The Effect of Chronic Cytomegalovirus Infection on Pneumococcal Vaccine Responses

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Background. Immune function declines with age and has been associated with reduced vaccine responsiveness. Chronic infection with cytomegalovirus (CMV) has been proposed as a contributor to poorer responses in older adults. A pneumococcal vaccine has been recommended in the United Kingdom for those aged >65 years since 2003 to prevent pneumococcal disease.

Methods. We evaluated the effect of age and CMV status on pneumococcal vaccine responses in 348 individuals aged 50–70 years.

Results. We found participant age to be associated with serotype-specific and functional antibody titers after pneumococcal vaccination, with a mean 6.2% (95% confidence interval, 2.9%–9.5%) reduction in postvaccination functional antibody titers per year. CMV status was not associated with serotype-specific immunoglobulin G concentrations or functional antibody titers after pneumococcal vaccination. However, CMV seropositivity was associated with higher levels of prevaccination functional antibody for 4 of 7 pneumococcal serotypes assessed.

Conclusions. These data imply that CMV infection is not directly responsible for the decline in pneumococcal vaccine responses seen with age but suggest that CMV-seropositive individuals differ in their natural exposure to pneumococci or have altered mucosal immune responses after colonization with this organism.

Human cytomegalovirus (CMV) is the largest known human herpesvirus, with a double-stranded linear DNA genome of about 235 kb [1]. In industrialized countries, adult CMV seropositivity rates exceed 50%, and this figure increases progressively with age [2, 3]. CMV rarely causes serious illness in immunocompetent individuals, but this virus has a number of mechanisms of immune subversion and establishes a lifelong infection [4]. In CMV-infected individuals a high proportion of memory T cells become devoted to this virus, and chronic infection has been linked with persistent changes to overall immune composition [5]. CMV seropositivity is associated with increased numbers of CD4+ and CD8+ T cells that lack the expression of the naive T-cell marker CD28, and MHC tetramer analysis has confirmed that a large proportion of these cells show CMV specificity [6–8]. CMV infection particularly drives a clonal expansion in CMV-reactive CD8+ T cells, which may account for >25% of all peripheral blood CD8+ T cells [8, 9]. A diminishing naive T-cell pool and the accumulation of oligoclonal memory T cells are also characteristics of an aging immune system and are believed to be detrimental to immune function [10]. CMV infection in the elderly has been associated with an altered ratio of CD8+ to CD4+ T cells, poor in vitro proliferative responses, and a higher 2-year mortality rate [11, 12]. Chronic viral infections, such as CMV infection, may exacerbate age-associated alterations in immune function by providing a persistent antigenic stimulus, driving T cells to a high-differentiated and senescent state [13].

Immune function deteriorates with age making the elderly more susceptible to infectious disease, as well as poorer responders to vaccination [14–18]. However,
there are conflicting data on the effect of CMV infection on the ability of the immune system to respond to vaccination [19–25]. In 3 recent studies, CMV seropositivity in older adults was associated with poorer responses to influenza vaccine [19–21]. However, a large study of elderly individuals in long-term care facilities did not show an association between CMV status and the magnitude of influenza vaccine responses [22]. CMV has a major impact on the T-cell repertoire of both the young and the elderly, and it has been suggested that these immunological modifications may alter immune responses before the onset of immunosenescence [26]. However, 2 studies in Gambian infants did not show decreased responses to *Haeomophilus influenzae* type b, tetanus toxoid, or serogroup C meningococcal or measles vaccination in CMV-seropositive infants [24, 25]. Conversely, these data suggested that the magnitude of T-cell responses to CMV in infancy positively correlated with postvaccination titers of measles virus hemagglutination-inhibiting antibodies [25].

The 23-valent plain polysaccharide vaccine (PPV23) has been recommended for all persons aged >65 years in United Kingdom since 2003, but it has shown only limited efficacy [27]. There are data suggesting that PPV23 is less immunogenic in the elderly than in young adults, with production of lower-avidity antibodies with less functional activity [28–30]. In the current study, we investigated the effect of aging and CMV status on immunological responses to pneumococcal vaccination in adults aged 50–70 years.

**MATERIAL AND METHODS**

**Subjects and Vaccinations**

Study participants were healthy 50–70-year-olds recruited from Oxford, United Kingdom, into a randomized clinical trial to receive pneumococcal vaccination. The details of this trial are described elsewhere [31]. In brief, participants who had no history of pneumococcal vaccination or disease were randomly assigned into 3 groups to receive PPV23 (Pneumovax II; Aventis Pasteur) after priming with 0, 1, or 2 doses of the 7-valent pneumococcal conjugate vaccine PCV7 (Prevenar; Wyeth Vaccines). This retrospective study assessed the influence of age and CMV status on immunological responses to the first dose of either PCV7 or PPV23 vaccine.

**Immunological Measures of Vaccine Immunity**

Blood samples were obtained at baseline and 1 month after vaccination. Serotype-specific immunoglobulin G (IgG) concentrations were measured for the 7 serotypes (4, 6B, 9V, 14, 18C, 19F, and 23F) present in both vaccines, using a validated bead-based multiplex assay [32]. In brief, serum samples were incubated with pneumococcal polysaccharides covalently attached to carboxylated fluorescent microspheres, with the addition of cell wall polysaccharide and 22F polysaccharide. Anti-human IgG was added before reading on the Bio-Plex (Bio-Rad, Hertfordshire, UK). Undetectable specific antibody concentrations were set to half the lower limit of detection.

Opsonophagocytic assay (OPA) titers, a measure of serotype-specific functional antibody, were measured for the 7 serotypes present in both vaccines on a subset of randomly selected participants. OPA titers were calculated as the interpolated reciprocal dilution of test serum that causes a 50% reduction of bacteria surviving the opsonophagocytic reaction incubation. A titer of 4 was allocated to serum samples with undetectable OPA titers. Cultured memory B-cell enzyme-linked immunospot assays were performed as described elsewhere [33].

**CMV Status**

Specific IgG antibodies to human CMV were measured in the serum samples of participants by immunoassay (Diaisorin Liaison CMV IgG II). CMV seronegativity was defined as a concentration of CMV-specific IgG of <12 IU/mL, and seropositivity as >14 IU/mL, with values between 12 and 14 IU/mL considered equivocal.

**Statistical Analysis**

A generalized linear model was used to test for an association between age, sex, and CMV status. Reciprocal OPA titers, memory B-cell spots and IgG concentrations were log_{10} transformed for analysis. The relationships between participant age, CMV status, and serotype-specific immunological measures were assessed using a linear regression model, adjusted for sex, vaccine administered (PCV7 or PPV23) and baseline antibody concentration, as appropriate. A combined serotype analysis was also undertaken for each immunological measure, using a mixed-effects model with participant as a random effect and serotype, age, sex, CMV status, vaccine administered, and baseline antibody concentration as fixed effects. Analyses of OPA titers were not adjusted for baseline values because the participants tested at visit 1 (baseline) were not the same as those tested at visit 2 (postvaccination); serial measures were not available. Combined serotype analyses were considered the primary analyses because they had the greatest statistical power. Statistical analyses were performed using the R (version 3.0.1) software environment for statistical computing and plotted using the ggplot2 package [34, 35]. Differences were considered statistically significant at *P* < .05.

**RESULTS**

**CMV Status**

Sera were available for measurement of specific IgG antibodies to human CMV for 333 of the 348 individuals recruited into the original pneumococcal vaccine trial. Two had equivocal CMV serology and were therefore excluded from analyses. Sensitivity analyses including these equivocal sample, as CMV seropositive or seronegative, did not alter the findings presented.
here. A summary of the demographic characteristics for the remaining 331 participants is presented in Table 1. In this cohort, 53% of individuals were CMV seropositive, and status did not differ significantly with sex or age (Table 1).

**Effect of Age on Serotype-Specific Pneumococcal IgG Concentrations and OPA Titers**

Older age was associated with lower baseline pneumococcal IgG concentrations, for 2 of 7 serotypes in individual analyses, as well as overall in the combined serotype analysis (Figure 1 and Supplementary Table 1). After vaccination, older age was associated with lower IgG antibody levels and OPA titers, equating to a 3.1% (95% confidence interval, 1.2%–5.1%) and 6.2% (2.9%–9.5%) decrease in IgG concentrations and OPA titers per year, respectively (Figure 1 and Supplementary Table 2). There were no statistically significant relationships between age at vaccination and pre- or postvaccination memory B-cell frequencies (Supplementary Table 1 and Supplementary Table 2).

**Effect of CMV Status on Serotype-Specific Pneumococcal IgG Concentrations and OPA Titers**

At baseline, OPA titers were significantly higher in CMV-seropositive individuals than in CMV-seronegative individuals for 4 of 7 serotypes, as well as in combined serotype analysis (Figure 1, Figure 2, and Supplementary Table 1). After vaccination, no statistically significant differences in OPA titers were observed between CMV-seropositive and CMV-seronegative individuals (Figure 1, Figure 2, and Supplementary Table 1). Only 1 of 7 serotypes had significantly higher serotype-specific IgG levels in CMV-seropositive participants after vaccination, but combined serotype analysis did not show an association (Figure 1 and Supplementary Table 1). CMV status was not associated with pre- or postvaccination memory B-cell frequencies (Supplementary Table 1 and Supplementary Table 2).

### Table 1. Overview of CMV Status in Healthy Adults Recruited into a Pneumococcal Vaccine Study in Oxford, United Kingdom

<table>
<thead>
<tr>
<th>Demographic Group</th>
<th>Participants, No. (%)</th>
<th>CMV Seropositive</th>
<th>CMV Seronegative</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group, y</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50–55</td>
<td>36 (45)</td>
<td>44 (55)</td>
<td>.22</td>
<td></td>
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<td>55–60</td>
<td>61 (55)</td>
<td>49 (45)</td>
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<td>60–65</td>
<td>65 (56)</td>
<td>51 (44)</td>
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<td>65–70</td>
<td>14 (56)</td>
<td>11 (44)</td>
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<tr>
<td>Sex</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>75 (50)</td>
<td>76 (50)</td>
<td>.25</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>101 (57)</td>
<td>76 (43)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All participants</td>
<td>176 (53)</td>
<td>155 (47)</td>
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Abbreviation: CMV, cytomegalovirus.

* A generalized linear model was used to assess the relationship between CMV status and age, adjusting for sex.

**DISCUSSION**

Here, we present what is to our knowledge the first study assessing the influence of age and CMV status on responses to pneumococcal vaccination. Although there was a negative relationship between age and pneumococcal vaccine responses, CMV status was not associated with the magnitude of immunological responses to pneumococcal vaccination. These data imply that CMV status does not contribute to the decline in pneumococcal vaccine responses seen with age.

We demonstrated an age-associated decline in serotype-specific and functional antibody titers after pneumococcal vaccination for 4 of 7 serotypes assessed. It is unclear why age had a statistically significant effect on immune responses to only some serotypes. However, this phenomenon may be partly due to (1) differences in vaccine capsular chemistry eliciting contrasting immunological constituents, (2) differing previous exposure to serotypes as a result of carriage, or (3) variability in serotype specific assay performance and analytical noise. Nevertheless, a significant decline is seen in both IgG and OPA titers in combined serotype analysis, suggesting a global age-related decline in responsiveness to the pneumococcal polysaccharides contained in PCV7.

A number of previous studies have assessed the influence of CMV seropositivity on influenza vaccine responses; however, these have typically evaluated a modest number of individuals and have presented conflicting data [19–23, 36]. Differences in study design may underlie these inconsistencies, particularly with regard to participant demography, sample size, and analytical methods. Several of these studies enrolled elderly individuals from nursing facilities, a population with higher rates of comorbid conditions than community-based older adults, potentially confounding interstudy comparisons [21]. These studies have also differed as to whether CMV status or titers of anti-CMV antibody were compared with vaccine responses,
with 2 studies reporting disparities between these approaches [23, 36].

The data presented here were used to evaluate the influence of CMV status on responses to pneumococcal vaccination in a large and healthy cohort of community-recruited 50–70-year-old adults. Participants in this study had no history of previous pneumococcal vaccination or pneumococcal infection; however, it is likely that they would have been exposed to nasopharyngeal colonization by various serotypes of pneumococci and were therefore not immunologically naïve [37]. It may be that the immunological changes associated with chronic CMV infection are chiefly detrimental in response to novel antigens [17]. Although, 2 studies of Gambian infants after primary immunization did not show CMV status to affect responses to neoantigens, this has not yet been assessed in the context of an aged thymus [24, 25]. It may remain true that CMV infection exacerbates the decline in vaccine responses seen in the elderly (aged >70 years). However, in our cohort of 50–70-year-olds, despite an age-associated decline in pneumococcal vaccine responses, there was no evidence to suggest a detrimental effect of CMV seropositivity. It has also been suggested that the concentration of anti-CMV IgG could be indicative of viral reactivations and may therefore be more informative markers of the effects of CMV infection on immunosenescence. However,

**Figure 1.** Heat map regression coefficient matrix, depicting the relationship and statistical association between age (left) and cytomegalovirus (CMV)–seropositive status (right) with pneumococcal specific immunoglobulin G (IgG) and opsonophagocytic (OPA) antibody titers at baseline (visit 1) and after vaccination (visit 2). *P < .05; †P < .01; ‡P < .001.
Figure 2. Top, Serotype-specific immunoglobulin G (IgG) antibody geometric mean concentrations for each of the 7 pneumococcal serotypes assessed at baseline (visit 1 [V1]) and after a single dose of vaccine (visit 2 [V2]), separated by cytomegalovirus (CMV) status. Bottom, geometric mean titers determined with opsonophagocytic assay (OPA) for each of the 7 pneumococcal serotypes assessed at baseline (V1) and after a single dose of vaccine (V2), separated by CMV. *P < .05; †P < .001.
when we accounted for the concentration of anti-CMV IgG antibodies, we found results consistent with serostatus, with a hint that pneumococcal responses may in fact increase with anti-CMV antibodies.

Interestingly, we found CMV seropositivity to be associated with increased pneumococcal specific functional antibody titers before vaccination. One possibility is that CMV-seropositive individuals differ in their natural exposure to pneumococci, particularly because the 2 organisms share a number of risk factors, such as household size and income [3, 38]. Alternatively, underlying CMV infection may alter mucosal immunity, increasing the tendency of CMV-infected individuals to respond to colonizing bacteria, such as pneumococci. Murine studies have shown herpesvirus latency heightens innate immunity, increases the tendency of CMV-infected individuals to respond to colo-

mococci between CMV-seropositive and CMV-seronegative individuals.

In conclusion, although we found that pneumococcal vaccine responses declined with age, CMV status was not independently associated with the magnitude of immunological responses. On the other hand, CMV seropositivity was strongly associated with higher levels of prevaccination serotype-specific functional OPA antibody, implying differences in natural exposure to pneumococci between CMV-seropositive and CMV-seronegative individuals.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Authorship contributions.** D. O. and J. T. had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Analysis and interpretation of data: D. O., J. T., M. V., and A. J. P. Drafting of the manuscript: D. O., J. T., R. L., E. A. C., M. V., K. J., and A. J. P. Acquisition of data: D. O., R. L., E. A. C., K. J., and A. J. P. Statistical analysis: D. O. and M. V. Obtaining funding: D. O., K. J., and A. J. P.

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**Potential conflicts of interest.** J. T. and R. L. have received financial assistance from vaccine manufacturers to attend scientific meetings. A. J. P. conducts clinical trials of vaccines for Oxford University, which receives grant funding from vaccine manufacturers and unrestricted educational grants for organization of courses and symposia, and does not receive any personal payments or support from vaccine manufacturers. A. J. P. is a member of the meningococcal subcommittee of the UK Department of Health’s Joint Committee on Vaccines and Immunisation and has previously advised the Department of Health on pneumococcal vaccines. All other authors report no potential 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**


