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

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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.
of Mexican origin. In addition, we assess whether the relationship between e4 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 (HhaI), as described elsewhere [20]. Allele frequencies are as follows: e2, 4.30% (n = 130); e3, 88.50% (n = 2678); and e4, 7.20% (n = 218). The APOE genotype was in Hardy-Weinberg equilibrium.

**Statistical analyses.** To compare means, t tests were used; to compare proportions, χ2 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 e4 (e2/4, e3/4, and e4/4) allele versus all other alleles (e2/3 and e3/3). General linear models were used to assess the relationship between e4 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 χ2 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 e4 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 e4 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 e4) 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 e4 and CMV levels, (2) the correlation between e4 and CRP levels, and (3) the effect of CRP on CMV levels controlling for e4. 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
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 $e^4$ was 13.7% (207/1513), and the proportion with no $e^4$ was 86.3% (1306/1513). The proportion of $e^3/e^3$ genotype was significantly higher among females than males, and $e^3/e^4$ 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 ($\geq 30$).

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 $e^4$ had high levels of CMV and HSV-1 antibodies but low levels of CRP; however the standard errors were high. Participants with $e^2/e^3$ or $e^3/e^3$ had low levels of CMV and HSV-1 antibodies but correspondingly higher levels of CRP.

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

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.

<table>
<thead>
<tr>
<th>Category</th>
<th>Total sample (n = 1561)</th>
<th>Female (n = 911)</th>
<th>Male (n = 643)</th>
<th>P (male/female difference)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Virus IgG antibody levels</td>
<td>CMV, mean ± SD</td>
<td>4.98 ± 2.04</td>
<td>5.36 ± 1.95</td>
<td>4.45 ± 2.02</td>
</tr>
<tr>
<td></td>
<td>HSV-1, mean ± SD</td>
<td>6.55 ± 2.24</td>
<td>6.51 ± 2.14</td>
<td>6.59 ± 2.36</td>
</tr>
<tr>
<td>ln-CRP levels, %b</td>
<td>Low (&lt;1)</td>
<td>18.42</td>
<td>13.08</td>
<td>25.97</td>
</tr>
<tr>
<td></td>
<td>Medium (1–3)</td>
<td>28.65</td>
<td>26.04</td>
<td>32.35</td>
</tr>
<tr>
<td></td>
<td>High (&gt;3)</td>
<td>52.93</td>
<td>60.88</td>
<td>41.68</td>
</tr>
</tbody>
</table>

NOTE. ln-CRP, natural log of CRP.

* t tests and χ² tests were conducted to assess whether sex differences were statistically significant.

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.
from pathogens [26]. For example, e4 appears to determine the outcome of HSV-1 infection, whereas e2/2 and e2/3 are protective for HCV and e2/2 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 e4-positive group, with e3/4 having the lowest CMV antibody levels, whereas e4/4 and e2/4 had similar CMV antibody levels. This variability pattern was unique to CMV. For HSV-1, the highest antibody levels were seen in e4/4, whereas e3/4 and e2/4 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 e4 allele, even though levels of CRP among e4-positive subjects was lower compared with non-e4 alleles. e4 carriers with high CRP levels were more than twice as likely to have high CMV levels than were non-e4 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 e4 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 e4 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 e4 and CMV.** Our mediation model demonstrated that CRP is a significant mediator of the relationship between e4 and CMV antibody levels. e4 is negatively associated with CRP, yet CRP was positively associated with CMV levels. It is likely that the relationship between e4 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 e4 and thus uncovered a stronger relationship between e4 and CMV. The influence of e4 appears to be specific to CMV, because our data showed that e4 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 e4 status.

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Figure 2. Apolipoprotein (APOE)–e4 and levels of cytomegalovirus (CMV) IgG antibodies, herpes simplex 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 e4 (homozygous or heterozygous for the e4 allele) vs. no e4 (e2/3 and e3/3).

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 e4 in the presence of HSV-1 renders cells more vulnerable to lytic infection and possibly apoptosis than do other alleles (e2 or e3) [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...
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.

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

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**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.**

<table>
<thead>
<tr>
<th>Category</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CMV</td>
</tr>
<tr>
<td>High ln-CRP(^a)</td>
<td>1.398 (1.071–1.824)</td>
</tr>
<tr>
<td>High ln-CRP among hetero/homozygous ε4 only(^b)</td>
<td>2.736 (1.352–5.535)</td>
</tr>
<tr>
<td>High ln-CRP among non-ε4(^c)</td>
<td>1.328 (0.983–1.794)</td>
</tr>
<tr>
<td>ln-CRP × ε4(^d)</td>
<td>2.133 (0.997–4.563)</td>
</tr>
</tbody>
</table>

*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) = \(\beta_0 + \beta_1\) × high ln-CRP + \(\beta_2\) × age + \(\beta_3\) × 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) = \(\beta_0 + \beta_1\) × high ln-CRP + \(\beta_2\) × age + \(\beta_3\) × sex + \(\beta_4\) × 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).

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**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 β 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. \(^*P < .05\).
the most important opportunistic infections in HIV-positive patients. It is related to numerous conditions in this group, including oculcar 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 e4 and enhanced risk of HIV-related dementia [3, 37, 38]. Our results suggest that e4 is a factor that influences CMV IgG antibody levels. Therefore, it is important to assess whether HIV-infected patients who are e4 carriers are at an even greater risk for CMV-associated conditions and mortality.

The presence of e4 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 e4 (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 e4 allele [42, 43]. Individuals who are homozygous for e4 (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 e4 to enhance the risk of dementia [18]. Further research is necessary to examine how the complex relationship between e4, 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 e4 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 isofrom structural differences. This is also the first study of which we are aware to demonstrate that CRP mediates the relationship between e4 and CMV antibody levels. This mediation may suggest that e4 subjects experience differential immune responsiveness to persistent infection with CMV. The results provide strong justification for further research into APOE-immunity-herpesvirus interactions.

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References