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Detection of cytomegalovirus infection during a vaccine clinical trial in healthy young women: Seroconversion and viral shedding

Changpin Zhang [a,*], Hannah Buchanan [a], William Andrews [c], Ashley Evans [d], Robert F. Pass [a,b]

[a] *Department of Pediatrics, UAB School of Medicine, Birmingham, AL, United States*
[b] *Department of Microbiology, UAB School of Medicine, Birmingham, AL, United States*
[c] *Department of Obstetrics and Gynecology, UAB School of Medicine, Birmingham, AL, United States*
[d] *College of Community Health Sciences, University of Alabama, Tuscaloosa, AL, United States*

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Abstract

Background: An antibody method based on absorption of serum with cytomegalovirus (CMV) glycoprotein B (gB) was developed for detection of infection during clinical trials of CMV gB vaccine. Previous study showed that this method detected the antibody response to infection and was negative with vaccine induced immunity.
Objectives: In an ongoing efficacy trial of CMV gB vaccine the ability of the gB-absorbed CMV IgG assay to detect CMV infection was assessed and compared with viral culture results.
Study design: Two hundred and ninety two healthy, seronegative young women in a phase II, double-blind, placebo-controlled, clinical trial of recombinant CMV gB vaccine (sanofi pasteur) with MF59 adjuvant (Chiron) were randomized to receive CMV gB vaccine or placebo (1:1) on a 0, 1 and 6 month schedule. Participants were screened every 3 months for CMV infection using the gB-absorbed CMV IgG assay, and a subgroup was also screened for infection with viral cultures. Viral culture (urine, vaginal swab and saliva) was used to confirm CMV infection in all subjects with a positive gB-absorbed CMV IgG result.
Results: Evidence of CMV infection (gB-absorbed CMV IgG levels $\geq$ 5.0 AU/ml) was found in 23/292 (7.88%) study participants. The gB-absorbed CMV IgG levels of their first positive serum ranged from 15.7 to 251.0 AU/ml with a mean of 77.0 AU/ml and a median of 44.9 AU/ml. Cytomegalovirus was isolated from all 23 of them from culture specimens collected after their first positive gB-absorbed CMV IgG. The time to first CMV positive culture from first positive gB-absorbed CMV IgG ranged from 0 to 12 weeks with a median of 2 weeks.
Conclusions: The gB-absorbed CMV IgG assay detects CMV infection in CMV gB vaccine clinical trials earlier and more rapidly than virus culture and does not reveal whether subjects received CMV gB vaccine or placebo.

Keywords: CMV vaccine; Glycoprotein B; Primary CMV infection

1. Introduction

An important issue in any cytomegalovirus (CMV) vaccine clinical trial is the detection of endpoint. Viral shedding and associated CMV disease were used successfully as endpoints in a clinical trial with Towne CMV live virus vaccine in renal transplant patients (Plotkin et al., 1991). However, clinical trials of vaccines for prevention of maternal and congenital CMV infection will involve healthy subjects, and primary CMV infection in healthy young women is clinically silent in around 95% of cases (Griffiths and Baboonian, 1984; Stagno et al., 1982). Thus efficacy trials of CMV vaccines in healthy young women will require laboratory methods for detection of infection. Very little is known about the natural history of CMV infection near the time of primary infection in healthy subjects; the data upon which to base the selec-

Abbreviations: CMV, cytomegalovirus; gB, glycoprotein B; UAB, University of Alabama at Birmingham; PBS, phosphate buffered saline; CPE, cytopathic effect; PCR, polymerase chain reaction

* Corresponding author at: UAB Department of Pediatrics, Children's Hospital CHB 116, 1600 7th Avenue South, Birmingham, AL 35233, United States. Tel.: +1 205 996 7750; fax: +1 205 996 2370.
E-mail address: cpz@uab.edu (C. Zhang).

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tion of methods for endpoint detection in clinical trials is lacking.

Measurement of CMV IgG response after absorption of sera with purified glycoprotein B (gB) masks vaccine induced antibody response and should make serologic detection of infection possible in clinical trials of CMV gB vaccine (Zhang and Pass, 2004). However, previous evaluation of the gB-absorbed CMV IgG assay involved sera from phase I and II clinical trials in subjects not considered at risk for CMV infection and in which viral cultures were not performed. We evaluated the ability of the gB-absorbed CMV IgG assay to detect CMV infection in an ongoing efficacy trial of CMV gB vaccine in which subjects were also screened for infection using viral culture and in which all serologically identified infections were subject to confirmation by viral culture.

2. Materials and methods

2.1. Study population

The study population included 292 healthy, seronegative young women participating in a phase II, randomized, double-blind, placebo-controlled, clinical trial of recombinant CMV gB vaccine (sanofi pasteur, Marcy l'Etoile, France) with MF59 adjuvant (Chiron Vaccines, Emeryville, CA). All subjects who were participating in the clinical trial between January 2003 and December 2004 were included; they were enrolled at the University of Alabama at Birmingham (UAB) and the University of Alabama College of Community Health Sciences in Tuscaloosa. Participants were randomized to receive CMV gB vaccine or placebo (1:1) on a 0, 1 and 6 month schedule, they were screened every 3 months for CMV infection. Each participant is followed for 3.5 years from date of enrollment (first vaccine). This clinical trial was reviewed and approved by the Institutional Review Board of each participating institution and all subjects signed approved consent forms.

2.2. gB-Absorbed CMV IgG

Serum IgG antibody to CMV was measured using a microparticle enzyme immunoassay (Axsym® system CMV IgG, Abbott Laboratories, Abbott Park, IL) after incubation of sera at room temperature with phosphate buffered saline (PBS) containing CMV gB for 1 h. Recombinant CMV gB (vaccine antigen) was provided by sanofi pasteur. The recombinant gB molecule is identical to that used in the vaccine clinical trial which is derived from Towne CMV gB. Sanofi pasteur gB was dissolved in PBS at a concentration of 100 μg/ml. The optimal ratio of gB solution to serum was empirically determined to be 3:1, yielding a final gB concentration of 75 μg/ml. Following incubation of serum with PBS/gB for 1 h, CMV IgG was measured using the Axsym® system. Results are expressed as antibody units/ml (AU/ml) of serum. Calibration curves were established for each batch of reagents and positive and negative controls were included with each assay. CMV gB-absorbed IgG levels of ≥5.0 AU/ml were considered positive (Zhang and Pass, 2004). Sera from vaccine clinical trial participants were tested blinded as to source of the serum. In addition, the vaccine trial remains under blind at the time of this report.

2.3. CMV culture

In order to confirm the presence of CMV infection, 25 cm^2 tissue culture flasks seeded with human foreskin fibroblasts at passage 7–12 were used for viral isolation. Specimens of urine, vaginal swab and saliva were collected from vaccine trial participants who had serologic evidence of CMV infection (gB-absorbed CMV IgG level ≥ 5.0 AU/ml). These specimens were collected monthly for 6 months after the visit at which serologic evidence of infection was first detected and then at each regular visit (every three months) through termination. One 25 cm^2 flask was used for each sample and 0.5 ml of sample was inoculated. Flasks were examined weekly for evidence of CMV cytopathic effect (CPE) using low power (40×) magnification with the inverted microscope (Nikon TMS). Foci of CPE numbers of ≥1 were considered positive. The initial positive culture from each subject was subcultured for propagation of virus and confirmation of CMV. All samples were held and examined for at least 4 weeks (28 days) before being signed out as negative and discarded.

Cytomegalovirus culture was also used to screen subjects for CMV infection prior to incorporation of the gB-absorbed CMV IgG assay into study protocol. Specimens of urine and mouth swab were collected at each quarterly follow-up visit on a subgroup ($n = 135$) of participants in this study.

3. Results

3.1. CMV infection screening by gB-absorbed CMV IgG

The gB-absorbed CMV IgG was performed on each of 292 vaccine trial participants at a mean frequency of 6. Subjects with gB-absorbed CMV IgG positive results were compared with subjects with negative results, Table 1. Serologic evidence of CMV infection was found in 23/292 (7.88%) study participants who had gB-absorbed CMV IgG levels > 5.0 AU/ml. The gB-absorbed CMV IgG levels of the first positive serum ranged from 15.7 to 251.0 AU/ml with a mean of 77.0 AU/ml and a median of 44.9 AU/ml. Overall 1147 serum samples were tested, 1093 were negative (gB-absorbed CMV IgG level <5.0 AU/ml, range 0.0–3.7 AU/ml). Every subject with a positive gB-absorbed CMV IgG was positive on repeat testing at their next study visit. The gB-absorbed CMV IgG levels of the first follow-up serum post infection on the 23 positive subjects ranged from 13.0 to 251.0 AU/ml with a mean of 95.5 AU/ml and a median of 75.6 AU/ml. The median interval from last negative to first