MAJOR ARTICLE

Cytomegalovirus Infection and Risk of Alzheimer Disease in Older Black and White Individuals

Lisa L. Barnes,[1,2,3] Ana W. Capuano,[1,2] Alison E. Aiello,[4] Arlener D. Turner,[1] Robert H. Yolken,[5] E. Fuller Torrey,[5] and David A. Bennett[1,2]

[1]Rush Alzheimer's Disease Center, [2]Department of Neurological Sciences, and [3]Department of Behavioral Sciences, Rush University Medical Center, Chicago, Illinois; [4]Department of Epidemiology, University of Michigan, Ann Arbor; and [5]The Stanley Medical Research Institute, Chevy Chase, Maryland

Background. Human cytomegalovirus (CMV) is prevalent in older adults and has been implicated in many chronic diseases of aging. This study investigated the relation between CMV and the risk of Alzheimer disease (AD).

Methods. Data come from 3 cohort studies that included 849 participants (mean age [±SD], 78.6 ± 7.2 years; mean education duration [±SD], 15.4 ± 3.3 years; 25% black).

Results. A solid-phase enzyme-linked immunosorbent assay was used for detecting type-specific immunoglobulin G antibody responses to CMV and herpes simplex virus type 1 (HSV-1) measured in archived serum samples. Of 849 participants, 73.4% had serologic evidence of exposure to CMV (89.0% black and 68.2% white; $P < .001$). During an average of 5.0 years of follow-up, 93 persons developed AD. CMV seropositivity was associated with an increased risk of AD (relative risk, 2.15; 95% confidence interval, 1.42–3.27) and a faster rate of decline in global cognition (estimate [±standard error], −0.02 ± 0.01; $P = .03$) in models that controlled for age, sex, education duration, race, vascular risk factors, vascular diseases, and apolipoprotein ε4 level. Results were similar in black and white individuals for both incident AD and change in cognitive function and were independent of HSV-1 status.

Conclusions. These results suggest that CMV infection is associated with an increased risk of AD and a faster rate of cognitive decline in older diverse populations.

Keywords. CMV; Alzheimer's disease; race; epidemiology.

Human cytomegalovirus (CMV) is prevalent in older adults and has been associated with cardiovascular disease and mortality [1, 2]. CMV is transmitted person-to-person by contact with body fluids of persons with or without symptomatic infection [3, 4] and, for most people, has no clinical manifestations. Seroprevalence rates of the virus, typically acquired in childhood, increase steadily with age. In a representative sample, the age-adjusted CMV seroprevalence in the United States was approximately 60%, with rates ranging from 36% in children to 90% in adults aged >80 years [5].

An increasing number of studies in the literature suggest that CMV is involved in the etiology of Alzheimer disease (AD) [6]. However, most studies focus on persons with vascular disease or vascular dementia [7, 8]. One study found associations of CMV-related immunologic and virologic characteristics with AD pathology and a trend with clinical diagnosis among deceased persons [9], but we are unaware of prospective studies that have examined the link between CMV and the risk of AD in living persons.

Studies of primary CMV infections have demonstrated racial disparities, with black individuals more likely to be infected than white individuals [10, 11]. The reasons for the disparities are unknown but include factors such as poverty and overcrowding, low socioeconomic status, and higher levels of stress [3, 12]. There is also a racial disparity in AD, with black individuals having a greater risk and prevalence than white individuals [13]. Given the high rates of CMV infection in black individuals and their greater risk of AD, we sought to determine whether CMV might contribute to racial differences in the risk of AD.

Received 2 April 2014; accepted 28 July 2014; electronically published 8 August 2014.

Correspondence: Lisa L. Barnes, PhD, Rush Alzheimer's Disease Center, Rush University Medical Center, 600 S Paulina St, Ste 1038, Chicago, IL 60612 (lbarnes1@rush.edu).

The Journal of Infectious Diseases® 2015;211:230–7

DOI: 10.1093/infdis/jiu437

In the current study, we examined racial differences in the frequency of CMV seropositivity and tested the association of CMV serostatus with incident AD and decline in cognitive function in a longitudinal study of older black individuals and white individuals examined annually for up to 17 years. We also tested whether race modified the association.

METHODS

Participants

Participants come from the Rush Memory and Aging Project (MAP), the Religious Orders Study (ROS), and the Minority Aging Research Study (MARS), 3 ongoing cohort studies approved by the Institutional Review Board of Rush University Medical Center. The studies have similar study designs, data collection techniques, and recruitment techniques, which facilitate merging the data [14]. Participants undergo annual clinical evaluations, including a medical history, neurological examination, cognitive testing, and donation of blood.

The MAP is a longitudinal clinical-pathologic cohort study of older adults without known dementia who agreed to annual clinical evaluations and brain autopsy at death [15]. Older persons from the Chicago metropolitan area were recruited from retirement communities and subsidized housing facilities. Recruitment for the MAP began in September 1997 and is ongoing. Between 1997 and July 2012, 1412 MAP participants had available blood specimens for analysis.

The ROS is an ongoing longitudinal clinical-pathologic study of aging and AD in older Catholic nuns, priests, and brothers [16]. Participants are without known dementia and have agreed to annual evaluation and organ donation. Recruitment began in January 1994 and is ongoing. Between 1994 and July 2012, 1104 ROS participants had available blood specimens for analysis.

The MARS [17] is a longitudinal community-based cohort study of risk factors for cognitive decline and has enrolled >500 older black individuals. Participants without known dementia were recruited from community-based organizations, churches, and senior-subsidized housing facilities in the metropolitan Chicago area. Recruitment for the MARS began in August 2004 and is ongoing. Between 2004 and July 2012, 373 MARS participants had available blood specimens for analysis.

We used a stratified sampling scheme to select a subset of individuals from each cohort. Black individuals from all 3 studies were included if they had available blood specimens, did not have dementia at the time of the blood specimen collection, and underwent at least 2 cognitive evaluations to assess cognitive decline (n = 210). A subset of remaining white participants (n = 639) was randomly selected from the MAP and the ROS, using the same criteria. Participants from all 3 cohorts were treated as 1 analytic cohort (n = 894).

Outcome Measures

Clinical Evaluation for AD

Each person underwent an annual uniform structured evaluation performed by examiners who were blinded to previously collected information [15–17]. On the basis of this evaluation, an experienced clinician classified subjects with respect to AD and other neurologic conditions according to criteria set forth by the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association [18].

Assessment of Cognitive Function

Eighteen cognitive performance tests were administered as part of each clinical evaluation. One test, the Mini-Mental State Examination (MMSE) [19] was used only for descriptive purposes, and another test, Complex Ideational Material [15], was used for diagnostic purposes. Analyses are based on findings from the remaining 16 tests. The following Seven tests assessed episodic memory: Word List Memory, Word List Recall, and Word List Recognition, East Boston Story (immediate and delayed), and Story A from Logical Memory (immediate and delayed); 2 tests assessed semantic memory: a 15-item version of Boston Naming Test, and Verbal Fluency test; 3 tests assessed working memory: Digit Span Forward, Digit Span Backward, and Digit Ordering; 2 tests assessed perceptual speed: Symbol Digits Modalities Test, and Number Comparison Test; and 2 tests assessed visuospatial ability: a 15-item version of Judgement of Line Orientation, and a 12-item version of Standard Progressive Matrices, as previously described [20].

To minimize floor and ceiling effects and use all available data, a global measure based on all 16 tests was used in analyses. Scores on each individual test were converted to z scores, using the baseline mean and standard deviation for the entire group, and were averaged to yield the global measure, as previously described [20].

Laboratory Assessment

Frozen serum samples (−80 C) from each participant were shipped to Stanley Laboratory of Developmental Neurovirology at the Johns Hopkins School of Medicine to measure levels of antibodies to CMV and herpes simplex virus type 1 (HSV-1). Methods for determining the antibody levels have been described previously [21–23]. Briefly, solid-phase immunoassay techniques were used to measure immunoglobulin G (IgG) antibodies to CMV in sera [24]. Assays were performed by the reaction of diluted aliquots of standard control serum samples to antigens immobilized onto a solid-phase surface, with the subsequent quantitation of IgG antibodies by reaction of bound antibodies with enzyme-labeled anti–human IgG and enzyme substrate. The optical density of the ensuing enzyme-substrate reaction was quantified by means of spectrophotometric instrumentation. Reagents for CMV assays were obtained

from IBL-America (http://www.ibl-america.com/). Assays of antibodies with specificity for HSV-1 were performed using a purified viral envelope glycoprotein gG-1 as the solid-phase antigen. Reagents for HSV-1 assays were obtained from Focus Diagnostics (http://www.focusdx.com/product-catalog/herpeselect). Specimens were classified as seronegative if the immune status ratio, determined by comparison with defined standards, was within the range of negative (<0.9) or positive (≥0.9); equivocal values were classified as positive. The samples were anonymized by means of coding system, with the researchers performing the assays having no information relating to the clinical characteristics of the participants.

Covariates

Participants reported years of education, and both race/ethnicity (non-Hispanic black vs non-Hispanic white) and sex were self-identified. Age was determined on the basis of the participants' date of birth. Participants reported vascular risk factors and conditions in the medical history. Composite measures of vascular risk factor burden (ie, calculated as the number of the following 3 risk factors that were present: hypertension, smoking, and diabetes mellitus) and vascular disease burden (ie, calculated as the number of the following 3 risk factors that were present: claudication, stroke, congestive heart failure, and heart attack) were computed on the basis of self-report questions and medication inspection, as previously described [25]. Genotyping of the gene encoding apolipoprotein ε4 (*APOE*) was done, and individuals were dichotomized into those with at least 1 copy of the ε4 allele and those without a copy, as previously described [26].

Statistical Analysis

We first examined differences between black individuals and white individuals on the basis of demographic factors, CMV status, and health conditions. Because black individuals were significantly younger and had less education in this sample, scores based on normalized inverse probabilities were used to weight models so that racial differences across key covariates were minimized. Cox proportional hazard models [27] were used to test the hypothesis that CMV serostatus is associated with higher risk of AD and to determine whether the risk differs between black individuals and white individuals. All models included terms for age, sex, education duration, and race. Subsequent models adjusted for vascular risk factors, vascular diseases, and *APOE* findings.

We used mixed-effects models [28] to test the hypothesis that CMV was associated with a faster rate of cognitive decline and to determine whether the relation differed between black individuals and white individuals. Each model had terms for time (measured as the number of years since blood specimens were collected); CMV serostatus (positive vs negative) to control for the relation of CMV to cognitive function at baseline; and the interaction of CMV with time, to test the association of CMV with linear change in cognitive function. Terms for the interaction of CMV and race and the interaction of CMV, race, and time were included to test whether there were racial differences in the relation of CMV to baseline cognition and change over time. Models included random effects for time, incorporated for individual baseline level of cognition and individual rate of change in performance. CMV and other covariates were entered as fixed effects. All models also included terms for age, sex, and education duration. Secondary models were repeated to adjust for vascular risk factors, vascular diseases, and *APOE* findings.

As a sensitivity analysis, models were repeated with HSV-1 status as the predictor, with adjustment for age, sex, education duration, and race, first in a model by itself and subsequently with HSV-1 status and CMV status in the same model. All analyses were conducted by using SAS software, version 9.3, of the SAS system for Linux. Models were graphically and analytically validated.

RESULTS

CMV and Demographic Characteristics

Results from the CMV IgG antibody tests were dichotomized as seronegative or seropositive. Among the 849 participants in the study, 623 (73.4%) tested positive for CMV infection. Black individuals had significantly higher levels of CMV antibody levels than white individuals (89.0% vs 68.2%; $P < .001$). The baseline characteristics of the sample are shown in Table 1. Participants had a mean age (±SD) of 78.6 ± 7.2 years, a mean education duration (±SD) of 15.4 ± 3.3 years, and a mean MMSE score (±SD) of 28.1 ± 2.5. The sample was 25% Black and 76% were women. Black individuals were younger, had less education, lower MMSE scores, and more vascular risk factors. In contrast, white individuals had more vascular diseases.

Table 2 shows CMV antibody levels, by race and mean age. Black individuals had higher levels at every age. There was an

Table 1. Sample Characteristics of Participants, by Race

Characteristics	White (n = 639)	Black (n = 210)	*P* Value
Age, y	80.5 ± 6.6	72.9 ± 5.9	<.001
Education duration, y	15.6 ± 3.2	14.9 ± 3.6	<.05
Female sex, participants, %	77.3	73.3	.24
MMSE score	28.4 ± 1.9	28.0 ± 2.0	<.05
CMV seropositivity, positive participants, %	68.2	89.0	<.001
Vascular risk factors,[a] no.	0.94 ± 0.80	1.36 ± 0.85	<.001
Vascular diseases, no.[a]	0.40 ± 0.64	0.22 ± 0.47	<.001

Data are mean ± SD, unless otherwise indicated.

Abbreviations: CMV, cytomegalovirus; MMSE, Mini-Mental State Examination; SD, standard deviation.

[a] Of 3 possible risk factors or 3 possible diseases.

Table 2. Levels of Antibody Against Cytomegalovirus, by Race and Age

	Antibody Level (AU/ml) by Age			
Race	69 y	76 y	81 y	88 y
Black	3.0 ± 1.3	2.8 ± 1.5	2.9 ± 1.3	3.4 ± 1.4
White	1.9 ± 1.7	2.3 ± 1.7	2.6 ± 1.8	2.8 ± 1.7

Values are mean ±SD.

Abbreviation: SD, standard deviation.

increase in antibody level with age among white individuals, but the pattern was not as clear among black individuals.

CMV and Incidence of AD

During an average of 5 years of observation, 93 persons developed AD. In a Cox proportional hazards model (discrete time) with a term for CMV seropositivity and terms to control for age, sex, education duration, and race, CMV was associated with increased incidence of AD (relative risk [RR], 2.15; 95% confidence interval [CI], 1.42–3.27; $P < .001$; Table 3). As shown in Figure 1, a person with CMV infection was >2 times as likely to develop AD than a person without infection. A greater percentage of black individuals had CMV, consistent with previous reports [3, 29]. Thus, we repeated analysis of the core model with additional terms for the interaction of CMV with race. CMV infection continued to be associated with increased incidence of AD (RR, 2.20; 95% CI, 1.45–3.36; $P < .001$), but there was no interaction with race (RR, 0.11; 95% CI, .01–2.39; $P = .16$; Table 3). Results were unchanged after control for vascular risk factors and diseases (data not shown). Genetic factors such as *APOE4* have been related to the risk of AD [30], and CMV antibody levels have been shown to vary by *APOE* genotype [31]. The addition of a term for the presence of any ε4 did not affect the association of CMV and the risk of AD (RR, 2.24; 95% CI, 1.48–3.39; $P < .001$).

To test the specificity of the effect, we repeated analysis of the core model after replacing CMV status with HSV-1 status. Of 849 participants, 43.6% had serologic evidence of HSV-1. HSV-1 was not related to the incidence of AD (RR, 0.84; 95% CI, .62–1.16; $P = .29$), and there was no interaction of HSV with race (RR, 0.32; 95% CI, .01–19.71; $P = .58$). Finally, we included both CMV and HSV-1 in the same model, and results were the same: there was an increased risk of AD for CMV but no relation with HSV-1 (CMV-associated RR, 2.19 [95% CI, 1.44–3.33; $P < .001$]; HSV-1–associated RR, 0.94 [95% CI, .69–1.29; $P = .72$]).

Table 3. Cox Modeling of the Relationship Between Cytomegalovirus (CMV) and the Risk of Alzheimer Disease

	Hazard Ratio (95% Confidence Interval)	
Covariate	Model 1	Model 2
Male sex	0.49 (.31–.78)	0.49 (.31–.78)
Age	1.12 (1.08–1.16)	1.12 (1.08–1.16)
Education duration	1.02 (.97–1.08)	1.02 (.97–1.08)
Black race	1.16 (.26–5.24)	7.39 (.56–97.70)
CMV	2.15 (1.42–3.27)	2.41 (1.53–3.78)
CMV × black race	. . .	0.11 (.01–2.39)

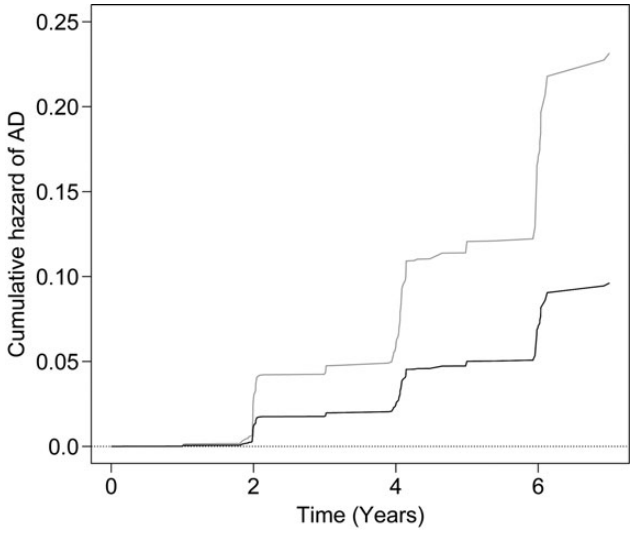

Figure 1. Risk of Alzheimer disease (AD) in persons infected with cytomegalovirus (CMV; top line) relative to those without CMV infection (bottom line), adjusted for age, sex, education duration, and race.

CMV and Change in Global Cognition

Because cognitive decline is the hallmark of AD, we tested the robustness of the finding with CMV by examining the relation of CMV with the decline in global cognition in a series of mixed-effects models that adjusted for age, sex, education duration, and race. In these models, the term for time indicates the mean annual change in global cognition. CMV was related to a faster rate of decline, as indicated by the term for CMV and time (Table 4). As shown in Figure 2, the cognition of persons infected with CMV infection declined faster than that of persons without CMV infection. Subsequent models included a term for the interaction of CMV and race. CMV continued to be related to a faster rate of decline in cognition, but there was no interaction with race (Table 4). Additional models adjusted for vascular risk factors and vascular diseases, and the results were essentially unchanged. In a model that included a term for the presence of any ε4, the magnitude of the effect of CMV on the change in global cognition was similar to that of the core model but only approached significance (estimate [±standard error] −0.02 ± 0.01; $P = .07$). Finally, in models that replaced CMV status with HSV-1 status, HSV-1 was not related to the level of or change in global cognition.

Table 4. Mixed-Effects Modeling of the Relationship Between Cytomegalovirus (CMV) Seropositivity and Annual Rate of Change in Cognition

	Model 1		Model 2		Model 3	
Model Term	Estimate ± SE	*P* Value	Estimate ± SE	*P* Value	Estimate ± SE	*P* Value
Time	−0.040 ± 0.009	<.01	−0.044 ± 0.009	<.01	−0.041 ± 0.009	<.01
Age, y, centered at 79 y	−0.015 ± 0.003	<.01	−0.027 ± 0.003	<.01	−0.027 ± 0.003	<.01
Age × time	−0.004 ± 0.001	<.01	−0.004 ± 0.001	<.01	−0.004 ± 0.001	<.01
Male sex	−0.144 ± 0.044	<.01	−0.130 ± 0.043	<.01	−0.132 ± 0.043	<.01
Male sex × time	0.013 ± 0.010	.207	0.014 ± 0.010	.171	0.013 ± 0.010	.191
Education duration, y, centered at 15 y	0.063 ± 0.006	<.01	0.057 ± 0.006	<.01	0.057 ± 0.006	<.01
Education duration × time	−0.001 ± 0.001	.310	−0.001 ± 0.001	.453	−0.001 ± 0.001	.502
CMV	−0.065 ± 0.043	.129	0.002 ± 0.043	.972	−0.014 ± 0.045	.761
CMV × time	−0.022 ± 0.010	.025	−0.020 ± 0.010	.046	−0.025 ± 0.010	.018
Black race	. . .		−0.441 ± 0.056	<.01	−0.524 ± 0.139	<.01
Black race × time	. . .		0.019 ± 0.015	.206	−0.028 ± 0.037	.439
Black race × CMV	. . .		. . .		0.098 ± 0.147	.503
Black race × CMV × time	. . .		. . .		0.054 ± 0.039	.161

DISCUSSION

To our knowledge, this is the first study to demonstrate a link between serological evidence of CMV exposure and the risk of AD in a community sample. Among 849 older black individuals and white individuals, we found that CMV seropositivity was higher in black individuals relative to white individuals and was associated with a 2-fold increase in the risk of AD and a faster rate of cognitive decline. The results were independent of demographic characteristics, vascular risk factors and diseases, and *APOE4* allele status. Although the relationship was similar in black individuals and white individuals, black individuals had a much higher frequency of CMV infection, suggesting that CMV may contribute to racial differences in the burden of AD. The relationships were independent of HSV-1 status, which showed no relationship with the risk of AD or cognitive decline.

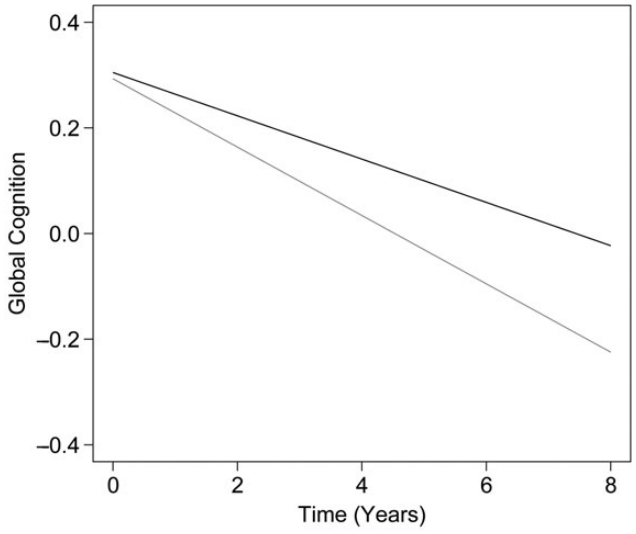

Figure 2. Predicted paths of change in global cognition for persons infected with cytomegalovirus (CMV; bottom line) relative to those without CMV infection (top line), adjusted for age, sex, education duration, and race.

CMV has been implicated in many chronic conditions, including atherosclerosis, endothelial dysfunction, and autoimmune diseases, in which it is thought to function as a proinflammatory agent replicating in sites of inflammation and sustaining the process of chronic inflammation [32–35]. Similar to other herpesviruses, CMV persists in a latent state until periodic reactivation in times of stress, times of immunosuppression due to chronic illness or medications, or during aging [36, 37]. Several studies have examined CMV and cognition either in older adults, in patients with specific clinical conditions, or among patients with mental illness [38–40]. Results have been variable. Most studies have been cross-sectional examinations that looked at either serostatus alone, antibody levels, or both. For example, one study did not find an association between CMV seropositivity and cognition in older adults, although it did find it in middle-aged adults [41]. Another study found that neither antibody level nor seropositivity was associated with cognitive impairment [35]. By contrast, a third study reported that both seropositivity and antibody level were associated with lower cognition [39]. To our knowledge, only 1 longitudinal study has reported that CMV is related to a faster rate of cognitive decline, but it was limited in cognitive measures [38].

The potential mechanisms linking CMV with the risk of AD is uncertain. Although CMV infection often remains undiagnosed because of the asymptomatic properties of the virus, it

remains in a persistent latent state in the immune system, with an increasing risk of reactivation during old age [42]. Thus, several factors suggest CMV may be associated with risk of AD as a result of its effects on the aging immune system. First, older adults have increased levels of IgG antibodies to CMV, compared with younger persons [43], and changes in cell-mediated immune parameters often occur with aging and can result in subclinical CMV reactivation [37]. Second, a relationship has been established between CMV and downregulation of cell-mediated immunity, resulting in an increase in cellular and inflammatory markers often associated with cognitive decline [42, 44]. For example, it has been shown that CMV-specific $CD8^+$ T cells have the ability to produce interferon γ [44], and increased levels of CMV IgG antibodies are significantly correlated with higher tumor necrosis factor α and interleukin 6 levels in older adults [12, 45]. Finally, a recent report found an association of CMV with AD neuropathology in the brains of deceased participants [9]. They also found a trend for an association with clinical diagnosis of AD, but results were limited because of the small sample size. Similarly, another pathologic study of 15 decedents found that CMV was present in a high proportion of brains among persons with vascular dementia, further suggesting a role of vascular disease and inflammation [7]. Together, these studies support a CMV-related immune and inflammatory pathway that is also implicated in AD and cognitive decline and may account for the association reported in the current study [46].

Another possible mechanism linking CMV to risk of AD is the strong pattern of the association of CMV antibody levels with race, income, and education level reported in previous meta-analyses and national representative studies [5, 11, 29, 47]. Race and factors associated with low socioeconomic status have all been shown to be independently associated with CMV seropositivity [3]. Interestingly, risk and prevalence of AD are also higher in the same demographic subgroups. Although the results in the current study are consistent with both of these trends, we did not find that the relationship of CMV with the risk of AD differed as a function of race. However, it is possible that the relative homogeneity of black individuals and white individuals with respect to measures of income level and education duration in the current study could have influenced the results. Also, we had a relatively small sample size for black individuals, compared with white individuals, which may have limited the power to detect an interaction of CMV with race. Future population-based studies in which the full spectrum of income level, education level, and race is represented, and sample sizes are sufficient to examine subgroup analyses are needed. Despite the lack of a differential effect by race, however, the results suggest that the overall burden of this relationship is greater among black individuals because they are much more likely to be infected with CMV. In fact, given the well-established racial difference in CMV prevalence and risk of AD, the current results represent a targetable health disparity for potential interventions, such as antiviral medications for CMV, to assess whether intervening on CMV reactivation may reduce risk of AD development and cognitive decline. For such a strategy to be most effective, however, it would need to be given as prophylaxis to high-risk groups.

Some studies have found that HSV-1 is related to AD, but the results have been inconsistent, and often driven by an interaction with *APOE4* status [38, 48]. We did not find an association between HSV-1 and the risk of AD, suggesting that the effect of CMV on the risk of AD and cognitive decline is relatively specific and not due to a general role of infection on immune dysfunction. Of note, rates of positivity for HSV-1 in our sample were much lower than those for CMV, and this might be a reason for the lack of associations with HSV. We are not aware of published data on frequency of HSV-1 in adults aged >65 years. Thus, future studies should determine whether the lower levels in our sample are due to the much higher ages of our cohort.

The study has limitations. We used seropositivity status, which indicates prior exposure to CMV, rather than when participants were infected. Existing cross-sectional data in the literature suggest that black individuals acquire CMV infection earlier than white individuals [5, 29], potentially because of racial differences in exposure to social stressors, including lower income level, education level, and occupational status. Whether or how time of acquisition might influence the relationship between CMV and cognitive function is not known but should be examined in future studies. Some studies have suggested that antibody levels are a more important and salient measure of immune response to CMV. Evidence of a relationship between persistent infection and risk of AD would strengthen the findings. In addition, analyses were conducted on selected participants with available blood specimens for analyses.

This study had several strengths. First, data come from cohort studies with high follow-up rates and well-characterized participants. Second, we considered important confounding factors, including demographic characteristics, vascular conditions, and *APOE* genotype. Third, we were able to test the specificity of the effect by examining the relation of another common herpesvirus that has been implicated in AD, HSV-1. Finally, we used a complementary outcome to AD, change in cognitive function, and found similar results, which further strengthens confidence in the relationship between CMV and AD.

Notes

Acknowledgments. We thank the participants of the Rush Memory and Aging Project, the Religious Order Study, and the Minority Aging Research Study, for their invaluable contributions; Charlene Gamboa, MPH, Tracy Colvin, MPH, Tracey Nowakowski, Barbara Eubeler, Karen Lowe-Graham, MS, and Karen Skish, MS, PA(ASCP) MT, for study recruitment and coordination; John Gibbons, MS, and Greg Klein, for data management; and the staff of the Rush Alzheimer's Disease Center. We would also like to thank Dr Glen Ford from VanPelt Biosciences for his contribution to the performance of the assays.

Financial support. This work was supported by the National Institute on Aging (grants R01AG22018, R01AG17917, and P30G10161), the Stanley Medical Research Institute, and the Illinois Department of Public Health.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Pawelec G, McElhaney JE, Aiello AE, Derhovanessian E. The impact of CMV infection on survival in older humans. Curr Opin Immunol **2012**; 24:507–11.
2. Simanek AM, Dowd JB, Pawelec G, Melzer D, Dutta A, Aiello AE. Seropositivity to cytomegalovirus, inflammation, all-cause and cardiovascular disease-related mortality in the United States. PLoS One **2011**; 6: e16103.
3. 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**; 50:1439–47.
4. Hamprecht K, Maschmann J, Vochem M, Dietz K, Speer CP, Jahn G. Epidemiology of transmission of cytomegalovirus from mother to preterm infant by breastfeeding. Lancet **2001**; 357:513–8.
5. Staras SA, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988–1994. Clin Infect Dis **2006**; 43:1143–51.
6. Honjo K, van Reekum R, Verhoeff NP. Alzheimer's disease and infection: do infectious agents contribute to progression of Alzheimer's disease? Alzheimers Dement **2009**; 5:348–60.
7. 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**; 9:82–7.
8. Strandberg TE, Pitkala KH, Linnavuori KH, Tilvis RS. Impact of viral and bacterial burden on cognitive impairment in elderly persons with cardiovascular diseases. Stroke **2003**; 34:2126–31.
9. Lurain NS, Hanson BA, Martinson J, et al. Virological and immunological characteristics of human cytomegalovirus infection associated with Alzheimer disease. J Infect Dis **2013**; 208:564–72.
10. Colugnati FA, Staras SA, Dollard SC, Cannon MJ. Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. BMC Infect Dis **2007**; 7:71.
11. Zajacova A, Dowd JB, Aiello AE. Socioeconomic and race/ethnic patterns in persistent infection burden among U.S. adults. J Gerontol A Biol Sci Med Sci **2009**; 64:272–9.
12. Roberts ET, Haan MN, Dowd JB, Aiello AE. Cytomegalovirus antibody levels, inflammation, and mortality among elderly Latinos over 9 years of follow-up. Am J Epidemiol **2010**; 172:363–71.
13. Tang MX, Cross P, Andrews H, et al. Incidence of AD in African-Americans, Caribbean Hispanics, and Caucasians in northern Manhattan. Neurology **2001**; 56:49–56.
14. Bennett DA, Barnes LL. Datasets for special series on cognitive reserve. J Int Neuropsychol Soc **2011**; 17:587–92.
15. Bennett DA, Schneider JA, Buchman AS, Barnes LL, Boyle PA, Wilson RS. Overview and findings from the rush Memory and Aging Project. Curr Alzheimer Res **2012**; 9:646–63.
16. Bennett DA, Schneider JA, Arvanitakis Z, Wilson RS. Overview and findings from the religious orders study. Curr Alzheimer Res **2012**; 9:628–45.
17. Barnes LL, Shah RC, Aggarwal NT, Bennett DA, Schneider JA. The Minority Aging Research Study: ongoing efforts to obtain brain donation in African Americans without dementia. Curr Alzheimer Res **2012**; 9:734–45.
18. McKhann G, Drachman D, Folstein M, Katzman R, Price D, Stadlan EM. Clinical diagnosis of Alzheimer's disease: report of the NINCDS-ADRDA Work Group under the auspices of Department of Health and Human Services Task Force on Alzheimer's Disease. Neurology **1984**; 34:939–44.
19. Folstein MF, Folstein SE, McHugh PR. "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res **1975**; 12:189–98.
20. Wilson RS, Barnes LL, Krueger KR, Hoganson G, Bienias JL, Bennett DA. Early and late life cognitive activity and cognitive systems in old age. J Int Neuropsychol Soc **2005**; 11:400–7.
21. Dickerson F, Stallings C, Sullens A, et al. Association between cognitive functioning, exposure to Herpes Simplex Virus type 1, and the COMT Val158Met genetic polymorphism in adults without a psychiatric disorder. Brain Behav Immun **2008**; 22:1103–7.
22. Dickerson F, Stallings C, Origoni A, et al. Association between Cytomegalovirus Antibody Levels and Cognitive Functioning in Non-Elderly Adults. PLoS One **2014**; 9:e95510.
23. Dickerson FB, Boronow JJ, Stallings C, Origoni AE, Ruslanova I, Yolken RH. Association of serum antibodies to herpes simplex virus 1 with cognitive deficits in individuals with schizophrenia. Arch Gen Psychiatry **2003**; 60:466–72.
24. Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH. Maternal infections and subsequent psychosis among offspring. Arch Gen Psychiatry **2001**; 58:1032–7.
25. Boyle PA, Wilson RS, Aggarwal NT, et al. Parkinsonian signs in subjects with mild cognitive impairment. Neurology **2005**; 65:1901–6.
26. Barnes LL, Arvanitakis Z, Yu L, Kelly J, De Jager PL, Bennett DA. Apolipoprotein E and change in episodic memory in blacks and whites. Neuroepidemiology **2013**; 40:211–9.
27. Cox DR. Regression models and life tables (with discussion). R Stat Soc Series B **1972**; 74:187–220.
28. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics **1982**; 38:963–74.
29. Dowd JB, Aiello AE, Alley DE. Socioeconomic disparities in the seroprevalence of cytomegalovirus infection in the US population: NHANES III. Epidemiol Infect **2009**; 137:58–65.
30. Evans DA, Bennett DA, Wilson RS, et al. Incidence of Alzheimer disease in a biracial urban community: relation to apolipoprotein E allele status. Arch Neurol **2003**; 60:185–9.
31. Aiello AE, Nguyen HO, Haan MN. C-reactive protein mediates the effect of apolipoprotein E on cytomegalovirus infection. J Infect Dis **2008**; 197:34–41.
32. Freeman RB Jr. The 'indirect' effects of cytomegalovirus infection. Am J Transplant **2009**; 9:2453–8.
33. Blum A, Giladi M, Weinberg M, et al. High anti-cytomegalovirus (CMV) IgG antibody titer is associated with coronary artery disease and may predict post-coronary balloon angioplasty restenosis. Am J Cardiol **1998**; 81:866–8.
34. Grahame-Clarke C. Human cytomegalovirus, endothelial function and atherosclerosis. Herpes **2005**; 12:42–5.
35. Mathei 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**; 59:2201–8.
36. Soderberg-Naucler C, Nelson JY. Human cytomegalovirus latency and reactivation - a delicate balance between the virus and its host's immune system. Intervirology **1999**; 42:314–21.
37. Stowe RP, Kozlova EV, Yetman DL, Walling DM, Goodwin JS, Glaser R. Chronic herpesvirus reactivation occurs in aging. Exp Gerontol **2007**; 42:563–70.
38. Aiello AE, Haan M, Blythe L, Moore K, Gonzalez JM, Jagust W. The influence of latent viral infection on rate of cognitive decline over 4 years. J Am Geriatr Soc **2006**; 54:1046–54.
39. Gow AJ, Firth CM, Harrison R, Starr JM, Moss P, Deary IJ. Cytomegalovirus infection and cognitive abilities in old age. Neurobiol Aging **2013**; 34:1846–52.
40. Shirts BH, Prasad KM, Pogue-Geile MF, Dickerson F, Yolken RH, Nimgaonkar VL. Antibodies to cytomegalovirus and Herpes Simplex Virus 1 associated with cognitive function in schizophrenia. Schizophr Res **2008**; 106:268–74.
41. Tarter KD, Simanek AM, Dowd JB, Aiello AE. Persistent viral pathogens and cognitive impairment across the lifecourse in the third

National health and nutrition examination survey. J Infect Dis **2014**; 209:837–44.
42. Koch S, Solana R, Dela RO, Pawelec G. Human cytomegalovirus infection and T cell immunosenescence: a mini review. Mech Ageing Dev **2006**; 127:538–43.
43. Weymouth LA, Gomolin IH, Brennan T, Sirpenski SP, Mayo DR. Cytomegalovirus antibody in the elderly. Intervirology **1990**; 31:223–9.
44. Almanzar G, Schwaiger S, Jenewein B, et al. Long-term cytomegalovirus infection leads to significant changes in the composition of the CD8+ T-cell repertoire, which may be the basis for an imbalance in the cytokine production profile in elderly persons. J Virol **2005**; 79:3675–83.
45. Schmaltz HN, Fried LP, Xue QL, Walston J, Leng SX, Semba RD. Chronic cytomegalovirus infection and inflammation are associated with prevalent frailty in community-dwelling older women. J Am Geriatr Soc **2005**; 53:747–54.
46. Itzhaki RF, Lin WR, Shang D, Wilcock GK, Faragher B, Jamieson GA. Herpes simplex virus type 1 in brain and risk of Alzheimer's disease. Lancet **1997**; 349:241–4.
47. Dowd JB, Aiello AE. Socioeconomic differentials in immune response. Epidemiology **2009**; 20:902–8.
48. Itzhaki RF, Dobson CB, Shipley SJ, Wozniak MA. The role of viruses and of APOE in dementia. Ann N Y Acad Sci **2004**; 1019:15–8.