Negative Association of Cognitive Performance With Blood Serum Neurotoxicity and Its Modulation by Human Herpes Virus 5 (HHV5) Seropositivity in Healthy Women

Neuroscience Insights
Volume 19: 1–8
© The Author(s) 2024
Article reuse guidelines:
sagepub.com/journals-permissions
DOI: 10.1177/26331055241258436

Lisa M James[1,2,3], Effie-Photini Tsilibary[1,2], Erik J Wanberg[1] and Apostolos P Georgopoulos[1,2,3,4]

[1]The Healthy Brain Aging Group, Brain Sciences Center, Department of Veterans Affairs Health Care System, Minneapolis, MN, USA. [2]Department of Neuroscience, University of Minnesota Medical School, Minneapolis, MN, USA. [3]Department of Psychiatry, University of Minnesota Medical School, Minneapolis, MN, USA. [4]Department of Neurology, University of Minnesota Medical School, Minneapolis, MN, USA.

ABSTRACT: Identification of early influences on cognitive decline is of paramount importance in order to stem the impacts of decrements in cognitive functioning and to potentially intervene. Thus, here we focused on 132 healthy adult women (age range 26-98 years) to (a) determine whether factors circulating in serum may exert neurotoxic effects in vitro, (b) evaluate associations between serum neurotoxicity and cognitive performance, and (c) assess the influence of human herpes virus (HHV) seroprevalence and other factors on apoptosis and cognitive performance. The results documented that the addition of serum from healthy adult women to neural cell cultures resulted in apoptosis, indicating the presence of circulating neurotoxic factors in the serum. Furthermore, apoptosis increased with age, and was associated with decreased cognitive performance. Stepwise regression evaluating the influence of 6 HHVs on apoptosis and cognitive function revealed that only HHV5 (cytomegalovirus; CMV) seropositivity was significantly associated with apoptosis and cognitive decline, controlling for age. These findings document neurotoxic effects of serum from healthy women across the adult lifespan and suggest a unique detrimental influence associated with CMV seropositivity.

KEYWORDS: Apoptosis, human serum, neurotoxicity, human herpes viruses, cytomegalovirus, neuroblastoma N2A cells

RECEIVED: December 21, 2023. **ACCEPTED:** May 15, 2024.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Partial funding for this study was provided by the University of Minnesota (the Anita Kunin Chair in Women's Healthy Brain Aging, the Brain and Genomics Fund, the McKnight Presidential Chair of Cognitive Neuroscience, and the American Legion Brain Sciences Chair) and the U.S. Department of Veterans Affairs. The sponsors had no role in the current study design, analysis or interpretation, or in the writing of this paper. The contents do not represent the views of the U.S. Department of Veterans Affairs or the United States Government.

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Lisa M James, Department of Neuroscience, University of Minnesota Medical School, 1 Veterans Dr BSC 11B, Minneapolis, MN 55417, USA. Email: lmjames@umn.edu.

Introduction

Humans are exposed to myriad natural and synthetic neurotoxins, several of which have been implicated in neurodegeneration.[1-8] Neurotoxins circulate in the blood and, therefore, are readily detected in blood and serum samples.[9] Under healthy conditions, the brain is protected from exposure to neurotoxins via the blood-brain barrier (BBB); however, breakdown of the BBB enables entry of harmful substances into the brain, ultimately contributing to neuronal dysfunction and neurodegeneration.[10,11] Several factors including sex, age, temperature, physical exercise, neurotransmitters, inflammatory substances, and inflammatory markers, among others, influence BBB permeability.[12,13] In addition, infection with neurotropic viruses results in structural and functional changes in several components of the BBB, thereby affecting permeability and permitting entry of viruses and other pathogens into the brain.[14]

Numerous viruses are neurotropic and therefore capable of entering the nervous system and causing immediate or delayed neuropathological changes and disease.[15] Human herpes viruses (HHVs) are among the most common viruses worldwide and are associated with numerous and varied human health conditions.[16-19] After initial infection, typically in childhood, HHVs establish latency and may be periodically reactivated by various factors including stress, fever, and UV exposure, as well as age-related immunological changes and conditions and/or treatments associated with immunosuppression.[20,21] HHVs vary in terms of cell tropism. Some HHVs are neurotropic and establish latency within the nervous system; several others establish latency elsewhere (eg, B cells, monocytes) but are capable of entering the brain via the bloodstream or peripheral nerves. Consequently, several HHVs have been documented in brain tissue,[15,22-26] and increasing evidence points to a role of HHVs in conditions affecting the brain.[19,25,27-37] HHVs are ubiquitous; consequently, nearly 100% of adult humans are seropositive for at least 1 type of HHV. Five HHVs are most commonly implicated in human disease—namely, herpes simplex virus type 1 (HSV/HHV1) and 2 (HSV/HHV2), varicella zoster virus (VZV/HHV3), Epstein-Barr virus (EBV/HHV4), cytomegalovirus (CMV/

Creative Commons Non Commercial CC BY-NC: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 License (https://creativecommons.org/licenses/by-nc/4.0/) which permits non-commercial use, reproduction and distribution of the work without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (https://us.sagepub.com/en-us/nam/open-access-at-sage).

HHV5).[21] Burgeoning evidence also links HHV6 and HHV7 to neurodegenerative diseases.[26,38-41]

Here, we bridge these separate lines of research implicating neurotoxins and HHVs in neuropathology by assessing the effects of serum obtained from healthy adult women on neural cell cultures and with regard to HHV seropositivity. Previous research in our lab and elsewhere has documented neurotoxic effects of serum from patient populations[42] and older adults[43] on neural and hippocampal progenitor cell cultures, respectively. It is not clear whether findings documenting the presence of harmful substances circulating in the blood in those populations extend to younger, healthy samples; however, since subtle neuropathological changes are evident decades before symptom onset and clinical diagnoses,[44] it is important to assess for the presence of potentially neurotoxic factors in healthy individuals across the lifespan. Thus, we first evaluated whether the addition of serum from healthy adult women to neural cell cultures resulted in apoptosis, and assessed the correspondence between apoptosis and cognitive performance. Then, in light of immense literature implicating HHVs in conditions affecting the brain, we evaluated apoptosis and cognitive performance with regard to seropositivity of neurotropic HHVs. Since apolipoprotein E and body mass index (BMI) have been shown to influence cognitive function,[45,46] we also evaluated and controlled for their influence in this sample.

Materials and Methods

Participants

A total of 132 healthy women participated in the study as paid volunteers. This data was obtained as part of an ongoing longitudinal study involving annual data acquisition; consequently, the number of annual visits varied for participants as follows: 60 participants completed 1 annual visit, 56 completed 2 annual visits, and 16 completed 3 annual visits, for a total of 220 visits. Women were excluded from participation if they had been diagnosed at any point in their lifetime with a neurological disorder, any autoimmune disorder associated with neurocognitive dysfunction (eg, systemic lupus erythematous, rheumatoid arthritis), any major medical condition affecting brain function (eg, brain cancer, head injury with cognitive sequelae), serious psychiatric diagnoses (eg, bipolar disorder, schizophrenia, any history of psychiatric hospitalization), or any recent/current medication or treatment known to affect brain functioning (eg, radiation, chemotherapy). Written informed consent was obtained from study participants. The institutional review board and relevant committees of the Minneapolis VA Health Care System approved the study protocol.

Cognitive assessment

The Montreal Cognitive Assessment (MoCA)[47] was administered to participants to screen for cognitive impairment. The MoCA assesses several domains of cognitive function including executive function, memory, language, and abstract reasoning, among others. Scores for each domain are added to reflect a total score ranging from 0 to 30; total scores greater than 25 are considered normal.[47] At recruitment, all participants had a total MoCA score (without the education point) of greater than 25. MoCA assessment was obtained during all but 1 visit, for a total of 219 MoCA assessments.

ApoE genotyping

DNA samples were genotyped using PCR amplification followed by restriction enzyme digestion as per recommendations.[48] Known apoE isoform standards were included in the analysis.

HHV seropositivity assays

We determined the seroprevalence of IgG antibodies against HHV1, HHV2, HHV3, HHV4, HHV5, and HHV6; we could not obtain reliable results from kits for HHV7 and HHV8. We employed standard method using commercially available indirect Enzyme-Linked Immunosorbent Assays (ELISA). The ELISA tests were performed as per the manufacturer's instructions and recommendations. The specimens were processed using a mini-automated 5-in-1 workstation (Crocodile cat. 84024-01; Berthold Technologies, Oak Ridge, TN, USA). The workstation includes the ELISA microtiter plate reader which was read at dual wavelengths for absorbance at 450 and 620 nm as reference wavelengths. Details of the virus-specific ELISA kits are as follows. (a) HHV1: Human Anti-Herpes simplex virus Type 1 IgG ELISA Kit (HSV1), Abcam Inc., Boston, MA USA, cat. ab 108737; (b) HHV2: Human Anti-Herpes simplex virus Type 2 IgG ELISA Kit (HSV2) Abcam cat. ab 108739; (c) HHV3: Human Anti-Varicella-Zoster virus IgG ELISA Kit (VZV) Abcam cat. ab 108782; (d) HHV4: Human Anti-Epstein Barr virus IgG ELISA Kit (EBV-VCA) Abcam cat. ab 108730; (e) Human Anti-Cytomegalovirus IgG ELISA Kit (CMV) Abcam cat. ab 108724; (f) Human Herpesvirus 6 IgG ELISA Kit cat. KA1457, Abnova, Taiwan. Seroprevalence for 1 or more HHVs were missing for 7 women.

Body mass index (BMI)

BMI was calculated from anthropometric measurements. Weight was not able to obtained for a small number of acquisitions ($n = 7$), precluding BMI calculations in those instances; BMI was available for 213 acquisitions and was used as a continuous measure in analyses.

Neural cell culture

Neuro-2A neuroblastoma (N2A) cells were cultured in Eagle's minimal essential medium (EMEM, ATCC, VA, USA) containing 10% fetal bovine serum (ThermoFisher Scientific, Waltham, MA) poly-D-lysine coated, 24-well plates at a concentration of 30 000 to 50 000/well for 48 to 72 hour. The medium was then changed to Neurobasal containing N2 supplement and L-glutamine (ThermoFisher Scientific, Waltham, MA), in the absence (medium control) or presence of human serum. For all experiments, human serum (10%) was added and the cells were cultured for 2 more days. All experiments were carried out in quintuplicate.

Cell apoptosis with terminal deoxynucleotidyl transferase mediated dUTP Nick End Labeling assay (TUNEL) assay

The extent of cell apoptosis of Neuro-2A cells incubated with women's serum was examined. All samples were examined using 4- and 8-chamber glass slides (ThermoFisher Scientific, Waltham, MA) coated with poly-D-lysine at 50 µg/ml as mentioned above. N2A cells were seeded at a concentration of 50 000 to 100 000 cells per chamber, in 1 ml of Neurobasal/ N2/ L glutamine medium for 2 days. In sequence, 10% of each sample of women's serum, incubated for 60 minutes at 37°C. At the end of the incubation period, the cells were examined for apoptosis. Apoptotic cells were detected using the In Situ Cell Death Detection Kit, TMR red (Terminal deoxynucleotidyl transferase (TdT) enzyme and fluorochrome labeling solution), according to the manufacturer's protocol. Briefly, the cells were fixed in ice-cold methanol for 10 minutes at room temperature, rinsed with PBS and permeabilized with 0.1% Triton X-100 in PBS for 3 minutes on ice. The cells were then incubated with 150 µl of TUNEL reaction mixture for 60 minutes at 37°C in the dark (Insitu Cell Death Detection Kit, TMR red, ThermoFisher scientific, Waltham, MA). The cells were then washed 3X with PBS and Diamond AntiFade mounting medium with 4´,6-diamidino-2-phenylindole (DAPI) stain (ThermoFisher Scientific, Waltham, MA) was used for visualization of nuclei, using the EVOS FL Cell Imaging System (ThermoFisher Scientific, Waltham, MA) or an Olympus 3000 confocal microscope. The 8 to 10 images were obtained from different fields from a minimum of 2 experiments with each different experimental condition. Apoptosis was then calculated with ImageJ software by measuring the number of TUNEL-labeled cells (red nuclei) relative to the total cell number (DAPI-stained nuclei). Since experiments were run in quintuplicate, there were 5 Media and 5 Test apoptosis values per run (A_M and A_T, respectively), for a total of 220 runs × 5/run = 1100 data values for each A_M and A_T. A_M served as the baseline for apoptosis in a particular run and, hence, was used as a covariate in all analyses on A_T.

Data Analysis

MoCA

MoCA values are counts and hence were square-root transformed, a transformation appropriate for the statistical analysis of counts[49]:

$$\text{sMoCA} = \sqrt{MoCA} \quad (1)$$

All MoCA-related statistical analyses were performed using sMoCA.

ApoE

With respect to apoE genotype, 3 apoE isoforms (E4, E3, and E2) are the result of cysteine-arginine interchanges at 2 sites: there are zero interchanges in E4, 1 interchange in E3, and 2 interchanges in E2. The resulting 6 apoE genotypes (E4/4, E4/3, E4/2, E3/3, E3/2, E2/2) yield 5 groups with respect to the number of cysteine residues per mole, as follows. ApoE4/4 contains zero cysteine residues per mole (0-CysR/mole), E4/3 contains 1 (1-CysR/mole), E4/2 and E3/3 each contain 2 (0-CysR/mole), E3/2 contains 3 (0-CysR/mole), and E2/2 contains 4 (0-CysR/mole). The use of the number of cysteine residues per mole to characterize the apoE molecule converts the categorical apoE genotype scale, consisting of 6 distinct genotypes above, to a 5-point continuous scale (0-4 cysteine residues per mole), a conversion that allows the use of statistical analyses suitable for continuous variables (eg, regression) to quantify the relations between various variables and apoE.[50,51] Therefore, we used the number of cysteine residues per mole (CysR/mole) as a covariate to evaluate the possible association of apoE with MoCA and apoptosis. In addition, as a measure complementary to CysR/mole above), we computed a binary variable, E4, based on the presence [apoE4(+) = 1] or absence [apoE4(−) = 0] of the apoE4 allele in the participant's apoE genotype and used it as a fixed group factor in statistical analyses below.

Implementation of statistical analyses

The IBM-SPSS Statistical package (version 29) was used to analyze the data, including Pearson correlation, partial correlation, and analysis of covariance (ANCOVA). All P-values reported are 2-tailed.

Results

Age, MoCA, apoE, BMI

The mean (±SEM) age of the study participants was 70.4 ± 0.79 years, (range 25.7-97.8 years, N = 220), the mean of the MoCA was 27.25 ± 0.16 (range 17-30, N = 219), and the mean BMI was 28.28 ± 0.44 (N = 213). The frequency distributions of age, MoCA, and BMI are shown in Figure 1. The frequency distribution of the apoE genotypes and the number of apoE CysR/mole are given in Tables 1 and 2, respectively;

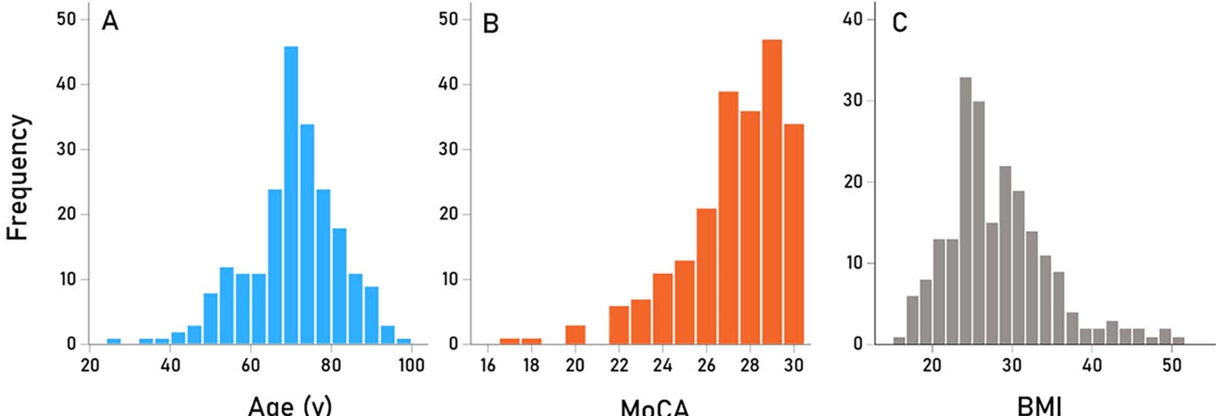

Figure 1. (A) Frequency distribution of ages at time of test. N = 220 participants. (B) Frequency distribution of MoCA scores at time of test. N = 220 participants. (C) Frequency distribution of BMI. N = 213 participants.

Table 1. Frequency distribution of apoE genotypes.

APOE GENOTYPE	FREQUENCY (%)
23	22 (10)
24	4 (1.8)
33	131 (59.8)
34	49 (22.4)
44	13 (5.9)
Total	219 (100)

Table 2. Frequency distribution of number of apoE CysR/mole.

N OF APOE CYSR/MOLE	APOE	FREQUENCY (%)
0	E4/E4	13 (5.9)
1	E3/E4	49 (22.4)
2	E2/E4, E3/E4	135 (61.6)
3	E2/E3	22 (10)
Total		219 (100)

there were no E2/E2 homozygotes. Analyses evaluating associations between sMoCA, apoE, BMI, and age revealed several significant associations. Age was associated with decreased sMoCA (Pearson $r = -.308$, $P < .001$, N = 219; Figure 2A) and decreased BMI ($r = -.373$, $P < .001$, N = 213). BMI was not significantly associated with sMoCA, controlling for age (partial correlation $r = -0.117$, $P = .097$, N = 213). Finally, sMoCA increased significantly with the number of apoE CysR/mole, controlling for age (sMoCA vs CysR/mole, partial correlation = .154, $P = .024$, N = 218) and was significantly higher in the apoE4(+) than the apoE4(−) group ($P = .042$, F-test in ANCOVA, where sMoCA was the dependent variable, apoE4 was a (binary) fixed factor, and age was a covariate).

Apoptosis

Next we evaluated the associations between sMoCA, apoE, BMI, and age with apoptosis. We found that apoptosis increased significantly with increasing age (Partial correlation A_T vs age = .106, controlling for A_M, $P < .001$, N = 1220; Figure 2B) and that sMoCA decreased significantly with apoptosis (Partial correlation sMoCA vs A_T = −0.092, controlling for A_M and age, $P = .002$, N = 1095; Figure 2C). Apoptosis was not significantly associated with BMI (partial correlation A_T vs BMI = −.002, controlling for A_M and age, $P = .941$, N = 1065) or apoE CysR/mole (partial correlation A_T vs number of apoE CysR/mole = .027, controlling for A_M and age, $P = .368$, N = 1095). Similarly, apoptosis A_T did not differ significantly between apoE4(+) and apoE4(−) groups ($P = .737$, F-test in ANCOVA, where A_T was the dependent variable, apoE4 was a binary fixed factor, and A_M and age were covariates).

HHV

The seroprevalence of HHV1-6 are given in Table 3. The effect of HHV seroprevalence on apoptosis was evaluated using a stepwise multiple linear regression, where A_T was the dependent variable, and HHV1, HHV2, HHV4, HHV5, and HHV6 seroprevalence status (coded as binary 0/1 values), age, and A_M were the independent variables. This analysis yielded only HHV5 seroprevalence as having a highly statistically significant positive effect on apoptosis ($P = .005$, *t*-test on the HHV5 partial regression coefficient), in addition to A_M and age (as expected). Collinearity diagnostics confirmed the absence of possible collinearities: all tolerance values were >0.9 and all variance inflation factor (VIF) <1.1. Detailed results of the regression analysis above are given in Table 4.

The HHV5 effect was quantified by performing an ANCOVA where A_T was the dependent variable, HHV5 serostatus was a fixed factor, and A_M, age, number of apoE CysR/mole, and BMI were covariates. The effect of HHV5

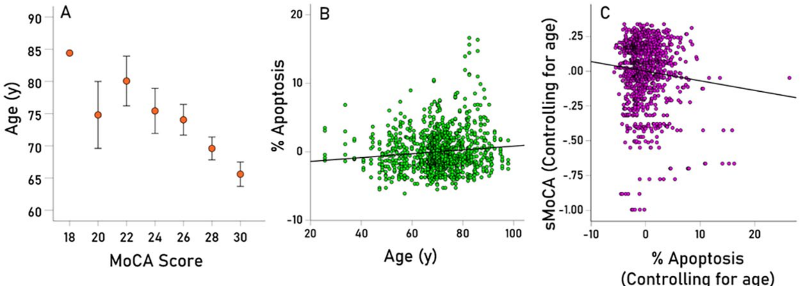

Figure 2. (A) mean (±SEM) MoCA scores are plotted against age. N = 220. (B) % apoptosis A_T (adjusted for A_M) is plotted against age. N = 1100. (C) sMoCA (adjusted for age) is plotted against % apoptosis A_T (adjusted for A_M and age). N = 1100. See text for details.

Table 3. Prevalence of seropositivity for the 6 HHV strains investigated. Numbers in the column Total indicate the number of tests that gave unambiguous results in the ELISA.

HHV STRAIN		N SEROPOSITIVE	TOTAL	PERCENT SEROPOSITIVE
HHV-1	HSV1	110	209	52.6
HHV-2	HSV2	46	216	21.3
HHV-3	VZV	216	216	100.0
HHV-4	EBV	211	218	96.8
HHV-5	CMV	101	218	46.3
HHV-6		200	207	96.6

Table 4. Results of the multiple regression analysis described in the text.

	STANDARDIZED β	*T*-VALUE	*P*-VALUE	TOLERANCE	VIF[a]
HHV-1	–.023	0.719	.472	0.946	1.057
HHV-2	–.018	0.564	.573	0.930	1.075
HHV-4	–.03	0.932	.351	0.953	1.049
HHV-5	**.094**	**2.941**	**.003**	**0.951**	**1.052**
HHV-6	.019	0.600	.548	0.984	1.017
Age (y)	.097	3.050	.002	0.963	1.038
Media (A_M)	.215	6.790	<.001	0.979	1.021

[a]VIF, variance inflation factor. Bold denotes significant effect on HHV seroprevalence on apoptosis.

seropositivity on apoptosis is illustrated in Figure 3. The mean (±SEM) A_T was 3.553% ± 0.119 for HHV5(–) versus 4.063% ± 0.128 for HHV5(+), a 14.3% increase in HHV5(+) over HHV5(–). No other HHV had a significant effect. Finally, HHV5 seropositivity had the following 2 additional MoCA-related effects. First, a significant decrease in MoCA with increasing apoptosis was observed only in the HHV5(+) group (partial correlation of A_T with sMoCA = –.100, *P* = .025, N = 505); for the HHV5(–) group this correlation was .058, *P* = .151, N = 580. And second, a positive association of MoCA with the number of apoE CysR/mole was observed in the HHV5(–) group (partial correlation, controlling for age = .231, *P* = .013, N = 116) but was not present in the HHV5(+) group (partial correlation = .017, *P* = .864, N = 101), indicating a negative HHV5-mediated modulation of the protective effect of apoE CysR/mole on MoCA.

Discussion

In this study we evaluated the effect of serum from healthy women on neural cell cultures and with regard to cognitive performance and HHV seroprevalence. The findings documented the presence of neurotoxic factors, unrelated to BMI or apoE, that resulted in apoptosis and influenced cognitive function. Previous studies have documented serum neurotoxicity in older

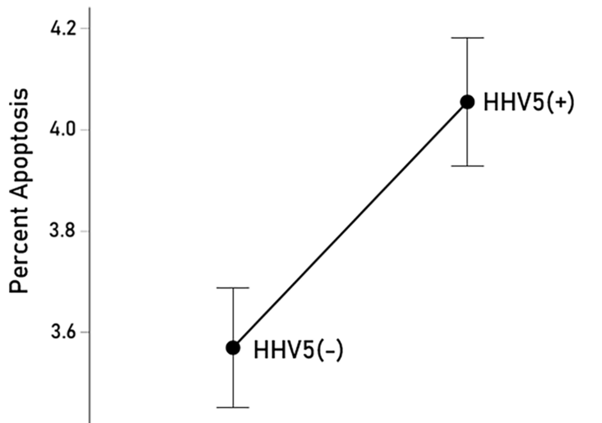

Figure 3. Mean % apoptosis A_T ± SEM (adjusted for A_M, age, apoE CysR/mole, and BMI) is plotted for HHV-5(–) and HHV-5(+) groups.

adults[43] and in certain patient populations[42]; here, we show neurotoxic factors resulting in in vitro apoptosis and decreased cognitive performance in healthy women across the adult lifespan. This finding highlights deleterious effects of neurotoxic factors circulating in the blood. Since HHVs have been widely implicated in conditions affecting the brain, we then evaluated the influence of seroprevalence of 6 HHVs, BMI, and apoE on apoptosis and cognitive performance. The findings documented a unique influence of HHV5/CMV seropositivity on increased apoptosis and decreased cognitive performance. Notably, apoE CysR/mole was associated with protective effects on cognitive performance in CMV seronegative women but not those who were seropositive. Taken together, the findings of this study point to neurotoxic factors in serum of healthy women that may be associated with CMV exposure, adding to the literature regarding the potential for harmful downstream effects of CMV infection on brain health.

CMV is a common infection worldwide[52] that is transmitted congenitally or through direct contact with body fluids. The seroprevalence of CMV increases with age such that approximately half of women of childbearing age and two-thirds of women age 70 to 79 are seropositive.[53,54] Like other human herpes viruses, CMV establishes lifelong persistence characterized by periods of latency and reactivation.[20] Age-related increases in chronic reactivation of CMV,[55] coupled with increased CMV viral load,[56] and CMV-specific adaptive immune system dysregulation (eg, inflation of memory T cells and low or inverted CD4/CD8 ratio)[57,58] exhaust the immune system over time.[57] These effects are thought to contribute to low-grade chronic inflammation (ie, "inflammaging"),[59] immunosenescence,[60] epigenetic aging,[61] and increased mortality.[62,63] To that end, CMV seropositivity has been associated with various chronic health conditions including atherosclerosis[64] and vascular dysfunction,[65] autoimmune disorders,[66] and cancer,[67] as well as age-related frailty[68] (cf Matheï et al[69]), functional impairment,[70] and cognitive decline.[71,72]

Here, we showed correspondence between neurotoxic effects of serum from CMV seropositive women and decrements in cognitive performance. These findings suggest that the presence of harmful agents circulating in the blood of CMV seropositive individuals may impact brain function. Previous studies have identified CMV as a risk factor for cognitive impairment and dementia[71-75] (cf, Matheï et al[69]). Furthermore, CMV is present in a high proportion of brains from vascular dementia patients,[76] and recently, CMV has been shown to induce tau pathology, a hallmark of Alzheimer's dementia.[77] The extent to which the neurotoxic effects of CMV seropositive serum observed in these healthy women is a predictor of future cognitive dysfunction remains to be determined and is an ongoing area of investigation in our lab.

These novel findings implicating CMV in the association between serum neurotoxicity and cognitive function must be considered within the context of study limitations. First, we used neural cell cultures as a proxy for the brain and it is not clear to what extent the deleterious in vitro effects correspond with in vivo effects in the human brain. Remarkably, the in vitro effects were associated with cognitive function suggesting some correspondence between in vitro and in vivo effects. Second, the present study focused exclusively on women. Since previous studies have documented sex-specific neurotoxic effects,[78] it is unknown whether the present findings generalize to men. Third, our analyses were limited to presence or absence of HHV antibodies, precluding evaluation of effects of titer concentration on apoptosis and cognitive performance. Finally, it is worth noting that the present findings do not preclude involvement of other potentially neurotoxic factors; nonetheless, the findings document serum neurotoxicity and decrements in cognitive performance in CMV seropositive women. Consequently, the development of CMV vaccines[79] may be beneficial in preventing CMV-related cognitive decline.

Acknowledgements

We would like to thank Laura Kalipe, Briana Pomonis, and Stacy Dolan for their contributions to data collection.

Author Contributions

APG: conceived the research; LMJ, EPT, and EJW contributed to data acquisition; APG, LMJ, and EPT wrote the paper; all authors edited and approved the final version of the paper.

Data Availability

Data will be available to qualified researchers by the corresponding author on a reasonable request.

ORCID iDs

Lisa M. James https://orcid.org/0000-0001-9021-0690
Apostolos P. Georgopoulos https://orcid.org/0000-0003-4412-725X

REFERENCES

1. Cao Y, Li B, Ismail N, et al. Neurotoxicity and underlying mechanisms of endogenous neurotoxins. *Int J Mol Sci*. 2021;22:12805.
2. Iqubal A, Ahmed M, Ahmad S, et al. Environmental neurotoxic pollutants: review. *Environ Sci Pollut Res*. 2020;27:41175-41198.
3. Pogue AI, Lukiw WJ. Natural and synthetic neurotoxins in our environment: from Alzheimer's disease (AD) to autism spectrum disorder (ASD). *J Alzheimers Dis Park*. 2016;6:249.
4. Richardson JR, Fitsanakis V, Westerink RHS, Kanthasamy AG. Neurotoxicity of pesticides. *Acta Neuropathol*. 2019;138:343-362.
5. Sharma S, Wakode S, Sharma A, et al. Effect of environmental toxicants on neuronal functions. *Environ Sci Pollut Res*. 2020;27:44906-44921.
6. Segura-Aguilar J. On the role of endogenous neurotoxins and neuroprotection in Parkinson's disease. *Neural Regen Res*. 2017;12:897-901.
7. Stokum JA, Cannarsa GJ, Wessell AP, et al. When the blood hits your brain: the neurotoxicity of extravasated blood. *Int J Mol Sci*. 2021;22:5132.
8. Zhao Y, Jaber VR, Pogue AI, et al. Lipopolysaccharides (LPSs) as potent neurotoxic glycolipids in Alzheimer's disease (AD). *Int J Mol Sci*. 2022;23:12671.
9. Cesbron A, Saussereau E, Mahieu L, et al. Metallic profile of whole blood and plasma in a series of 106 healthy volunteers. *J Anal Toxicol*. 2013;37:401-405.
10. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. *Neuron*. 2008;57:178-201.
11. Sweeney MD, Sagare AP, Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat Rev Neurol*. 2018;14:133-150.
12. Almutairi MM, Gong C, Xu YG, Chang Y, Shi H. Factors controlling permeability of the blood-brain barrier. *Cell Mol Life Sci*. 2016;73:57-77.
13. Zhao Y, Gan L, Ren L, et al. Factors influencing the blood-brain barrier permeability. *Brain Res*. 2022;1788:147937.
14. Chen Z, Li G. Immune response and blood-brain barrier dysfunction during viral neuroinvasion. *Innate Immun*. 2021;27:109-117.
15. Ludlow M, Kortekaas J, Herden C, et al. Neurotropic virus infections as the cause of immediate and delayed neuropathology. *Acta Neuropathol*. 2016;131: 159-184.
16. Khalesi Z, Tamrchi V, Razizadeh MH, et al. Association between human herpesviruses and multiple sclerosis: a systematic review and meta-analysis. *Microb Pathog*. 2023;177: 106031.
17. Piotrowski SL, Tucker A, Jacobson S. The elusive role of herpesviruses in Alzheimer's disease: current evidence and future directions. *Neuroimmune Pharmacol Ther*. 2023;2:253-266.
18. Smatti MK, Cyprian FS, Nasrallah GK, et al. Viruses and autoimmunity: a review on the potential interaction and molecular mechanisms. *Viruses*. 2019; 11:762.
19. Wouk J, Rechenchoski DZ, Rodrigues BCD, Ribelato EV, Faccin-Galhardi LC. Viral infections and their relationship to neurological disorders. *Arch Virol*. 2021;166:733-753.
20. Cohen JI. Herpesvirus latency. *J Clin Investig*. 2020;130:3361-3369.
21. Grinde B. Herpesviruses: latency and reactivation - viral strategies and host response. *J Oral Microbiol*. 2013;5:22766.
22. Lin WR, Wozniak MA, Cooper RJ, Wilcock GK, Itzhaki RF. Herpesviruses in brain and Alzheimer's disease. *J Pathol*. 2002;197:395-402.
23. Moreno MA, Or-Geva N, Aftab BT, et al. Molecular signature of Epstein-Barr virus infection in MS brain lesions. *Neurol Neuroimmunol Neuroinflamm*. 2018;5:e466.
24. Olsson J, Lövheim H, Honkala E, et al. HSV presence in brains of individuals without dementia: the TASTY brain series. *Dis Model Mech*. 2016;9:1349-1355.
25. Santpere G, Telford M, Andrés-Benito P, Navarro A, Ferrer I. The presence of human herpesvirus 6 in the brain in health and disease. *Biomolecules*. 2020;10:1520.
26. Zavala-Vega S, Castro-Escarpulli G, Hernández-Santos H, et al. An overview of the infection of CMV, HSV 1/2 and EBV in Mexican patients with glioblastoma multiforme. *Pathol Res Pract*. 2017;213:271-276.
27. Bruno F, Abondio P, Bruno R, et al. Alzheimer's disease as a viral disease: revisiting the infectious hypothesis. *Ageing Res Rev*. 2023;91:102068.
28. Itzhaki RF. Corroboration of a major role for herpes simplex virus type 1 in Alzheimer's disease. *Front Aging Neurosci*. 2018;10:e00324.
29. Warren-Gash C, Forbes HJ, Williamson E, et al. Human herpesvirus infections and dementia or mild cognitive impairment: a systematic review and metaanalysis. *Sci Rep*. 2019;9:4743.
30. Eimer WA, Vijaya Kumar DK, Navalpur Shanmugam NK, et al. Alzheimer's disease-associated β-amyloid is rapidly seeded by Herpesviridae to protect against brain infection. *Neuron*. 2018;99:56-63.e3.
31. Readhead B, Haure-Mirande JV, Funk CC, et al. Multiscale analysis of independent Alzheimer's cohorts finds disruption of molecular, genetic, and clinical networks by human herpesvirus. *Neuron*. 2018;99:64-82.e7.
32. Zhou L, Miranda-Saksena M, Saksena NK. Viruses and neurodegeneration. *Virol J*. 2013;10:172.
33. Watson AM, Prasad KM, Klei L, et al. Persistent infection with neurotropic herpes viruses and cognitive impairment. *Psychol Med*. 2013;43:1023-1031.
34. Komaroff AL, Pellett PE, Jacobson S. Human herpesviruses 6a and 6b in brain diseases: association versus causation. *Clin Microbiol Rev*. 2020;34:e00143-NaN20.
35. Athanasiou E, Gargalionis AN, Boufidou F, Tsakris A. The association of human herpesviruses with malignant brain tumor pathology and therapy: two sides of a coin. *Int J Mol Sci*. 2021;22:2250.
36. Donati D. Viral infections and multiple sclerosis. *Drug Discov Today Dis Models*. 2020;32:27-33.
37. James LM, Charonis SA, Georgopoulos AP. Schizophrenia, human leukocyte antigen (HLA), and herpes viruses: immunogenetic associations at the population level. *Neurosci Insights*. 2023;18:1-22.
38. Hogestyn JM, Mock DJ, Mayer-Proschel M. Contributions of neurotropic human herpesviruses herpes simplex virus 1 and human herpesvirus 6 to neurodegenerative disease pathology. *Neural Regen Res*. 2018;13:211-221.
39. Carbone I, Lazzarotto T, Ianni M, et al. Herpes virus in Alzheimer's disease: relation to progression of the disease. *Neurobiol Aging*. 2014;35:122-129.
40. Charonis S, James LM, Georgopoulos AP. In silico assessment of binding affinities of three dementia-protective human leukocyte antigen (HLA) alleles to nine human herpes virus antigens. *Curr Res Transl Med*. 2020;68:211-216.
41. James LM, Charonis SA, Georgopoulos AP. Association of dementia human leukocyte antigen (HLA) profile with human herpes viruses 3 and 7: an in-silico investigation. *J Immunol Sci*. 2021;5:7-14.
42. Georgopoulos A, Tsilibary EP, Souto EP. Adverse effects of Gulf war illness (GWI) serum on neural cultures and their prevention by healthy serum. *J Neurol Neuromed*. 2018;3:19-27.
43. de Lucia C, Murphy T, Maruszak A, et al. Serum from older adults increases apoptosis and molecular aging markers in human hippocampal progenitor cells. *Aging Dis*. 2021;12:2151-2172.
44. Sperling RA, Aisen PS, Beckett LA, et al. Toward defining the preclinical stages of Alzheimer's disease: recommendations from the National Institute on Aging-Alzheimer's association workgroups on diagnostic guidelines for Alzheimer's disease. *Alzheimers Dement*. 2011;7:280-292.
45. Qu Y, Hu HY, Ou YN, et al. Association of body mass index with risk of cognitive impairment and dementia: a systematic review and meta-analysis of prospective studies. *Neurosci Biobehav Rev*. 2020;115:189-198.
46. Wisdom NM, Callahan JL, Hawkins KA. The effects of apolipoprotein E on non-impaired cognitive functioning: a meta-analysis. *Neurobiol Aging*. 2011;32:63-74.
47. Nasreddine ZS, Phillips NA, Bédirian V, et al. The Montreal Cognitive Assessment, MoCA: a brief screening tool for mild cognitive impairment. *J Am Geriatr Soc*. 2005;53:695-699.
48. Reymer PW, Groenemeyer BE, van de Burg R, Kastelein JJ. Apolipoprotein E genotyping on agarose gels. *Clin Chem*. 1995;41:1046-1047.
49. Tukey JW. *Exploratory Data Analysis*. Pearson; 1977.
50. Leuthold AC, Mahan MY, Stanwyck JJ, Georgopoulos A, Georgopoulos AP. The number of cysteine residues per mole in apolipoprotein E affects systematically synchronous neural interactions in women's healthy brains. *Exp Brain Res*. 2013;226:525-536.
51. James LM, Engdahl BE, Georgopoulos AP. Apolipoprotein E: the resilience gene. *Exp Brain Res*. 2017;235:1853-1859.
52. Zuhair M, Smit GSA, Wallis G, et al. Estimation of the worldwide seroprevalence of cytomegalovirus: A systematic review and meta-analysis. *Rev Med Virol*. 2019;29:e2034.
53. Bate SL, Dollard SC, Cannon MJ. Cytomegalovirus seroprevalence in the United States: the National Health and Nutrition Examination Surveys, 1988-2004. *Clin Infect Dis*. 2010;50:1439-1447.
54. Lachmann R, Loenenbach A, Waterboer T, et al. Cytomegalovirus (CMV) seroprevalence in the adult population of Germany. *PLoS One*. 2018;13:e0200267.
55. Stowe RP, Kozlova EV, Yetman DL, et al. Chronic herpesvirus reactivation occurs in aging. *Exp Gerontol*. 2007;42:563-570.
56. Parry HM, Zuo J, Frumento G, et al. Cytomegalovirus viral load within blood increases markedly in healthy people over the age of 70 years. *Immun Ageing*. 2016;13:1.
57. Fülöp T, Larbi A, Pawelec G. Human T cell aging and the impact of persistent viral infections. *Front Immunol*. 2013;4:271.
58. Klenerman P, Oxenius A. T cell responses to cytomegalovirus. *Nat Rev Immunol*. 2016;16:367-377.
59. Franceschi C, Bonafè M, Valensin S, et al. Inflamm-aging. An evolutionary perspective on immunosenescence. *Ann N Y Acad Sci*. 2000;908:244-254.
60. Weltevrede M, Eilers R, de Melker HE, van Baarle D. Cytomegalovirus persistence and T-cell immunosenescence in people aged fifty and older: a systematic review. *Exp Gerontol*. 2016;77:87-95.
61. Poloni C, Szyf M, Cheishvili D, Tsoukas CM. Are the healthy vulnerable? Cytomegalovirus seropositivity in healthy adults is associated with accelerated epigenetic age and immune dysregulation. *J Infect Dis*. 2022;225: 443-452.

62. Simanek AM, Dowd JB, Pawelec G, et al. Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States. *PLoS One*. 2011;6:e16103.
63. Savva GM, Pachnio A, Kaul B, et al.; Medical Research Council Cognitive Function and Ageing Study. Cytomegalovirus infection is associated with increased mortality in the older population. *Aging Cell*. 2013;12:381-387.
64. Cristescu CV, Alain S, Ruță SM. The role of CMV infection in primary lesions, development and clinical expression of atherosclerosis. *J Clin Med*. 2022;11:3832.
65. Grahame-Clarke C, Chan NN, Andrew D, et al. Human cytomegalovirus seropositivity is associated with impaired vascular function. *Circulation*. 2003;108:678-683.
66. 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.
67. Nauclér CS, Geisler J, Vetvik K. The emerging role of human cytomegalovirus infection in human carcinogenesis: a review of current evidence and potential therapeutic implications. *Oncotarget*. 2019;10:4333-4347.
68. Schmaltz HN, Fried LP, Xue QL, et al. Chronic cytomegalovirus infection and inflammation are associated with prevalent frailty in community-dwelling older women. *J Am Geriatr Soc*. 2005;53:747-754.
69. Matheï C, Vaes B, Wallemacq P, Degryse J. Associations between cytomegalovirus infection and functional impairment and frailty in the BELFRAIL cohort. *J Am Geriatr Soc*. 2011;59:2201-2208.
70. Aiello AE, Haan MN, Pierce CM, Simanek AM, Liang J. Persistent infection, inflammation, and functional impairment in older Latinos. *J Gerontol A Biol Sci Med Sci*. 2008;63:610-618.
71. Aiello AE, Haan M, Blythe L, et al. The influence of latent viral infection on rate of cognitive decline over 4 years. *J Am Geriatr Soc*. 2006;54:1046-1054.
72. Stebbins RC, Noppert GA, Yang YC, et al. Association between immune response to cytomegalovirus and cognition in the health and retirement study. *Am J Epidemiol*. 2021;190:786-797.
73. Barnes LL, Capuano AW, Aiello AE, et al. Cytomegalovirus infection and risk of Alzheimer disease in older black and white individuals. *J Infect Dis*. 2015; 211:230-237.
74. Gow AJ, Firth CM, Harrison R, et al. Cytomegalovirus infection and cognitive abilities in old age. *Neurobiol Aging*. 2013;34:1846-1852.
75. Lee KH, Kwon DE, Do Han K, La Y, Han SH. Association between cytomegalovirus end-organ diseases and moderate-to-severe dementia: a population-based cohort study. *BMC Neurol*. 2020;20:216-219.
76. Lin WR, Wozniak MA, Wilcock GK, Itzhaki RF. Cytomegalovirus is present in a very high proportion of brains from vascular dementia patients. *Neurobiol Dis*. 2002;9:82-87.
77. Mody PH, Marvin KN, Hynds DL, Hanson LK. Cytomegalovirus infection induces Alzheimer's disease-associated alterations in tau. *J Neurovirol*. 2023; 29:400-415.
78. Gade M, Comfort N, Re DB. Sex-specific neurotoxic effects of heavy metal pollutants: epidemiological, experimental evidence and candidate mechanisms. *Environ Res*. 2021;201:111558.
79. Anderholm KM, Bierle CJ, Schleiss MR. Cytomegalovirus vaccines: current status and future prospects. *Drugs*. 2016;76:1625-1645.