical implications for the aging population. Given the observational nature of this study, these findings should be confirmed through longitudinal or interventional studies.

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Writing – Review & Editing; M. M.: Writing – Review & Editing; R. G.: Supervision, Conceptualization, Software, Writing – Review & Editing. All authors revised and approved the contents of the manuscript, contributed to the article, and approved the submitted version.

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Data availability Data will be available under reasonable request to the corresponding author.

Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval All data adhered to the principles outlined in the Declaration of Helsinki. Ethical approval for the MARK-AGE project was granted by the Local Research Ethics Committees of the respective recruiting centers and the study was retrospectively registered with the German Clinical Trials Register (DRKS00007713; Ethics Committee No.: 2008-075-f, Ethik-Kommission bei der Landesärztekammer Baden-Württemberg). Informed consent was obtained from all participants in the study. Informed consent was obtained from all subjects involved in the study.

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