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## Impact of human cytomegalovirus (CMV) infection on immune response to pandemic 2009 H1N1 influenza vaccine in healthy adults

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### Abstract

Human cytomegalovirus (CMV) infection has been implicated in immunosenescence. To examine the influence of CMV on ability of healthy adults to respond to a novel influenza antigen, the rate of seroconversion and the magnitude of titers to pandemic 2009 H1N1 vaccine was assessed. The clinical trial was stratified by age; 52 persons aged 18–64 and 55 aged 65 and older were enrolled. Among the younger group, 33% had CMV antibody compared with 62% among the older group. No differences by CMV seropositivity in the proportion of participants achieving a seroprotective titer 21 days following the second immunization were noted. However, the geometric mean titer in hemagglutination inhibition assay was significantly higher among CMV seronegative younger participants compared with CMV seropositive younger participants (385 vs 142,  $p=0.013$ ). In contrast, among the older group, CMV serostatus was not associated with differential antibody titers (53 vs 63,  $p=0.75$ ). These data suggest that CMV may shape immune response to neoantigens among younger persons; these groups should be included in future studies of immunosenescence and CMV.

### Keywords

immunosenescence; influenza vaccine; cytomegalovirus; antibody titer

### Introduction

Aberrant host response to human cytomegalovirus (CMV) infection has been implicated in immunosenescence, the dysfunction of the immune system associated with aging [Brunner et al., 2010; Pawelec et al., 2010]. Impaired immune response to neoantigens, including pathogens and vaccines, is considered a hallmark of immunosenescence. Poor response to influenza vaccine among the elderly has been used as an example of immunosenescence but studies that investigated the association between CMV infection and poor response to

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influenza vaccine have had inconsistent findings [Powers and Belshe, 1993; Trzonkowski et al., 2003; den Elzen et al., 2011; Moro-Garcia et al., 2012]. To evaluate the effect of CMV infection on the response to pandemic 2009 H1N1 influenza vaccine, sera were tested for CMV antibody from adult participants in a double-blind, randomized, clinical trial of p2009 H1N1 influenza.

## Methods

A multi-site study of pH1N1 influenza vaccine compared immune response to 15ug and 30ug of antigen administered twice 21 days apart; the study population was stratified by age (18–64 and ≥65). The details of the parent trial that was sponsored by Vaccine and Treatment Evaluation Network have been published [Chen et al., 2012]. Briefly, healthy adults were randomized to two different doses of the study product made by bioCSL (Victoria, Australia; previously CSL Biotherapies), and followed closely for reactogenicity for the first 7 days after each immunization. Blood was drawn at baseline, day 8, 21, 29 and 42 following the initial immunization for assessment of immunogenicity. In August 2009, the University of Washington site enrolled 131 participants, of which 105 provided consent to use their stored sera remaining after testing for immune response to pH1N1 influenza. Immunogenicity of the influenza vaccine was assessed by hemagglutination inhibition assay, at a central laboratory, as previously reported [Chen et al., 2012]. Immunogenicity was defined as reaching a seroprotective titer (geometric mean titer >1:40) or seroconversion (4-fold or greater increase in titer). Baseline sera from participants at University of Washington site were tested for CMV antibody using a commercial CMV IgG assay (Wampole Laboratories, Princeton, NJ, USA). Associations between CMV serostatus and age group, and CMV serostatus and seroconversion to pH1N1 were examined using Fisher's exact test. Mann-Whitney was used to compare the geometric mean titers to p2009H1N1 by group. In all results, 2-sided p value of ≤0.05 were considered statistically significant. University of Washington Human Subjects Review Committee approved both this analysis and the parent vaccine study. The primary analysis focused on day 21 results after 2<sup>nd</sup> vaccination.

## Results

One hundred and five participants provided consent for future use of stored sera; 8 were excluded from the analysis as their baseline sera showed protective levels of pH1N1 antibodies. The remaining 97 participants were enrolled in 2 strata, 18–64 years of age and ≥65 and older. In the younger stratum of 42 participants, the mean age was 44, of whom 23 (55%) were women and 37 (88%) were white. In the older stratum of 55 participants, the mean age was 70, of whom 26 (47%) were women and 47 (85%) were white. Among younger participants, 40% were CMV seropositive compared with 62% of older participants ( $p = 0.04$ ). All 97 persons received both injections and completed the study protocol.

At 21 days following the second vaccine dose, 63 (65%) achieved seroprotective titer (geometric mean titer >1:40) or seroconverted (4-fold or greater increase in titer). Among younger participants, 88% of 42 persons had seroconverted to pH1N1 compared with 47% among older participants ( $p < 0.001$ ). No differences in seroconversion were noted by antigen dose. However, among the younger stratum, the geometric mean titers in hemagglutination inhibition assay at 21 days post the second vaccination were 385 for CMV seronegative vs 142 for CMV seropositive. In contrast, no differences in pH1N1 seroconversion rate ( $p = 0.99$ ) or geometric mean titer in hemagglutination inhibition assay ( $p = 0.75$ ) were noted by CMV status among older adults (fig 1 and 2). Following initial immunization, the geometric mean titer rose in both age and CMV serostatus groups but the rise among the younger CMV seronegative group was substantially steeper and the higher titers were sustained until the end of study. Results were similar when age groups were stratified at 50

years of age, instead of 65 ( $p=0.017$  for comparison of 18–49 year old CMV negative vs CMV positive participants). Analyses could not be further restricted by age, as only 15 persons were aged younger than 40.

## Discussion

This exploratory analysis of the role of prior CMV infection on the immune response to influenza vaccine suggested that CMV infection is associated with a dampened humoral response among younger persons but appeared to have no effect on antibody titers among the elderly. In this study, CMV serostatus had no effect on the proportion of subjects achieving a protective antibody titer at 21 weeks after vaccination, either overall or separately by age group. This is in contrast to previously published literature showing that CMV seropositivity may be associated with a poor response to vaccines among the elderly [Trzonkowski et al., 2003]. However, the geometric mean titer in hemagglutination inhibition assay was significantly higher among CMV seronegative younger participants compared with CMV seropositive younger participants while no such differences were seen among the older group. These findings are consistent with recognized poorer response of older persons to immunizations [Simonsen et al., 2007; Brunner et al., 2010], but, surprisingly, suggest that CMV serostatus may have an effect preferentially among younger individuals. Several reasons could potentially be responsible for this differential result between younger and older persons. One potential reason for the inconsistent results in the literature and in this study is that CMV serostatus is a rather crude measure of CMV exposure and the immune status, and CMV reactivation may be more biologically relevant factor. A recent large review indicated that CMV shedding is more common than generally assumed but specific data on shedding patterns by age are not available [Cannon et al., 2011]. Studies are needed to examine the impact of CMV shedding on vaccine responses and immune correlates of aging. Another possible reason for the expected results is that the study included a relatively small number of participants and potential unknown factors that may have affected immunogenicity. However, all patients were healthy with no major comorbidities and good nutrition. Finally, we assumed the persons identified as CMV seronegative at study entry remained so throughout the study, because the short duration of the study made it unlikely to provide many opportunities for CMV acquisition.

In conclusion, this study suggested that CMV serostatus affected the strength of the immune response to H1N1 vaccination in younger individuals but not older. Larger cohorts will be required to confirm these observations. The results suggest that studies examining the role of CMV infection in shaping immunity should include younger population, in addition to the elderly, to discern the impact of CMV infection among this age group.

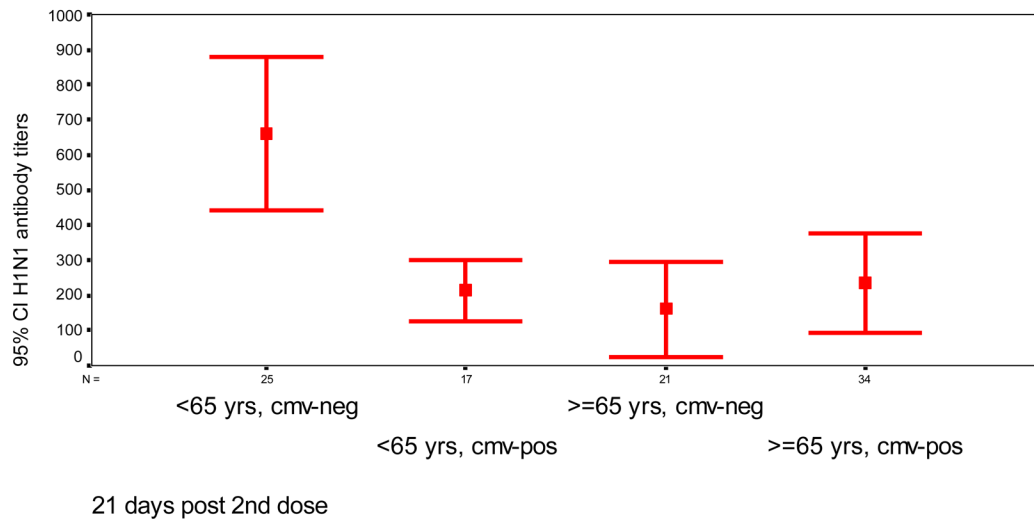
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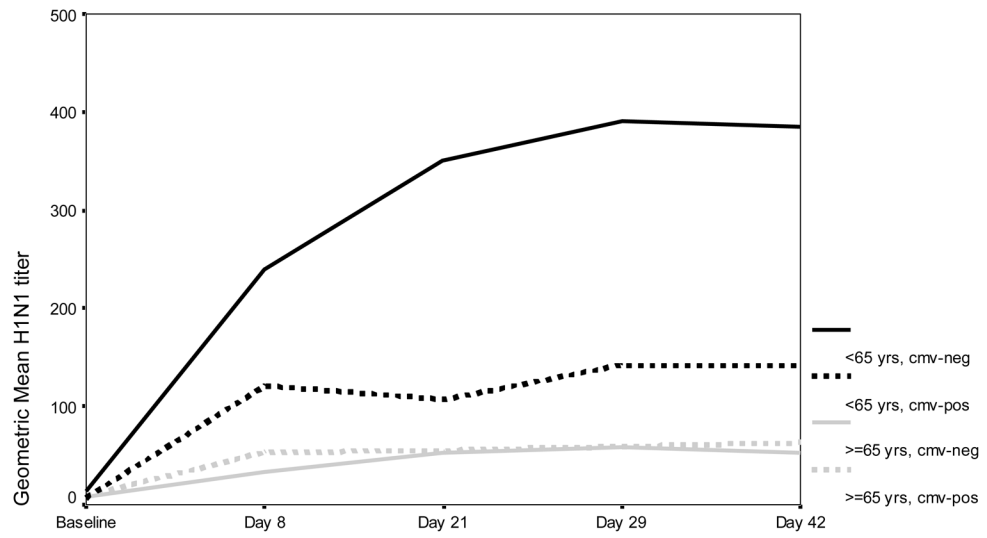
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**Figure 1.** Geometric mean titer of hemagglutination inhibition antibody at 21 days post 2<sup>st</sup> immunization by age and CMV status.



**Figure 2.** Geometric mean titer of hemagglutination inhibition antibody throughout the study by age and CMV serostatus.