MAJOR ARTICLE

# C-Reactive Protein Mediates the Effect of Apolipoprotein E on Cytomegalovirus Infection

**Allison E. Aiello,[1,2] Hoang-Oanh T. Nguyen,[1,2] and Mary N. Haan[2]**

[1]Center for Social Epidemiology and Population Health and [2]Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor

***Background.*** Although the apolipoprotein (APOE)–ε4 allele has been shown to determine the outcome of several infections, its relationship with cytomegalovirus (CMV) has not been explored. We examine whether APOE determines CMV and herpes simplex virus type 1 (HSV-1) antibody levels and assess whether C-reactive protein (CRP) mediates any observed relationships.

***Methods.*** We conducted a cross-sectional analysis of a randomly selected subset ($n = 1561/1789$) of participants aged 60–101 in the Sacramento Area Latino Study on Aging. Blood samples were tested for APOE genotype, CRP, and immunoglobulin G antibodies to CMV and HSV-1. Multivariate logistic regression was used to examine the association between ε4 and CMV and HSV antibody levels. We also assessed whether CRP mediates the effects of any observed associations between ε4 and viral antibody levels.

***Results.*** CMV antibody and CRP levels varied significantly by APOE genotype. The association between CRP and CMV antibody was strengthened in the presence of ε4. In contrast, this effect was not observed in HSV-1. We found that APOE-ε4 carriers had significantly lower levels of CRP yet significantly higher levels of CMV antibodies, suggesting a mediating pathway.

***Conclusions.*** APOE-ε4 carriers may experience immunological aberrations that lead to lower levels of CRP and correspondingly higher CMV antibody levels.

Apolipoprotein E (APOE) is a 299-aa protein involved in the packaging of cholesterol and other lipids. Three APOE alleles, APOE-ε2, -ε3, and -ε4 (henceforth referred to as ε2, ε3, and ε4, respectively), give rise to 3 protein isoforms, apoE2, apoE3, and apoE4, which have different affinities to lipoprotein receptors. The ε4 allele has been shown to determine the outcome of several infections, including herpes simplex virus type 1 (HSV-1), hepatitis C virus (HCV), HIV, and *Chlamydia pneumoniae* [1–6]. For example, HIV-infected individuals possessing the ε4 allele had increased oxidative stress of the central nervous system and were twice more likely to have dementia than HIV-positive individuals without ε4 allele [2, 3]. In other studies, the presence of ε4 was significantly more frequent among HSV-1–seropositive individuals with cold sores than were HSV-1–seropositive individuals without cold sores [5, 7]. On the other hand, possessing an ε4 allele may be protective against liver damaged caused by HCV [1, 6].

C-reactive protein (CRP) is an important component of the innate immune system, in which it acts as an opsonin and activates the classical complement pathway [8, 9]. A high CRP response plays a protective role against active bacterial and viral infections and also been found to be moderately elevated in the presence of persistent herpesvirus infections [10–15]. Several recent studies [16–18] have indicated that the presence of any ε4 allele is associated with lower levels of CRP and that the levels vary depending on the ε4 allele combination.

The present study sought to examine whether APOE genotype influences antibody response to herpesvirus infections (HSV-1 and cytomegalovirus [CMV]) in a large, elderly, community-based sample of individuals

Received 19 April 2007; accepted 6 July 2007; electronically published 5 December 2007.

Potential conflicts of interest: none reported.

Financial support: National Institute of Aging, National Institutes of Health (NIH; grant AG12975 to M.N.H.); National Institute of Diabetes and Digestive and Kidney Diseases, NIH (grant NIH5P60 DK60753 to M.N.H.); Chemistry Core of the Michigan Diabetes Research and Training Center (grant NIH5P60 DK20572 to M.N.H.); Robert Wood Johnson Health and Society Scholars Program at the University of Michigan (grant 045823 to fund the viral testing to A.E.A.); University of Michigan–Medical School, Institute of Gerontology (fellowship support support to A.E.A.).

Reprints or correspondence: Dr. Allison E. Aiello, Assistant Prof. of Epidemiology, Dept. of Epidemiology, Center for Social Epidemiology and Population Health, University of Michigan School of Public Health, 3659 SPH Tower, 109 Observatory, Ann Arbor, MI 48109-2029 (aielloa@umich.edu).

**The Journal of Infectious Diseases 2008; 197:34–41**

0022-1899/2008/19701-0007$15.00
DOI: 10.1086/524144

of Mexican origin. In addition, we assess whether the relationship between ε4 and antibody response to herpesvirus infections is mediated by CRP.

## METHODS

***Study design.*** The study subjects were derived from the Sacramento Area Latino Study on Aging (SALSA), an ongoing prospective cohort study of Mexican Americans aged 60–100 (in 1998/1999) living in the community. From the overall SALSA cohort ($n = 1789$), we drew a subpopulation of 1561 subjects who had available results for CMV, HSV-1, APOE, and CRP testing. The details of the SALSA study have been discussed elsewhere [19]. All study subjects signed an informed consent form, and the study was approved by the Institutional Review Board at the University of Michigan.

Baseline data collection for the SALSA study began in 1998, with a 2-h interview of participants in their homes. Information on lifestyle, health, duration, date of diagnoses for 35 health conditions, and demographic factors were collected during the participant interviews.

***Laboratory analyses.*** The level (expressed as optical density units) of IgG reactive to HSV-1 and CMV in aliquots of continuously frozen (−70°C) serum samples was assessed using a commercially available ELISA IgG detection system (Wampole Laboratories). In accordance with the manufacturer's instructions, samples were analyzed for the level of IgG antibody signal as expressed by the mean optical density value. The sensitivity and specificity of the assay for HSV-1 are reported to be 97.1% and 96.8%, respectively; for CMV, the sensitivity and specificity are reported to be 96.4% and 93.3%, respectively. Each 96-well commercial ELISA kit contained controls and internal calibrations.

CRP was analyzed using the CRP Ultra Wide Range Reagent Kit latex-enhanced immunoassay (Equal Diagnostics). The manufacturer's instructions were followed, and CRP levels were determined using an automated analyzer and a prepared calibration curve. With the highly sensitive method, the kit provides a range of measurable CRP from 0.05 to 80.0 mg/L. When results fell outside of the range, samples were diluted, retested, and multiplied by the dilution factor.

The 1513 samples for APOE genotyping were obtained from serum samples for 1122 participants and from buccal cell swabs for 391 participants. DNA was extracted using the PureGene DNA Extraction Kit (Gentra Systems), and APOE genotype was determined by polymerase chain reaction amplification followed by restriction enzyme digestion (*Hha*I), as described elsewhere [20]. Allele frequencies are as follows: ε2, 4.30% ($n = 130$); ε3, 88.50% ($n = 2678$); and ε4, 7.20% ($n = 218$). The APOE genotype was in Hardy-Weinberg equilibrium.

***Statistical analyses.*** To compare means, *t* tests were used; to compare proportions, $\chi^2$ or Fisher's exact tests were used. Measured CRP levels in milligrams per liter were skewed, so a natural logarithmic transformation (ln-CRP) was applied to maintain a normal distribution. We graphed the means and SEs of antibody levels and ln-CRP levels by different APOE genotype, to assess how genotype affects these levels.

Next, APOE genotype was categorized as any ε4 (ε2/4, ε3/4, and ε4/4) allele versus all other alleles (ε2/3 and ε3/3). General linear models were used to assess the relationship between ε4 status and CMV antibody levels, HSV-1 antibody levels, and ln-CRP levels. Logistic regression was used to assess the effect of CRP on antibody levels (CMV and HSV-1) after adjusting for age and sex. Other covariates including place of birth, education, the number of health conditions (diabetes mellitus, hypertension, myocardial infarction, angina pectoris, heart failure, atrial fibrillation, rheumatic fever/heart valve problem, cancer, and stroke) and body mass index (BMI) were assessed as potential confounders. Confounding may be present when the following criteria are met: (1) the covariate is related to the outcome of interest; (2) the distribution of the covariate is significantly different among the exposure groups; (3) and the covariate is not hypothesized to be in the pathway between the exposure and the outcome of interest. Correspondingly, *t* tests and $\chi^2$ tests were used to examine whether each of the covariates was associated with ln-CRP levels and whether the distribution of the covariates varied by any ε4 allele versus all other alleles and by levels of antibody to each of the viruses (HSV-1 and CMV). None of the baseline health covariates met the criteria and therefore were not included in the final models. Sex met the criteria of a confounder and was included in the final models. Although age did not meet all of the criteria of confounding, it was included in the final models because age is known to be associated with CRP levels, APOE distribution, and CMV.

We explored the possible mediation effects of CRP on the relationship between ε4 and antibody levels by use of the steps suggested by Baron and Kenny [21]. A factor that represents a step in the hypothesized pathway linking an exposure/trait (carriage of ε4) and an outcome (CMV IgG levels) is considered an "intermediate" factor that "mediates" the relationship between the 2 variables. Further discussion regarding mediation and testing for mediation has been discussed by Shrout and Bolger [22]. Briefly, these steps involved 3 models to assess (1) the correlation between ε4 and CMV levels, (2) the correlation between ε4 and CRP levels, and (3) the effect of CRP on CMV levels controlling for ε4. We also used the β coefficients (slope) and SEs from the models for 2 and 3 above to conduct a Sobel test for mediation. All analyses were conducted using procedures in SAS (version 9.1; SAS Institute).

## RESULTS

***Demographic characteristics.*** The demographic characteristics of subjects who were included in the analyses ($n = 1561$) are shown in table 1, stratified by sex. There were no differences in

**Table 1. Descriptive statistics for the Sacramento Area Latino Study on Aging cohort analysis sample.**

| Characteristic | Total sample ($n = 1561$) | Female ($n = 911$) | Male ($n = 643$) | $P$ (male/female difference)[a] |
|---|---|---|---|---|
| Age at baseline, mean ± SD, years[b] | 70.5 ± 7.0 | 70.7 ± 7.2 | 70.2 ± 6.8 | .19 |
| Place of birth Mexico or other Latin American country, %[c] | 49.30 | 51.04 | 46.81 | .10 |
| No. of baseline health conditions, mean ± SD[d] | 1.3 ± 1.1 | 1.31 ± 1.2 | 1.4 ± 1.1 | .34 |
| APOE genotype, %[e] | | | | |
| $\varepsilon 2/\varepsilon 3$ | 7.90 | 8.00 | 7.77 | .87 |
| $\varepsilon 2/\varepsilon 4$ | 0.73 | 0.79 | 0.65 | .75 |
| $\varepsilon 3/\varepsilon 3$ | 78.42 | 80.29 | 75.73 | .03 |
| $\varepsilon 3/\varepsilon 4$ | 12.22 | 10.47 | 14.72 | .01 |
| $\varepsilon 4/\varepsilon 4$ | 0.73 | 0.45 | 1.13 | .13 |
| Any $\varepsilon 4$ | 13.70 | 11.71 | 16.50 | .008 |
| Education, % | | | | |
| Grade 0–3 | 31.15 | 31.72 | 30.33 | .56 |
| Grade 4–11 | 38.29 | 40.50 | 35.15 | .03 |
| Grade ⩾12 | 30.57 | 27.77 | 34.53 | .004 |
| LDLC, mg/dL | . . . | 123.93 ± 35.2 | 121.97 ± 33.5 | .54 |
| BMI, %[f] | | | | |
| <25 | 20.18 | 21.29 | 18.62 | .20 |
| 25–30 | 38.48 | 35.12 | 43.19 | .0013 |
| ⩾30 | 41.34 | 43.59 | 38.18 | .03 |

**NOTE.** APOE, apolipoprotein; BMI, body mass index; LDLC, low-density lipoprotein cholesterol.

[a] $t$ tests and $\chi^2$ tests were conducted to assess whether sex differences were statistically significant.

[b] Information on age at baseline was not available for 7 individuals.

[c] Information on place of birth was not available for 7 individuals.

[d] Total no. of health conditions included reported diabetes mellitus, hypertension, myocardial infarction, angina pectoris, heart failure, atrial fibrillation, rheumatic fever/heart valve problem, cancer, and stroke.

[e] Information on APOE genotype was not available for 48 individuals.

[f] Information on BMI was not available for 25 individuals.

age, place of birth (Mexico or other Latin American country vs. United States), and number of baseline health conditions between males and females. Overall, the proportion of those with any $\varepsilon 4$ was 13.7% (207/1513), and the proportion with no $\varepsilon 4$ was 86.3% (1306/1513). The proportion of $\varepsilon 3/3$ genotype was significantly higher among females than males, and $\varepsilon 3/4$ was significantly higher among males than females. None of the other genotypes showed significant sex differences. The differences in proportions between male and female in terms of education were significant for grade level beyond fourth grade, with more women with highest level of education under the 11th grade and more men completing at least the 12th grade and beyond. There was a higher proportion of men who had a BMI between 25 and 30, whereas women had higher proportion with the highest BMI level (≥30).

Using the clinical cut point designated by the ELISA test kits, 97% (1507/1560) of the study subjects showed signs of prior infection with CMV, and 98% (1528/1560) showed signs of prior infection with HSV-1. Mean levels of CMV and HSV-1 IgG antibodies and ln-CRP are shown in table 2. Women had significantly higher mean levels of CMV IgG antibodies than men ($P < .0001$). In contrast, there was no difference between mean levels of HSV-1 IgG comparing males and females ($P = .4930$). More than 50% of the study subjects had CRP levels in the highest category, and CRP levels varied significantly by sex ($P < .0001$)

***Effects of APOE on infections and CRP.*** The levels of CMV, HSV-1 IgG antibodies, and CRP varied with APOE genotype (see figure 1). The variability pattern of CRP diverged from those of CMV and HSV-1 antibody levels. Subjects who were homozygous for $\varepsilon 4$ had high levels of CMV and HSV-1 antibodies but low levels of CRP; however the standard errors were high. Participants with $\varepsilon 2/3$ or $\varepsilon 3/3$ had low levels of CMV and HSV-1 antibodies but correspondingly higher levels of CRP.

We also assessed variability by any $\varepsilon 4$ status (homozygous or heterozygous) versus all other alleles (figure 2). Individuals with at least one $\varepsilon 4$ allele had significantly higher levels of CMV antibodies yet significantly lower levels of CRP than did subjects without an $\varepsilon 4$ allele. Although there were similar observed patterns in HSV-1 antibody by $\varepsilon 4$ allele status, the variability was not statistically significant.

**Table 2. Levels of cytomegalovirus (CMV) antibodies, herpes simple virus type 1 (HSV-1) antibodies, and proportions of C-reactive protein (CRP) among study subjects.**

| Category | Total sample ($n$ = 1561) | Female ($n$ = 911) | Male ($n$ = 643) | $P$ (male/female difference)[a] |
|---|---|---|---|---|
| Virus IgG antibody levels | | | | |
| CMV, mean ± SD | 4.98 ± 2.04 | 5.36 ± 1.95 | 4.45 ± 2.02 | <.0001 |
| HSV-1, mean ± SD | 6.55 ± 2.24 | 6.51 ± 2.14 | 6.59 ± 2.36 | .49 |
| ln-CRP levels, mean ± SD | 1.11 ± 1.20 | 1.32 ± 1.16 | 0.80 ± 1.20 | <.0001 |
| ln-CRP levels, %[b] | | | | |
| Low (<1) | 18.42 | 13.08 | 25.97 | <.0001 |
| Medium (1–3) | 28.65 | 26.04 | 32.35 | .0068 |
| High (>3) | 52.93 | 60.88 | 41.68 | <.0001 |

**NOTE.** ln-CRP, natural log of CRP.

[a] $t$ tests and $\chi^2$ tests were conducted to assess whether sex differences were statistically significant.

[b] Information on ln-CRP levels was not available for 8 individuals.

***Association between CRP and ε4 with antibody levels.*** Individuals with CRP levels above the median value had significantly higher levels of CMV antibody, adjusting for age and sex (table 3). The odds ratio for HSV-1 was also elevated but not statistically significant. The association between CRP and CMV antibody levels increased by 2-fold in the presence of at least one ε4 allele. The interaction term between CRP and ε4 also shows a trend indicating a stronger association with increased levels of CMV antibody but was of borderline statistical significance ($P = .051$). Higher levels of CRP were not associated with higher levels of HSV-1 antibody levels, nor did ε4 increase the association to a significant level.

***CRP mediation of ε4 and CMV.*** Figure 3 shows the results of linear regression models used to assess possible CRP mediation of ε4 and CMV relationship. Each path on the diagram represents the standardized $\beta$ coefficient for the following relationships: (1) between ε4 and CRP, (2) between CRP and CMV, (3) between ε4 and CMV, (4) between ε4 and CMV adjusted for CRP (estimate in parentheses). The estimate in parentheses shows that, after adjusting for CRP, the relationship between ε4 and CMV antibody levels is significantly increased. Conducting the Sobel test for mediation on the results shown in figure 3 yielded $z = -2.83$ and $P = .005$, suggesting significant mediation by CRP.

## DISCUSSION

This is the first study of which we are aware to demonstrate that APOE is significantly associated with variability in CMV antibody levels. It is also the first study to demonstrate that, through its suppressive effects on CRP, ε4 is related to higher levels of circulating CMV IgG antibody, even after controlling for age and sex. In contrast, variability in HSV-1 IgG antibody levels by APOE status was not statistically significant.

Most of the earlier research on the relationship between ε4 and infection has focused on HSV-1 [23–25], but a few other pathogens, such as HIV, HCV, and malarial parasites, have also

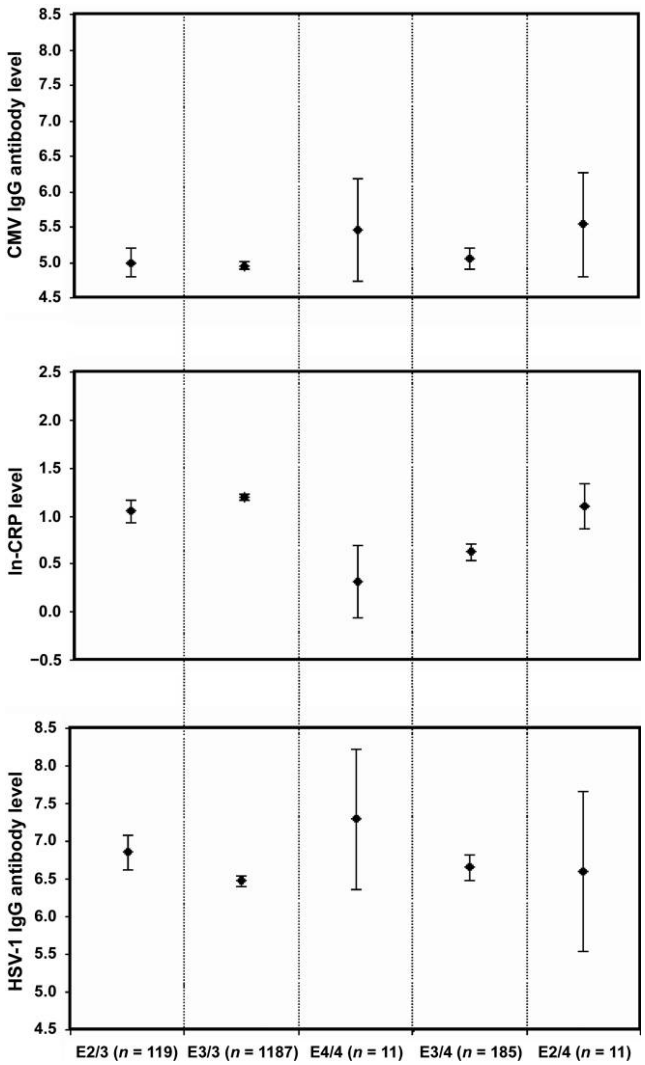


**Figure 1.** Apolipoprotein (APOE) genotypes and levels of cytomegalovirus (CMV) IgG antibodies, herpes simple virus type 1 (HSV-1) IgG antibodies, and C-reactive protein (CRP). Means and SEs of levels of CMV and HSV-1 IgG antibodies and the natural log of CRP (ln-CRP) were calculated and plotted by APOE genotype.

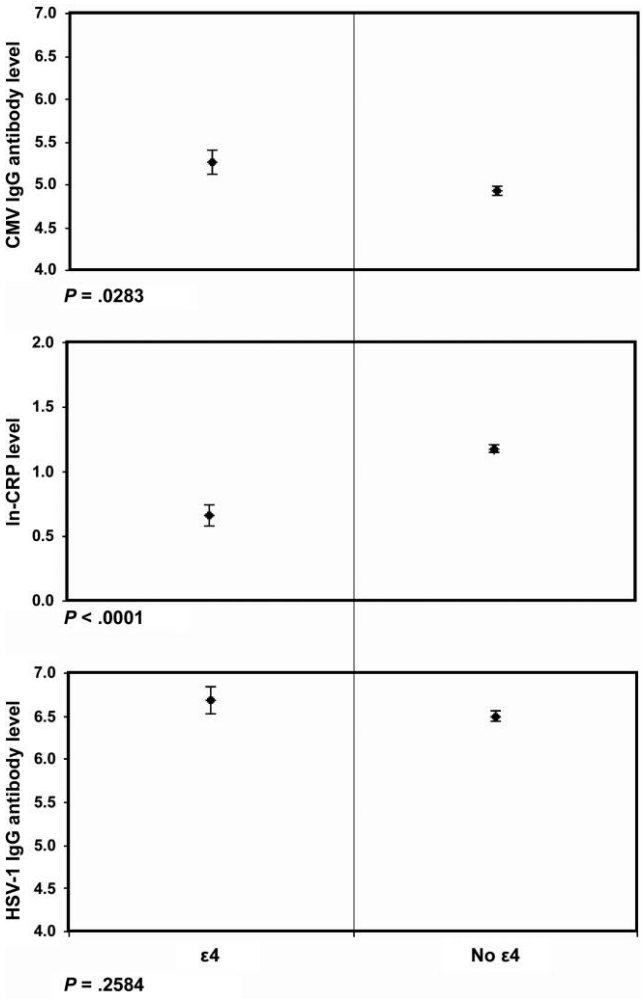


**Figure 2.** Apolipoprotein (APOE)–ε4 and levels of cytomegalovirus (CMV) IgG antibodies, herpes simple virus type 1 (HSV-1) IgG antibodies, and C-reactive protein (CRP). General linear models were used to generate least-square means of levels of CMV and HSV-1 IgG antibodies and natural log of CRP (ln-CRP) stratified by any apolipoprotein ε4 (homozygous or heterozygous for the ε4 allele) vs. no ε4 (ε2/3 and ε3/3).

been examined [1, 3, 6, 26]. Wozniak et al. [26] have suggested that APOE may compete with these pathogens for entry into cells because these pathogens also use the same cell-entry mediators as those of APOE, such as surface-binding heparan sulphate proteoglycans (HSPG) and/or low-density lipoprotein receptors. A recent mouse study by Miller and Federoff supports such a proposed pathway for HSV-1 by demonstrating that carriage of ε4 in the presence of HSV-1 renders cells more vulnerable to lytic infection and possibly apoptosis than do other alleles (ε2 or ε3) [27]. HSPG engagement is also integral to CMV entry [28]. Therefore, it is possible that a similar relationship exists between CMV and APOE during cellular entry processes.

It has also been suggested that differing structures of the APOE genotypes may lead to variations in binding affinity for pathogen-specific cellular entry sites or receptors, ultimately influencing entry, spread, cellular damage, or even protection from pathogens [26]. For example, ε4 appears to determine the outcome of HSV-1 infection, whereas ε2/4 and ε2/3 are protective for HCV and ε2/2 appears to be a risk factor for malarial infection at an earlier age of onset [1, 5, 6, 23–25, 29]. Correspondingly, we observed that CMV antibody levels varied within the ε4-positive group, with ε3/4 having the lowest CMV antibody levels, whereas ε4/4 and ε2/4 had similar CMV antibody levels. This variability pattern was unique to CMV. For HSV-1, the highest antibody levels were seen in ε4/4, whereas ε3/4 and ε2/4 had comparable antibody levels. Further studies assessing how variations in isoform-binding affinities may influence CMV pathogen cellular entry, spread, and damage are warranted.

We observed not only that the higher levels of CRP were associated with higher levels of CMV but that this association was strengthened in the presence of at least one ε4 allele, even though levels of CRP among ε4-positive subjects was lower compared with non-ε4 alleles. ε4 carriers with high CRP levels were more than twice as likely to have high CMV levels than were non-ε4 carriers with high CRP levels, after adjusting for age and sex. CMV entry is associated with activation of the innate immune system, and infection is a strong trigger of antipathogen response, including heightened levels of interleukin (IL)–6, which modulates CRP activation [30]. Persistent CMV IgG seropositivity in adults and/or elderly persons has been associated with higher levels of IL-6 and CRP, suggesting that these infection may elicit a persistent low-level inflammatory response [12–15, 31]. Recent studies have shown that primary astrocytes in mice expressing ε4 had significantly lower secretion of both tumor necrosis factor (TNF)–α and IL-6 compared with other APOE isoforms after lipopolysaccharide (LPS) activation [32]. The opposite relationship, however, has been reported in a mouse model examining LPS stimulation of macrophages, whereby mice with ε4 had higher levels of TNF-α and IL-6 [33]. Further research is needed to examine whether APOE determines the levels of IL-6 in human populations and whether this accounts for the variability in CRP antibody levels that we and other researchers have observed aging study populations [16–18].

***CRP in the pathway between ε4 and CMV.*** Our mediation model demonstrated that CRP is a significant mediator of the relationship between ε4 and CMV antibody levels. ε4 is negatively associated with CRP, yet CRP was positively associated with CMV levels. It is likely that the relationship between ε4 and CMV antibody levels was reduced in the presence of CRP because mediation can occur in the case when the mediator (CRP) acts as a pathway-suppressor variable [34]. The adjustment for CRP in step 3 of the mediation analysis suppresses (removes) the unwanted variance in ε4 and thus uncovered a stronger relationship between ε4 and CMV. The influence of ε4 appears to be specific to CMV, because our data showed that ε4 and CRP did not have the same effects on HSV-1 antibody levels. We did not find any significant effect of CRP on HSV-1 levels, whether independently or when stratified by ε4 status.

**Table 3. Association between C-reactive protein (CRP) and apolipoprotein (APOE)–ε4 on cytomegalovirus (CMV) and herpes simplex virus type 1 (HSV-1) antibody levels.**

| | Odds ratio (95% CI) | |
|---|---|---|
| Category | CMV | HSV-1 |
| High ln-CRP[a,b] | 1.398 (1.071–1.824) | 1.226 (0.938–1.603) |
| High ln-CRP among hetero/homozygous ε4 only[c] | 2.736 (1.352–5.535) | 1.803 (0.918–3.542) |
| High ln-CRP among non-ε4[c] | 1.328 (0.983–1.794) | 1.153 (0.852–1.560) |
| ln-CRP × ε4[d] | 2.133 (0.997–4.563) | 1.470 (0.715–3.026) |

**NOTE.** Logistic regression was used to assess the independent effect of high levels of the natural log of CRP (ln-CRP) on levels of CMV and HSV-1 for all subjects, which was then stratified by APOE-ε4 status. All models were adjusted for age and sex.

[a] High ln-CRP refers to ln-CRP values above the second quartile.

[b] Model: logit (probability of high antibody levels among subjects hetero/homozygous ε4 only) = $\beta_0 + \beta_1 \times$ high ln-CRP + $\beta_2 \times$ age + $\beta_3 \times$ sex.

[c] Same model as b but among non-ε4 subjects only.

[d] Interaction between CRP and ε4; model: logit (probability of high antibody levels) = $\beta_0 + \beta_1 \times$ high ln-CRP + $\beta_2 \times \varepsilon4 + \beta_3 \times$ age + $\beta_4 \times$ sex + $\beta_5 \times$ high ln-CRP × ε4. Odds ratio of the interaction term = exp(maximum likelihood estimate); 95% confidence interval (CI) = exp(maximum likelihood estimate ± 1.96 × SE).

Before examining mediation, we also explored the effect of ε4 and CRP interaction on CMV and HSV-1 antibody levels. The interaction term did not have a statistically significant effect on levels of CMV or HSV-1 antibodies. This interaction might be biologically plausible and have an effect specific to CMV antibody levels, as indicated by the trend shown in our data (table 3). However, our study did not have enough power to test for this interaction for HSV-1 or CMV given the small number of ε4 carriers ($n = 207$). Further research should explore interactions between ε4 and CRP on the effect of these herpesvirus antibody levels in larger cohorts.

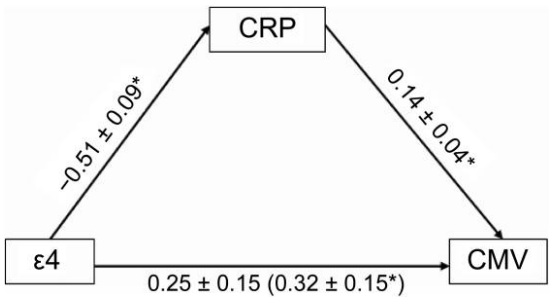


**Figure 3.** C-reactive protein (CRP) as a mediator of the relationship between apolipoprotein (APOE)–ε4 and cytomegalovirus (CMV) antibody levels. Linear regression models were used to assess the following path associations: (1) the relationship between ε4 and CRP, (2) the relationship between CRP and CMV, (3) the relationship between ε4 and CMV, and (4) the relationship between ε4 and CMV adjusting for CRP (estimate in parentheses). Each estimate along the path represents the standardized $\beta$ coefficient from the linear regression model. The bottom path estimate in parentheses shows that, after adjusting for CRP, the relationship between ε4 and CMV antibody levels is significantly increased. Conducting the Sobel test for mediation on the results shown in figure 3 yielded $z = -2.83$ and $P = .005$, suggesting significant mediation by CRP. All models were adjusted for age and sex. *$P < .05$.

Our study sample consisted of elderly Mexican Americans. As shown in NHANES III, Mexican Americans have a significantly higher prevalence of CMV infection (81.7%) than do non-Hispanic white persons (51.2%) and non-Hispanic black persons (75.8%) [35]. Furthermore, CMV seroprevalence also increased with age: 90.8% in those aged ≥80 years [35]. Thus, our study population would be expected to have a higher CMV seroprevalence than the general population, which may have enhanced the associations we observed.

Our analysis is cross-sectional, where genotype, levels of persistent virus antibody levels, and expression of biomarkers were measured at the same time. This limited our ability to assess the temporal relationship between CRP and antibody levels. Given the mediation effect of CRP on the relationship between ε4 and CMV IgG antibody levels, we would expect low levels of CRP to precede an increase in CMV IgG antibody levels. However, a prospective study design is needed to test this hypothesis. We assessed antibody levels in a population-based study of individuals living in the community setting. Our findings do not reflect clinical infection; therefore, it is unclear what these relationships imply for clinically apparent infection with HSV-1 and CMV. This study demonstrates an association with concurrent persistent CMV IgG antibody levels. For these reasons, it is not possible to evaluate whether the differences in IgG reflect heightened risk of subclinical reactivation of the virus among ε4 carriers.

***Clinical implications.*** The tripartite relationship of APOE, CRP, and CMV might play a role in predicting several health conditions, including susceptibility to HIV-associated CMV infection, dementia, and cardiovascular diseases. CMV is among

the most important opportunistic infections in HIV-positive patients. It is related to numerous conditions in this group, including ocular CMV disease, immune reconstitution syndrome, gastrointestinal disease, encephalitis and pneumonia, and mortality [36]. Furthermore, there is increasing evidence of an association between the presence of ε4 and enhanced risk of HIV-related dementia [3, 37, 38]. Our results suggest that ε4 is a factor that influences CMV IgG antibody levels. Therefore, it is important to assess whether HIV-infected patients who are ε4 carriers are at an even greater risk for CMV-associated conditions and mortality.

The presence of ε4 has also been associated with increased risk of cognitive decline [39], dementia [40–42], and Alzheimer disease in animal models [43, 44]. Most work supports the notion that individuals who are heterozygous for ε4 (ε2/4, ε3/4) have an increased risk of AD and vascular dementias and worse cognitive impairment than do those who do not carry any ε4 allele [42, 43]. Individuals who are homozygous for ε4 (ε4/4) are at even higher risk for these impairments [45, 46]. Our earlier work has shown that CMV significantly influences the rate of cognitive decline among aging individuals [12]. CRP has also been implicated as a risk factor for cognitive decline and dementia, and CRP appears to interact with ε4 to enhance the risk of dementia [18]. Further research is necessary to examine how the complex relationship between ε4, CRP, and infection might influence dementia and other chronic health conditions. Last, both CRP and CMV appear to aggravate atherosclerosis in APOE knockout mice [47, 48]. Thus, further studies are needed to assess whether the CRP-mediated influence of ε4 on antibody levels is also implicated in atherosclerosis given the suggested roles of persistent CMV in this disease [41].

***Conclusion.*** Our results underscore the important effects of APOE and CRP on CMV antibody levels. Because CMV antibody levels varied significantly by APOE genotype, it is possible that 3 alleles had different effects on the virus due to isoform structural differences. This is also the first study of which we are aware to demonstrate that CRP mediates the relationship between ε4 and CMV antibody levels. This mediation may suggest that ε4 subjects experience differential immune responsiveness to persistent infection with CMV. The results provide strong justification for further research into APOE-immunity-herpesvirus interactions.

## Acknowledgments

We thank Lynn Blythe, Kari Moore, and Dr. Oveta Fuller for their contribution to the laboratory testing and the study participants for their contributions to the study.

## References

1. Wozniak MA, Itzhaki RF, Faragher EB, James MW, Ryder SD, Irving WL. Apolipoprotein E-epsilon 4 protects against severe liver disease caused by hepatitis C virus. Hepatology **2002**; 36:456–63.
2. Turchan-Cholewo J, Liu Y, Gartner S, et al. Increased vulnerability of ApoE4 neurons to HIV proteins and opiates: protection by diosgenin and L-deprenyl. Neurobiol Dis **2006**; 23:109–19.
3. Corder EH, Robertson K, Lannfelt L, et al. HIV-infected subjects with the E4 allele for APOE have excess dementia and peripheral neuropathy. Nat Med **1998**; 4:1182–4.
4. Gerard HC, Wildt KL, Whittum-Hudson JA, Lai Z, Ager J, Hudson AP. The load of *Chlamydia pneumoniae* in the Alzheimer's brain varies with APOE genotype. Microb Pathog **2005**; 39:19–26.
5. 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.
6. Price DA, Bassendine MF, Norris SM, et al. Apolipoprotein epsilon3 allele is associated with persistent hepatitis C virus infection. Gut **2006**; 55:715–8.
7. Lin WR, Shang D, Wilcock GK, Itzhaki RF. Alzheimer's disease, herpes simplex virus type 1, cold sores and apolipoprotein E4. Biochem Soc Trans **1995**; 23:594S.
8. Du Clos TW. Function of C-reactive protein. Ann Med **2000**; 32:274–8.
9. Du Clos TW, Mold C. C-reactive protein: an activator of innate immunity and a modulator of adaptive immunity. Immunol Res **2004**; 30: 261–77.
10. Szalai AJ, VanCott JL, McGhee JR, Volanakis JE, Benjamin WH Jr. Human C-reactive protein is protective against fatal *Salmonella enterica* serovar Typhimurium infection in transgenic mice. Infect Immun **2000**; 68:5652–6.
11. ter Borg EJ, Horst G, Limburg PC, van Rijswijk MH, Kallenberg CG. C-reactive protein levels during disease exacerbations and infections in systemic lupus erythematosus: a prospective longitudinal study. J Rheumatol **1990**; 17:1642–8.
12. Aiello A, 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.
13. Zhu J, Quyyumi AA, Norman JE, Csako G, Epstein SE. Cytomegalovirus in the pathogenesis of atherosclerosis: the role of inflammation as reflected by elevated C-reactive protein levels. J Am Coll Cardiol **1999**; 34: 1738–43.
14. Roivainen M, Viik-Kajander M, Palosuo T, et al. Infections, inflammation, and the risk of coronary heart disease. Circulation **2000**; 101:252–7.
15. Miller GE, Freedland KE, Duntley S, Carney RM. Relation of depressive symptoms to C-reactive protein and pathogen burden (cytomegalovirus, herpes simplex virus, Epstein-Barr virus) in patients with earlier acute coronary syndromes. Am J Cardiol **2005**; 95:317–21.
16. Rontu R, Ojala P, Hervonen A, et al. Apolipoprotein E genotype is related to plasma levels of C-reactive protein and lipids and to longevity in nonagenarians. Clin Endocrinol (Oxf) **2006**; 64:265–70.
17. Kahri J, Soro-Paavonen A, Ehnholm C, Taskinen MR. ApoE polymorphism is associated with C-reactive protein in low-HDL family members and in normolipidemic subjects. Mediators Inflamm **2006**; 12587.
18. Haan MN, Aiello AE, West NA, Jagust WJ. C-reactive protein and rate of dementia in carriers and non carriers of apolipoprotein APOE4 genotype. Neurobiol Aging **2007**; 29 May (electronically published ahead of print).
19. Haan MN, Mungas DM, Gonzalez HM, Ortiz TA, Acharya A, Jagust WJ. Prevalence of dementia in older latinos: the influence of type 2 diabetes mellitus, stroke and genetic factors. J Am Geriatr Soc **2003**; 51:169–77.
20. Kuller LH, Lopez OL, Newman A, et al. Risk factors for dementia in the cardiovascular health cognition study. Neuroepidemiology **2003**; 22:13–22.
21. Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol **1986**; 51:1173–82.
22. Shrout PE, Bolger N. Mediation in experimental and nonexperimental studies: new procedures and recommendations. Psychol Methods **2002**; 7:422–45.
23. Itzhaki RF, Dobson CB, Lin WR, Wozniak MA. Association of HSV1 and apolipoprotein E-varepsilon4 in Alzheimer's disease. J Neurovirol **2001**; 7:570–1.

24. Lin WR, Wozniak MA, Esiri MM, Klenerman P, Itzhaki RF. Herpes simplex encephalitis: involvement of apolipoprotein E genotype. J Neurol Neurosurg Psychiatry **2001**; 70:117–9.
25. Burgos JS, Ramirez C, Sastre I, Valdivieso F. Effect of apolipoprotein E on the cerebral load of latent herpes simplex virus type 1 DNA. J Virol **2006**; 80:5383–7.
26. Wozniak MA, Riley EM, Itzhaki RF. Apolipoprotein E polymorphisms and risk of malaria. J Med Genet **2004**; 41:145–6.
27. Miller RM, Federoff HJ. Isoform-specific effects of ApoE on HSV immediate early gene expression and establishment of latency. Neurobiol Aging **2006**; 11 November (electronically published ahead of print).
28. Reddehase MJ, ed. Cytomegaloviruses: Molecular Biology and Immunology. Wymondham, UK: Caister Academic Press, **2006**.
29. Wozniak MA, Faragher EB, Todd JA, Koram KA, Riley EM, Itzhaki RF. Does apolipoprotein E polymorphism influence susceptibility to malaria? J Med Genet **2003**; 40:348–51.
30. Soderberg-Naucler C. Does cytomegalovirus play a causative role in the development of various inflammatory diseases and cancer? J Intern Med **2006**; 259:219–46.
31. 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.
32. Maezawa I, Maeda N, Montine TJ, Montine KS. Apolipoprotein E-specific innate immune response in astrocytes from targeted replacement mice. J Neuroinflammation **2006**; 3:10.
33. Tsoi LM, Wong KY, Liu YM, Ho YY. Apoprotein E isoform-dependent expression and secretion of pro-inflammatory cytokines TNF-alpha and IL-6 in macrophages. Arch Biochem Biophys **2007**; 460:33–40.
34. Cohen J, Cohen, P, West, SG, Aiken LS. Applied multiple regression/correlation analysis for the behavioral sciences. Mehwah, NJ: Lawrence Erlbaum Associates, **2003**.
35. 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.
36. Steininger C, Puchhammer-Stockl E, Popow-Kraupp T. Cytomegalovirus disease in the era of highly active antiretroviral therapy (HAART). J Clin Virol **2006**; 37:1–9.
37. Pocernich CB, Sultana R, Hone E, et al. Effects of apolipoprotein E on the human immunodeficiency virus protein Tat in neuronal cultures and synaptosomes. J Neurosci Res **2004**; 77:532–9.
38. King CA, Baillie J, Sinclair JH. Human cytomegalovirus modulation of CCR5 expression on myeloid cells affects susceptibility to human immunodeficiency virus type 1 infection. J Gen Virol **2006**; 87:2171–80.
39. Tilvis RS, Kahonen-Vare MH, Jolkkonen J, Valvanne J, Pitkala KH, Strandberg TE. Predictors of cognitive decline and mortality of aged people over a 10-year period. J Gerontol A Biol Sci Med Sci **2004**; 59:268–74.
40. Forsell Y, Corder EH, Basun H, Lannfelt L, Viitanen M, Winblad B. Depression and dementia in relation to apolipoprotein E polymorphism in a population sample age 75+. Biol Psychiatry **1997**; 42:898–903.
41. Zhu J, Quyyumi AA, Norman JE, et al. Effects of total pathogen burden on coronary artery disease risk and C-reactive protein levels. Am J Cardiol **2000**; 85:140–6.
42. Davidson Y, Gibbons L, Purandare N, et al. Apolipoprotein E epsilon4 allele frequency in vascular dementia. Dement Geriatr Cogn Disord **2006**; 22:15–9.
43. Corder EH, Saunders AM, Strittmatter WJ, et al. Gene dose of apolipoprotein E type 4 allele and the risk of Alzheimer's disease in late onset families. Science **1993**; 261:921–3.
44. Cedazo-Minguez A, Cowburn RF. Apolipoprotein E: a major piece in the Alzheimer's disease puzzle. J Cell Mol Med **2001**; 5:254–66.
45. Myers RH, Schaefer EJ, Wilson PW, et al. Apolipoprotein E epsilon4 association with dementia in a population-based study: the Framingham study. Neurology **1996**; 46:673–7.
46. Stevens M, van Duijn CM, de Knijff P, et al. Apolipoprotein E gene and sporadic frontal lobe dementia. Neurology **1997**; 48:1526–9.
47. Paul A, Ko KW, Li L, et al. C-reactive protein accelerates the progression of atherosclerosis in apolipoprotein E-deficient mice. Circulation **2004**; 109:647–55.
48. Vliegen I, Duijvestijn A, Grauls G, Herngreen S, Bruggeman C, Stassen F. Cytomegalovirus infection aggravates atherogenesis in apoE knockout mice by both local and systemic immune activation. Microbes Infect **2004**; 6:17–24.