Differences in cognitive performance between cytomegalovirus-infected and cytomegalovirus-free students

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Abstract: Cytomegalovirus (CMV) is the herpetic virus, which infects 45–100% of people worldwide. Many reports suggest that CMV could impair the cognitive functions of infected subjects. Here we searched for indices of effects of CMV on infected subjects' intelligence and knowledge. The Intelligence Structure Test I-S-T 2000 R was used to compare the cognitive functions of 148 CMV-infected and 135 CMV-free university students. Infected students expressed higher cognitive performance ($p<0.001$; $\eta^2=0.085$), specifically verbal intelligence ($p=0.039$; $\eta^2=0.015$) and verbal knowledge ($p<0.001$; $\eta^2=0.049$). Paradoxically, the cognitive performance, especially verbal ($p = 0.024$, Tau = 0.110), numeric ($p = 0.041$, Tau = 0.097), fluid ($p = 0.021$, Tau = 0.114), and general ($p = 0.040$, Tau = 0.098) intelligence of the infected students, decreased with decreasing concentration of anti-CMV antibodies (a proxy of the time passed from the moment of infection). The observed higher cognitive performance of infected subjects suggests that more intelligent people, who have more social and sexual contacts, might have a higher risk of encountering CMV infection. On the other hand, the positive correlation between cognitive performance and concentration of anti-CMV antibodies suggests that latent infection with CMV has a cumulative negative effect on the cognitive performance of humans. The prevalence of CMV infection in all countries is very high, sometimes approaching 90%. Therefore, the total impact of CMV on human cognitive performance may be large.

Keywords: intelligence; IQ; I-S-T 2000 R; CMV; virus; cognitive functions; permutation tests

Introduction

Cytomegalovirus (CMV) is a ubiquitous virus belonging to the *Herpesviridae* family with high prevalence rates; 45–100% of women of reproductive age are infected worldwide (1). In immunocompetent patients, this infection is assumed to be asymptomatic; it, however, causes dramatic complications in immunocompromised subjects. Congenital cytomegalovirus infection is considered to be the main infectious cause of brain damage, cognitive delay, and sensorineural hearing loss worldwide (2). Following primary infection, CMV establishes lifelong latent infection with possible reactivation and reinfection. The latent phase in immunocompetent individuals is usually considered to be asymptomatic. However, infection with CMV has been associated with immunosenescence, functional impairment, frailty, cardiovascular disease, and Alzheimer's disease (3-8). Postnatal acquired CMV infection has also been associated with impaired cognition, predominantly in specific populations, i.e., in the elderly with cardiovascular disease (9), healthy elderly (10, 11), schizophrenics (12, 13), and AIDS patients (14). The association of asymptomatic CMV infection with mental health cognitive performance in healthy individuals has come into research focus only recently (15-18).

The main aim of the present study was to search for indices of impaired cognitive functions in university students with anamnestic anti-CMV antibodies. We measured the intelligence of a cohort of 283 biology students of the Faculty of Science, Charles University. In the double-blind study, we searched for possible differences in cognitive performance between CMV-infected and CMV-free

students and for possible correlations between this performance and concentration of anti-CMV IgG antibodies using The Intelligence Structure Test I-S-T 2000 R.

The first version of this paper was published in Scientific Reports in 2018 (19) and was later (2022) retracted by the authors because of an error in the permutation test for contaminated data that led to the wrong biological interpretation of higher cognitive performance in CMV-infected students (20). The first version of the article concluded that the better performance of CMV-infected students is an artifact caused by the presence of false-negative subjects (CMV infected, but anti-CMV antibodies low or free) in the CMV-negative subpopulation. However, when the permutation test is done properly, it rejects this hypothesis.

Materials and methods

Subjects

All undergraduate students who enrolled in courses of Evolutionary biology and Practical Methodology of Science in 2010–2012 were invited by e-mail to participate voluntarily in the research projects studying the effects of parasites on human behavior, performance, and personality. About 60–70% of invited students signed an informed consent form and provided 5 ml of blood (taken by medical personnel) for serological analysis. Testing of intelligence proceeded about 6 months after recruitment in 2010 and 2012. At the time of IQ testing, neither the subjects nor the researchers were aware of the CMV status of particular subjects. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the IRB of the Faculty of Science, Charles University (2013/07). All subjects were adults and all signed a written informed consent form approved by the IRB.

Intelligence testing

Testing of intelligence was performed with the Czech version of The Intelligence Structure Test I-S-T 2000 R (21, 22). The test consists of a basic module, which is comprised of three verbal, three numerical, and three abstract figural reasoning subtests and a knowledge test. The knowledge test is focused on questions from geography/history, business, arts/culture, mathematics, science, and daily life. Using both the basic module and the test of knowledge enabled us to obtain a broad spectrum of results: verbal (VI), numerical (NI), and figural intelligence (FigI), and also verbal (VK), numerical (NK), figural (FigK) and general knowledge (GK); fluid (FI), crystallized (CI) and general intelligence (GI). The test was administered on computers to groups of 7–12 individuals in the same room and at the same time (9:15 am). The total length of the test was about 145 minutes including a 15 minute break before administering the second module with the knowledge test.

Immunological tests for CMV

A sandwich ELISA method with an inactivated CMV AD 169 strain antigen (ETI-CYTOK-G PLUS, DiaSorin, Saluggia, Italy) was used for the determination of the CMV status of students (CMV-infected vs CMV-free). This method enables quantitative detection of CMV IgG from cut-off value 0.4 IU/ml to 10 IU/ml and expresses excellent specificity and very good sensitivity (23). All subjects with ambiguous results of the serological test, i.e., with IgG concentration 0.36–0.44 IU/ml were excluded from the analyses.

Statistical analysis

The SPSS 21® program was used for statistical testing (frequency tables, general linear models) and to check statistical tests' assumptions. Nonparametric tests were calculated with partial Kendall test (24, 25) using R 3.3.1 (26), the package ppcor (27) and our package of scripts Explorer 1.0 (https://doi.org/10.6084/m9.figshare.14685825.v1) (28).

In the descriptive statistics, including in the figures, we used age-standardized IQ scores. However, in all statistical tests, we used raw scores (sums of the correct answers) as the dependent variables and controlled for the effect of age (and sex and the age-sex interaction in the parametric tests) by including these confounding variables as predictors in our statistical models. This is the number one approach when specific populations (e.g. students, people of specific narrow age strata, etc.), and not the general population, are being studied. Even if population norms for a specific population under study are available (which is not true for biology students), it is always better to use raw data (and control for age and sex statistically) in cross-sectional studies to avoid potential problems with cohort effects and to solve the problem of continuously changing norms in our rapidly changing social and technical environment.

The nonparametric test, the partial Kendall correlation test, included the age of a subject as a covariate. We controlled for sex by performing separate analyses for male and female students. In the parametric tests – MANCOVA and ANCOVA general linear model (GLM) tests – with the components of intelligence as the dependent (outcome) variables (continuous) and CMV seropositivity status (binary variable) or the concentration of anti-CMV IgG antibodies (continuous variable) as the focal predictor, and sex (binary), age (continuous), and the sex-age interaction as the confounding variables. As the MANCOVA test with all ten components of cognitive performance showed a significant association between cognitive performance and CMV infection, we did not use any correction for multiple tests in the follow-up ANCOVA tests. The ten correlations between cognitive performance components and age was studied with general linear models (ANCOVA). The false discovery rate was controlled with the Benjamini-Hochberg procedure (29).

Permutation tests

Permutation tests were performed using the R script (30) available at https://github.com/costlysignalling/Permutation_test_for_contaminated_data.

Availability of materials and data

The datasets generated during and/or analyzed during the current study are available in the Figshare repository, https://figshare.com/s/97b37051afb7545819b8.

Results

a) *Descriptive statistics*

Two hundred eighty-three students of the Faculty of Science, Charles University, 197 women (21.2 years, S.D.=1.9) and 86 men (21.5 years, S.D.=2.0) were tested for specific immunity against CMV. The prevalence rates of CMV infection was 52.2% for all, 49.7% in women and 58.1% in men (Chi^2=1.69, p=0.194). The prevalence of CMV infection fluctuated depending on the size of settlements where subjects spent their childhood from 47% to 59%. However, these differences were not significant (all: Chi^2=2.25, p=0.689, women: Chi^2=4.87, p=0.300, men: Chi^2=3.05, p=0.548). Similarly, no relation was observed between intelligence and the size of settlements where subjects spent their childhood (all: $F_{1,467}$=0.010, p=0.919, women: $F_{1,292}$=0.472, p=0.493, men: $F_{1,172}$=0.187, p=0.174). Average standard IQ scores for each component of intelligence are shown in Table 1. We detected significant negative (women) and positive (men) associations between age and some components of intelligence (Table 2).

Table 1. Means and standard deviations of all components of intelligence in standard scores for women and men analyzed together and women and men analyzed separately.

	Total sample (N=283)		**Women (N=197)**		**Men (N=86)**	
	Mean	Std. Dev.	Mean	Std. Dev.	Mean	Std. Dev.
Verbal intelligence	131.32	7.85	130.89	7.21	132.30	9.12
Numerical intelligence	110.88	12.23	109.36	11.40	114.36	13.37
Figural intelligence	111.21	13.22	110.95	13.49	111.80	12.62
Crystallized intelligence	133.12	9.70	131.25	9.23	137.40	9.44
Fluid intelligence	123.65	12.47	122.77	11.95	125.65	13.45
General intelligence	120.16	10.00	119.20	9.59	122.35	10.62
Verbal knowledge	119.20	9.65	117.76	9.35	122.51	9.55
Numerical knowledge	120.99	12.05	119.02	11.30	125.51	12.56
Figural knowledge	120.34	10.04	118.69	10.20	124.13	8.61
General knowledge	122.73	9.37	120.84	8.89	127.05	9.05

Table 2. Correlations between IQs and age.

	Total sample (N=283)			Women (N=197)			Men (N=86)		
	p	η^2	B	p	η^2	B	p	η^2	B
Verbal intelligence	0.453	0.002	0.104	0.837	0.000	0.032	0.370	0.010	0.254
Numerical intelligence	0.241	0.005	-0.341	**0.027**	0.025	-0.739	0.385	0.009	0.495
Figural intelligence	0.100	0.010	-0.383	**0.032**	0.023	-0.617	0.783	0.001	0.109
Crystallized intelligence	0.658	0.001	0.678	0.460	0.003	-1.365	0.068	0.039	4.963
Fluid intelligence	0.094	0.010	-3.097	**0.008**	0.036	-5.711	0.499	0.005	2.387
General intelligence	0.212	0.006	-0.620	**0.023**	0.026	-1.324	0.362	0.010	0.858
Verbal knowledge	0.092	0.010	0.147	0.396	0.004	0.090	0.086	0.035	0.267
Numerical knowledge	0.428	0.002	-0.078	**0.006**	0.038	-0.316	0.022	0.061	0.422
Figural knowledge	0.729	0.000	-0.030	0.501	0.002	-0.074	0.646	0.003	0.063
General knowledge	0.854	0.000	0.039	0.240	0.007	-0.301	0.044	0.047	0.752

The table shows the values of p, η^2, B (the slope of a regression line, positive for the positive relations between cognitive performance and age) computed with GLM. The results significant after the correction for multiple tests are printed in bold. The False Discovery Rate (FDR) for the Benjamini Hochberg procedure was pre-set to 0.20% - this means that one out of our five significant results was probably a false-positive result.

b) Effect of CMV infection on intelligence – multiple multivariate tests

Multiple multivariate GLM analysis, with independent variables CMV, age, sex, sex-CMV, and sex-age interactions and verbal, numerical, figural intelligence, verbal, numerical, figural knowledge as dependent variables showed a significant positive association between CMV and intelligence ($p<0.001$; $\eta^2=0.085$). The *post hoc* MANCOVA tests performed separately for each sex showed that this association was significant in women ($p=0.010$; $\eta^2=0.084$) and nearly significant in less numerous men ($p=0.057$; $\eta^2=0.142$). Besides that, an association between sex and intelligence ($p=0.047$; $\eta^2=0.045$) and between the interaction sex-age and intelligence ($p=0.023$; $\eta^2=0.052$) was observed in the total sample. The *post hoc* test showed a significant negative correlation between age and intelligence ($p=0.007$; $\eta^2=0.089$) in women.

c) Effects of CMV on ten components of cognitive function – exploratory post hoc tests

Ten simple multivariate GLM analyses were performed with ten components of cognitive function as dependent variables and CMV and age (and also sex, sex-CMV and sex-age interactions when the total sample was analysed) as independent variables, Fig. 2. Because of the positive result of the previous multiple multivariate test, no correction for multiple statistical tests was performed. These *post hoc* tests revealed significantly higher scores in verbal intelligence ($p=0.039$; $\eta^2=0.015$) and verbal knowledge ($p<0.001$; $\eta^2=0.049$) in CMV-infected subjects compared to CMV-free subjects in the total

sample. The results also showed that women scored worse than men on numerical knowledge ($p=0.002$; $\eta^2=0.035$). In the total sample, age was positively associated with verbal knowledge scores ($p=0.045$; $\eta^2=0.014$); interaction sex-age was associated with numerical knowledge ($p<0.001$; $\eta^2=0.044$), general knowledge ($p=0.024$; $\eta^2=0.018$), fluid intelligence ($p=0.043$; $\eta^2=0.015$) and general intelligence ($p=0.046$; $\eta^2=0.014$). Visual inspection of data showed that these scores decreased with the age in women and increased with the age in men. When analyzed separately for women and men, CMV-infected women scored significantly higher than CMV-free women in verbal knowledge ($p=0.005$; $\eta^2=0.040$) and CMV-infected men obtained higher scores than controls in verbal knowledge ($p=0.009$; $\eta^2=0.080$).

Certain components of intelligence were not normally distributed even after transformations (Shapiro-Wilks test of Log transformed variables: verbal intelligence: $p<0.001$; verbal knowledge: $p=0.009$). Therefore, we also tested the influence of CMV on intelligence with the partial Kendall test, the nonparametric test allowing control for confounding variables. Ten models included CMV and age as independent variables and ten components of intelligence as dependent variables. The results obtained using these models were similar to those obtained using GLM. There were significant positive associations between CMV and verbal intelligence ($p=0.036$; $\tau=0.084$), verbal knowledge ($p<0.0005$; $\tau=0.195$), crystallized intelligence ($p=0.020$; $\tau=0.093$), and general knowledge ($p=0.018$; $\tau=0.094$) in the total sample. In the post hoc tests performed separately for women and men, CMV was positively associated with verbal knowledge in women ($p=0.001$; $\tau=0.160$) and men ($p=0.001$; $\tau=0.240$). CMV-infected subjects achieved higher scores on these facets of the cognitive performance compared to uninfected controls.

Table 3. The cognitive functions of CMV infected and CMV-free subjects.

	Total sample (N=283)		Women (N=197)		Men (N=86)	
	CMV-free	CMV-infected	CMV-free	CMV-infected	CMV-free	CMV-infected
Verbal intelligence	130 (8.4)	132 (7.2)	130 (7.0)	131 (7.4)	132 (11.6)	134 (6.6)
Numerical intelligence	110 (12.0)	112 (12.4)	108 (11.0)	114 (11.8)	110 (13.6)	115 (13.3)
Figural intelligence	111 (12.7)	111 (13.7)	111 (12.6)	113 (14.4)	111 (13.1)	111 (12.4)
Crystallized intelligence	132 (9.6)	134 (9.7)	131 (9.2)	135 (9.3)	132 (10.2)	139 (8.7)
Fluid intelligence	123 (12.3)	124 (12.6)	122 (11.6)	126 (12.3)	124 (13.8)	126 (13.3)
General intelligence	119 (9.7)	121 (10.2)	118 (9.1)	122 (10.1)	120 (11.0)	123 (10.4)
Verbal knowledge	117 (9.2)	121 (12.1)	116 (9.0)	119 (9.4)	120 (9.6)	125 (8.9)
Numerical knowledge	121 (12.0)	121 (12.1)	119 (11.1)	124 (11.6)	119 (13.9)	127 (11.5)
Figural knowledge	121 (10.1)	120 (10.1)	119 (10.0)	124 (10.6)	118 (9.7)	124 (7.8)
General knowledge	122 (9.2)	124 (9.4)	120 (8.6)	125 (9.2)	121 (10.1)	128 (8.1)

The table shows means and standard deviations (in parentheses).

d) Correlation between intelligence and concentration of anti-CMV antibodies

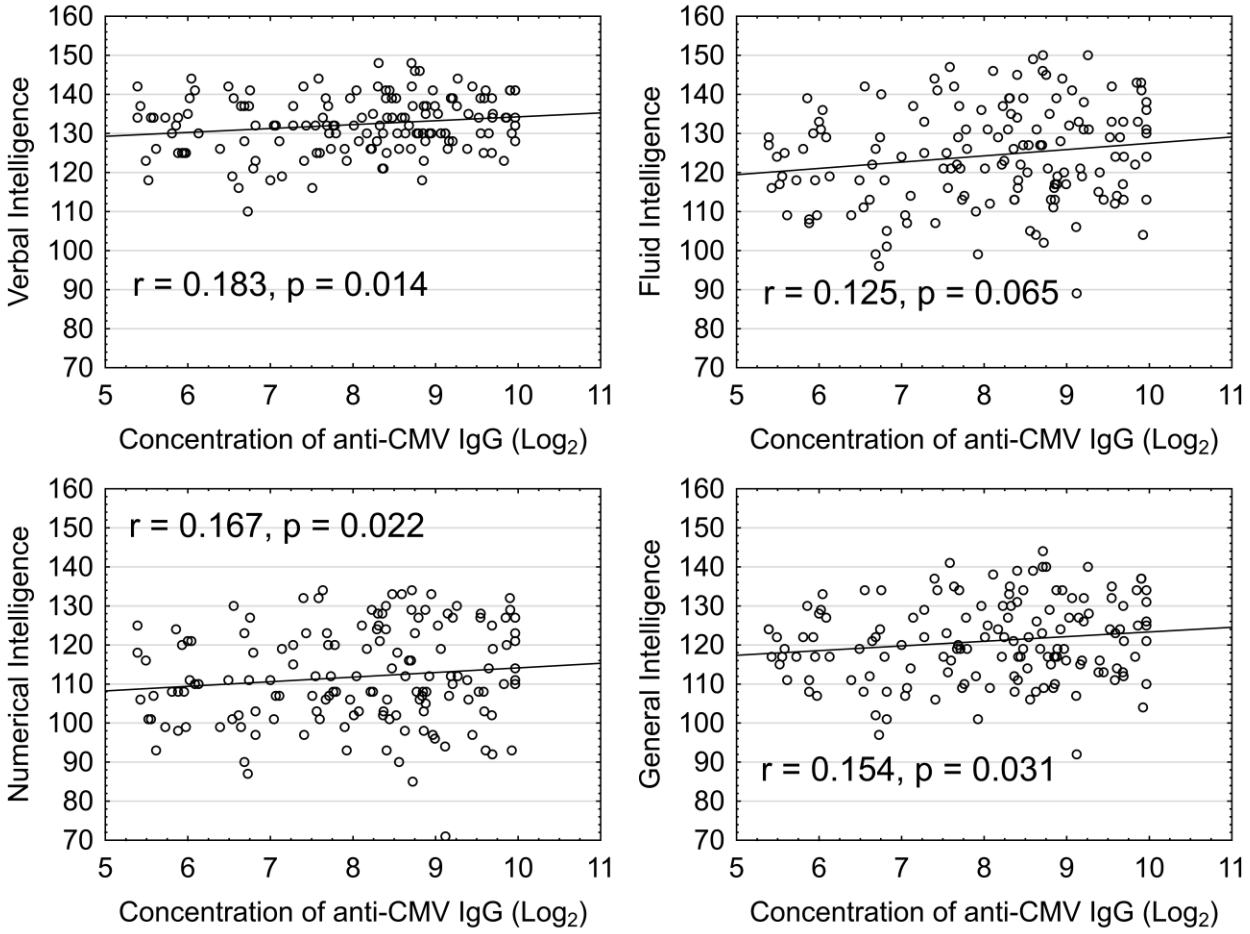
The level of specific IgG antibodies fluctuates in time depending on the physiological status of an individual and environmental factors. However, it generally declines with time from infection even in pathogens with dormant stages in nervous tissue (31). Therefore, it can be assumed (statistically) that the university students infected a long time ago would have on average lower levels of specific antibodies than the subjects infected recently. If changes in intelligence are caused by the infection, differences in IQ scores between CMV-infected and CMV-free subjects should gradually increase with the decline in the level of antibodies. Correlations of the level of specific IgG anti-CMV antibodies with each component of intelligence were analyzed with a partial Kendall test. Models included concentration of specific IgG anti-CMV antibodies and age as independent variables and dependent variables always were one of the ten components of intelligence. Four of ten positive correlations between the level of IgG antibodies and scores obtained were observed in the CMV-infected subsample (Fig. 1, Table 4). In the CMV-free subsample, there was no significant correlation between the level of IgG antibodies and IQs. We did not search for correlations between the detected concentration of anti-CMV antibodies in the whole, CMV-unsorted population as two different phenomena, mostly positive correlations of IQ with the concentration of specific anti-CMV IgG antibodies and mostly negative, non-significant correlations of IQ with the concentration of cross-reacting antibodies of an unknown specificity were observed in CMV seropositive and CMV seronegative subpopulations, respectively.

Table 4 Correlation of ten components of cognitive functions with the concentration of anti-CMV antibodies in CMV seropositive and CMV seronegative subjects.

		Verbal Intelig.	Numer. Intelig.	Figural Intelig.	Crystal. Intelig.	Fluid Intelig.	General Intelig.	Verbal Knowl.	Numer. Knowl.	Figural Knowl.	General Knowl.
CMV-positive	Tau	**0.110**	**0.097**	0.047	-0.050	**0.114**	**0.098**	-0.051	-0.024	0.018	-0.031
	p	0.024	0.041	0.199	0.369	0.021	0.040	0.358	0.661	0.376	0.573
CMV-negative	Tau	0.014	0.060	-0.057	0.002	0.024	0.016	-0.056	0.002	0.000	-0.012
	p	0.812	0.307	0.327	0.970	0.676	0.785	0.338	0.971	0.994	0.843

The table shows the values of a partial Kendall Tau (age controlled) and corresponding one-sided p. The results that were significant in a two-sided test after the correction for multiple (ten) tests by the Benjamini-Hochberg method were printed in bold. The FDR was pre-set to 0.25% - this means that one of our four significant results was probably a false-positive result. The False Discovery Rate (FDR) was pre-set to 0.25% - this means that one out of our four significant results was probably a false-positive result.

Figure 1. Correlation between antibody titre and cognitive functions in CMV-positive subjects.



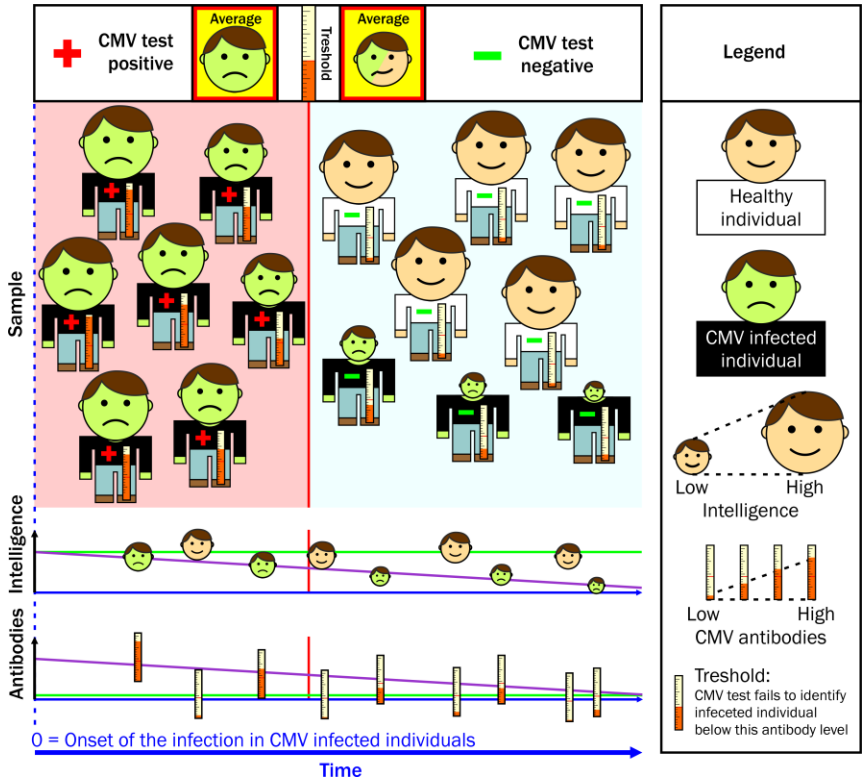
The abscissa shows the standard scores of intelligence (IQ) and the ordinate shows the level of specific anti-CMV IgG antibodies (logarithm of arbitrary units defined by the manufacturer of the ELISA kit). Here, the results (Pearson r and one-sided p) were calculated with parametric tests (Pearson correlation) instead of more proper partial Kendall Tau tests (Table 4).

e) Differences in IQ between CMV-positive and CMV-negative subjects estimated by permutation tail probability test with data reassignment

The reported decrease of specific antibodies with time from infection increases the risk of false-negative test results in students with an old CMV infection, e.g., in individuals infected in early childhood. Our subsample of CMV-free subjects could be therefore contaminated with an unknown number of misdiagnosed CMV-positive individuals that had been infected a long time ago (32, 33). This subpopulation of CMV-infected but CMV seronegative subjects could be the most influenced by CMV and could have the lowest IQ scores because of their long duration of infection or because of

their infection in the early stages of ontogenesis, see Fig. 2. That could explain the observed paradox, i.e., that the infected subjects have on average higher IQ scores and at the same time, the intelligence of infected subjects declines with the length of infection assessed by the level of IgG antibodies. To check the main premise of this model, we examined the IgG titres of all 24 CMV-seropositive subjects who had been tested by us two times within an interval 5-83 months. In 8 subjects, the concentration of specific anti-CMV IgG antibodies increased, while in 16 subjects it decreased. The nonparametric Wilcoxon matched-pairs test showed that this decrease was significant (T = 72, Z = 2.23, p = 0.026). One of these 24 subjects turned seronegative between the first and the second test. Contamination of the CMV-free subsample with false-negative individuals can be revealed and its impacts on the results of statistical tests eliminated by permutation tests with the reassignment of suspect cases between subsamples (32, 33).

Figure 2 The model explaining the paradox of seemingly higher intelligence in CMV infected subjects and a positive correlation between the concentration of anti-CMV antibodies and IQ.



The concentration of anti-CMV antibodies fluctuates over time and differs between subjects depending on many genetic and environmental factors, however, statistically, the level of these antibodies decreases with time from the infection in young people (see the lowest graph). In parallel, the intelligence of infected subjects decreases with time due to unknown cumulative effects of the chronic infection. Due to these two processes, the infected subjects with the lowest intelligence have also the lowest level of antibodies and many of them, therefore, score negatively in the ELISA test (upper part of the figure). Therefore, the mean intelligence of CMV-seropositive subjects is higher than that of CMV-seronegative subjects (who represent the mixture of CMV-free and CMV-very-long-infected subjects).

The results of the permutation tests with reassignment 0, 5, 10, 15, 20, and 25 percent of CMV-seronegative subjects with the lowest intelligence from the CMV-free to CMV-infected set is shown in Tab. 5. The results refuted our hypothetical model – they showed no evidence of the presence of false-negative subjects in our CMV-free set.

Table 5. Differences between CMV-positive and CMV-negative subjects (women and men taken together) analyzed by permutation one-tail probability test with data reassignment.

	0%	5%	10%	15%	20%	25%
Verbal intelligence	0.984	0.970	0.963	0.963	0.955	0.953
Numerical intelligence	0.886	0.879	0.882	0.890	0.902	0.904
Figural intelligence	0.569	0.603	0.617	0.605	0.591	0.606
Verbal knowledge	1.000	1.000	1.000	1.000	1.000	1.000
Numerical knowledge	0.647	0.630	0.649	0.681	0.684	0.693
Figural knowledge	0.383	0.377	0.388	0.397	0.419	0.456
General knowledge	0.954	0.958	0.954	0.952	0.957	0.960
Crystallized intelligence	0.962	0.966	0.960	0.960	0.965	0.970
Fluid intelligence	0.813	0.800	0.776	0.776	0.772	0.772
General intelligence	0.923	0.924	0.908	0.899	0.904	0.908

The column headings show the fraction of reassigned cases and the rest of the table shows the statistical significance (p) of one-sided permutation tests. The very high p values suggest that the validity of the hypothesis "Infected students have lower intelligence than CMV-free subjects." is improbable.

Discussion

Students with anamnestic anti-CMV antibodies expressed higher cognitive performance, especially higher verbal knowledge and verbal intelligence. At the same time, the intelligence of CMV-positive subjects declined with the decrease in the concentration of specific antibodies, which can be used (statistically, not in a clinical praxis) as a proxy of the time since the infection. The results of the permutation test suggest that this paradox cannot be caused by the presence of false-negative subjects in the CMV-seronegative subset.

The higher cognitive performance in the infected students was not expected *a priori*, however, it agreed with the results of a previous study performed on military personnel (17). This study, performed on 533 conscripts, demonstrated a higher intelligence (IQ = 97.8 vs 96.3, nonsignificant) of CMV-infected subjects and a positive correlation between the concentration of anti-CMV antibodies and the intensity of changes in personality in the infected conscripts. In that study, only the Otis test of verbal intelligence (a standard verbal intelligence test that consists of 32 questions focused on the

understanding of the given relationships, linguistic sensitivity, and vocabulary skills) was used and the age of subjects was not controlled.

The existence of a statistical association between two factors, here CMV infection and intelligence, can be explained in two principally different ways. Either the subjects with lower and higher intelligence differ in the probability of acquiring CMV infection or CMV infection influences, here increases, the intelligence of infected subjects. The existence of a positive correlation between specific anti-CMV antibodies and the intelligence of CMV-positive subjects when the age of the subject is statistically controlled, as well as many already published results showing the negative effects of CMV infection on human cognitive function, however, suggests that the latter explanation, i.e., the positive effect of CMV infection on human intelligence, is less parsimonious. Therefore, we suggest that more intelligent subjects who have more social and sexual contacts – CMV is transmitted by close contacts, e.g. by kissing (34) – might have a higher risk of encountering a CMV infection. It should be noted, however, that no cross-sectional study can exclude the third alternative, namely, that both the probability of CMV infection and IQ are independently influenced by some unknown factor, e.g. the socio-economic situation of a subject.

The observed decrease of intelligence with the concentration of anti-CMV antibodies, the proxy of the time passed since the CMV infection, suggests that CMV negatively affects general, fluid, numeric, and verbal intelligence. Therefore, the influence of CMV on the intelligence of our subjects is relatively nonspecific. It suggests that the worse performance of CMV-infected subjects in the intelligence tests might be caused by the same general defects, such as attention, learning, and recall, which were already observed in healthy CMV seropositive middle-age adults (16). We cannot even exclude the possible effects of CMV infection on the motivation of subjects or their cooperativeness. It has been, for example, already reported that CMV-infected subjects have lower novelty seeking and changed harm avoidance measured with the Cloninger Temperament and Character Inventory (17). It is important to measure these variables in future studies to distinguish whether the intelligence or just the performance in the IQ test differs between CMV-infected and CMV-free subjects.

Our results are in agreement with results obtained with another pathogen, the *Toxoplasma gondii*. The life cycles of *Toxoplasma* and CMV differ, however, postnatally acquired infection with both *Toxoplasma* and CMV results in a latent, but most probably life-long, infection of certain subpopulations of cells (probably including glial cells and monocytes in the case of CMV). Both infections were considered harmless or even asymptomatic for a long time. Many studies published in the past 20 years, nevertheless, have shown that at least latent toxoplasmosis induces specific changes in the behaviour of infected animals (35-37) and in the behaviour and personality of infected humans (38). The personality changes observed in *Toxoplasma*-infected humans were searched for and observed also in the CMV-infected humans. It was, for example, observed that both *Toxoplasma*- and CMV-infected subjects have lower Cloninger's novelty seeking. In *Toxoplasma*-infected subjects, the decrease of this factor is most probably caused by an increased concentration of dopamine (39-42). Both *Toxoplasma* (32, 43, 44) and CMV (17) were suggested to influence also the intelligence of infected subjects. Here, however, the results for Toxoplasma and CMV are distinct. Latent toxoplasmosis has usually been associated with decreased intelligence in men and sometimes with

increased intelligence in women. In the case of toxoplasmosis, the permutation test confirmed the existence of false-negative subjects with the most decreased intelligence and the most decreased level of anamnestic antibodies in the studied female population. In the case of CMV, the same test provided a negative result. Still, even here such subjects probably exist. Even in our small set of 24 subjects who had been tested for CMV two or more times, we found one individual who was seropositive in the first, but seronegative in the last test. Results of previous studies that used the permutation test for contaminated data, observed a decrease of seroprevalence of toxoplasmosis in the male population after the age of 36 (45) as well as observations of conversion of seropositive to seronegative individuals (46), indicate that such subpopulation always exists in large experimental samples and can qualitatively influence the results of observational studies (32, 33).

Previous studies investigating the relationship between CMV infection and cognitive functions were done predominantly on populations of children, elderly adults, schizophrenics, and HIV patients. The main body of literature concerns congenitally acquired CMV infection in children with symptoms after delivery. These symptoms include microcephaly, lethargy, seizures, paralysis, chorioretinitis, and hearing loss (47). Forty to 58% of symptomatic children have permanent sequelae, such as cognitive deficit or mental retardation (48). However, even 6.5% of children who are asymptomatic at birth can develop some type of cognitive or neurological impairment (48), as both case-control studies (49, 50), and one longitudinal study (51) showed. The incidence of congenital CMV infection is very low in the Czech Republic and is estimated to be lower than 1%. The incidence of subjects with congenital CMV infection is probably even lower in university students than in the general population. Therefore, the maximal expected occurrence of 2–3 such individuals among the participants of this study cannot be responsible for the observed statistical associations.

No association between CMV and cognition was usually observed in asymptomatically infected subjects (52-55). Temple et al. (56) observed differences between infected and controls only in the group of younger children, but not in the group of older children. Early postnatal infected subjects performed worse than controls both in very preterm infants (57) and in term infants (58). Even if there were not any association between cognition and CMV in asymptomatically infected children as some authors indicated, subjects may develop sequel later in life as the research on elderly adults suggests. Individuals with higher levels of anti-CMV IgG antibodies experienced a more rapid decline in cognition over 4 years compared to subjects with lower levels of antibodies in a large group of CMV-infected elderly adults (11). In this case, higher levels of antibodies refer more probably to more frequent reactivations of the infection over the life course (4) rather than to a recently acquired infection. Similarly, in the seropositive subsample of older adults, a higher level of antibodies to CMV was associated with lower general cognitive ability and processing speed. Moreover, CMV-positive subjects had lower cognitive ability than CMV-free controls (10). Other studies showed that cognitive functioning decreases with increasing viral burden including cytomegalovirus (9, 59).

Our results are in agreement with those obtained on populations with pre-existing clinical conditions, e.g. schizophrenia and AIDS patients. CMV-infected individuals scored worse than those CMV-free in the Trail Making Test in a set of schizophrenic patients (12). Similarly, in a combined group of schizophrenics and controls, the CMV-infected subjects performed worse than those

uninfected as measured by the Wisconsin Card Sorting Test (13). Goplen et al. (14) conclude that CMV can act as a cause or an important cofactor of dementia symptoms in AIDS patients. Moreover, Lin et al. (60) found a much higher percentage of CMV DNA in the brains of individuals with vascular dementia compared to controls in an elderly sample.

Watson et al. (15) compared the performance of patients with schizophrenia/schizoaffective disorder, their unaffected relatives, and healthy controls. The authors observed worse performance of CMV-infected subjects compared to uninfected controls in all three groups, moreover, the differences were more pronounced in individuals with multiple infections (herpes simplex virus (HSV) 1, HSV-2, CMV). Cognitive impairment was also reported for healthy middle-age either CMV or HSV-1 seropositive adults (16).

The number of experimental subjects (283) was relatively large; however, because of the highly imbalanced sex ratio among the Charles University biology students, the number of men was only 86. This increased the risk of Type II errors and also decreased the efficiency of controlling for potential confounding variables like the size of settlements where subjects spent their childhood, BMI, smoking, family background, or other (e.g. HSV-1, EBV, VZV, *Toxoplasma*, *Borrelia*, *Chlamydia*, *Candida*) infections. Most importantly, the effect of Rhesus D (RhD) phenotype is known to strongly influence the effect of latent toxoplasmosis on human performance and personality (44, 61, 62). Effects of RhD, as well as effects of age and smoking (63, 64) were not controlled in the present study. The Czech population contains only about 16% RhD negative subjects. A much larger sample is thus necessary for searching for an RhD phenotype-CMV interaction or for controlling a broader spectrum of potential confounders to avoid the problem of over-parametrization of the models and increased risk of false-positive or false-negative results of corresponding statistical tests.

In the present study, we used the concentration of anti-CMV IgG antibodies as a proxy for the time passed since the original CMV infection. This approach has already been used in other studies on different pathogens and is widely used in clinical praxis, e.g. as an auxiliary screening technique for the prevention of congenital toxoplasmosis. It must be emphasized that the antibody level depends (possibly more strongly) also on many other factors (the infection dose, the virulence, and antigenicity of the strain of a pathogen, number of successful or unsuccessful reinfections, the genetically or environmentally determined susceptibility of the host to the infection or the resulting disease, etc.). Therefore, the relation between the concentration of antibodies and time passed since the infection holds only statistically and probably only in certain phases of the infection. It must be emphasized that all previously mentioned factors, except the time passed since the infection, would result in the existence of a negative correlation between the level of antibodies and intelligence. In the present study, however, we detected a positive correlation between the antibody level and cognitive performance of the students, which suggests that the cognitive impairment progresses from the onset of infection when the antibody level is expected to be the highest. This suggests, but of course does not prove, that the antibody level in the biology students most likely (statistically) reflects the time passed since the infection, and not, for example, the intensity or frequency of past (re)infections.

Last but not least, the biology students of the most prestigious Czech university, Charles University in Prague, are not typical representatives of the general Czech population. Therefore, it is not clear to

what extent the observed phenomena can be generalized. We could expect to detect a much stronger effect of CMV on an "unsorted" population that had not passed recently through a sieve of relatively severe entrance examinations. This sieve probably eliminated a larger fraction of low-IQ subjects than that of high-IQ subjects. If some infection negatively affects IQ, only the individuals who had a very high IQ before the infection could successfully pass through the examinations. In contrast, among the infection-free, also the less gifted subjects could pass the same examinations. If the entrance examinations work properly, the cognitive performance of CMV seropositive and seronegative students would be very similar immediately after the entrance examination but the representation of CMV seropositive individuals would be higher in successful examinees (which could be tested in future studies). It will therefore be more effective to study the effect of CMV seropositivity (or of any other environmental factors) on the representatives of a general population or at least on subjects before, not after, the entrance examinations or any similar "sieves".

Also, the existence of the strict sieve effect of the entrance examinations could explain the observed difference in intelligence between male and female students. The mean intelligence of men and women in the general population is approximately the same, however, the spread of the intelligence is larger in men than in women. In contrast to the situation in the general population, higher mean intelligence in women than men can be therefore expected in any subpopulation of low intelligence subjects (e.g. students of less prestigious schools), and higher mean intelligence in men than women can be expected in any subpopulation of high intelligence subjects (e.g. students of the most prestigious universities).

An unknown third factor, e.g. socioeconomic situation of a subject, could correlate both with the probability of CMV infection and cognitive functions. The Czech population, and especially the university students, have extremely low socioeconomic stratification. Still, a broader spectrum of potential confounding variables should be monitored and controlled for in future studies.

Conclusions

The present study performed on nearly three hundred university students showed that CMV infection is associated with higher intelligence measured with The Intelligence Structure Test I-S-T 2000 R. The increased cognitive performance of infected students is probably caused by the increased probability of infection with CMV in more intelligent people, who have more social and sexual contacts. The positive correlation between four components of intelligence and the concentration of anti-CMV antibodies suggests that the cognitive performance of CMV infected subjects decreases with time since the infection. In accordance with the Bradford-Hill 5th criterion of causality, this positive correlation between intelligence and concentration of anti-CMV antibodies also suggests, but, of course, does not definitively prove, that the CMV-intelligence association is the effect rather than the cause of the CMV infection. CMV infection in both developing and developed countries is very high; most of the world's population is probably infected with this herpetic virus. Therefore, the total impact

of CMV on human intelligence, and secondarily on all the other aspects of human life, including quality of life and economy, may be extremely high.

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Authors' contributions

JF designed the study and wrote the article, VC performed research, analyzed the data, and wrote the article, BS, PT, and LP and HH performed research and participated in writing the article.

Conflict of Interest

The authors declare no conflict of interest. The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results.

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