Varicella-Zoster Virus–Specific Antibody Responses in 50–59-Year-Old Recipients of Zoster Vaccine

Myron J. Levin,1 Kenneth E. Schmader,2 John W. Gnann,3 Shelly A. McNeil,4 Timo Vesikari,5 Robert F. Betts,6 Susan Keay,5 Jon E. Stek,6 Nickoya D. Bundick,6 Shu-Chih Su,6 Yanli Zhao,6 Xiaoming Li,6 Ivan S. F. Chan,6 Paula W. Annunziato,6 and Janie Parrino3

1University of Colorado Denver Anschutz Medical Campus, Aurora, 2Duke University, Durham, North Carolina, 3Medical University of South Carolina, Charleston, 4University of Rochester, New York, 5Veterans Affairs Maryland Health Care System, Baltimore, Maryland, and 6Merck & Co., Inc., Whitehouse Station, New Jersey

Prevention and 6-week postvaccination samples from the immunogenicity substudy (n = 2269) of the zoster vaccine (ZV) efficacy trial (N = 22,439) in 50–59-year-old subjects were examined for varicella-zoster virus–specific antibody responses to vaccination. The varicella-zoster virus geometric mean titer (GMT) and geometric mean fold rise were higher in ZV recipients than in placebo recipients (GMT, 660.0 vs 293.1 glycoprotein enzyme-linked immunosorbent assay units/mL [P < .001], respectively; geometric mean fold rise, 2.31 vs 1.00 [P < .025]). In each group there was a strong inverse correlation between postvaccination GMT and risk of subsequent herpes zoster. Although these data provide strong evidence that relates ZV-induced antibody and the risk of herpes zoster, a protective threshold was not determined.

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Varicella-Zoster Virus (VZV)–specific antibody responses were examined in 50–59-year-old subjects vaccinated with the live attenuated herpes zoster (HZ) vaccine (zoster vaccine [ZV]; Zostavax™; Merck & Co., Inc.) was recommended by The Advisory Committee on Immunization Practices in the United States in 2008 for immunocompetent individuals aged ≥60 years, based on a large randomized, placebo-controlled trial, the Shingles Prevention Study (SPS) [1, 2]. There is strong clinical evidence that varicella-zoster virus (VZV)–specific cell-mediated immunity (VZV CMI) is both necessary and sufficient to prevent HZ and that ZV prevents HZ because it stimulates VZV CMI [3–6]. It is believed that there is a causal inverse relationship between the loss of VZV CMI that occurs with aging and the age-related increase in HZ. In contrast, VZV-specific immunoglobulin G antibody (VZV antibody) does not decline with age [3, 7]. Nevertheless, the SPS demonstrated that a measure of VZV antibody, in addition to 2 measures of VZV CMI, correlated with protection against HZ, although no quantitative measure of any of these responses reliably predicted the extent of protection [8].

A subsequent efficacy trial of ZV in 22,439 subjects 50–59 years old demonstrated an efficacy of 69.8% for preventing HZ [9]. Ten percent of subjects were randomly assigned to an immunology substudy/subcohort that measured VZV antibody response to ZV. The substudy objectives were to determine if ZV in 50–59-year-olds is immunogenic (as evaluated by glycoprotein enzyme-linked immunosorbent assay [gpELISA]) and to assess the association between antibody response at week 6 after vaccination and the risk of HZ.

METHODS

Study Design
The methods for this event-driven, randomized, double-blind, placebo-controlled, multicenter study (NCT00532428) are published elsewhere [10]. Subjects were randomized (1:1 ratio) to receive either ZV or placebo. To evaluate the correlation of vaccine-induced ZV antibody responses and subsequent protection against HZ, serum samples were collected from study subjects before and 6 weeks after vaccination, with VZV antibody concentrations measured by gpELISA in (1) the immunology subcohort population (10% protocol-prespecified, randomized subcohort) and (2) the case-cohort population (immunology subcohort plus all subjects in whom suspected HZ developed).

Study Population
Healthy subjects aged 50–59 years with a history of varicella or residence in a VZV-endemic area for ≥30 years, were enrolled. Exclusion criteria have been described elsewhere and included evidence of immunocompromise [9]. The protocol was conducted in accordance with principles of good clinical practice and approved by the ethical review committee of each participating site; written informed consent was obtained from each subject before study entry.
Intervention
Lyophilized ZV and placebo were supplied in 0.7-mL single-dose vials and stored at −15°C or colder. Placebo contained the same stabilizers as ZV, but no live virus or virus components. ZV and placebo were reconstituted with sterile water immediately before administration. All subjects received a single 0.65-mL subcutaneous injection of either ZV or placebo in the deltoid area.

Follow-up
Subjects were educated regarding the signs and symptoms of HZ and instructed to call their study site if HZ symptoms occurred. Contact by an interactive voice response system was undertaken monthly until study completion to ensure that suspected HZ was reported. Suspected HZ cases were evaluated by a site investigator. Initiation of treatment with antiviral therapy and pain medication was determined by the treating physician.

Assessment of Suspected HZ Cases
All HZ rash characteristics were recorded, and lesion swab samples were submitted for detection of VZV, herpes simplex, and human β-globin DNA using a polymerase chain reaction (PCR) assay (performed at PPD Vaccines and Biologics) [10].

Determination of Confirmed HZ Cases
Suspected HZ cases were defined as “confirmed HZ” if VZV DNA was present in skin lesion samples. If the PCR assay was positive for β-globin or herpes simplex virus DNA, and negative for VZV DNA, then the case was defined as “not HZ.” When there was no specimen or the specimen was inadequate, case determination was decided by a clinical evaluation committee [9].

VZV-Specific Antibody Assay
The gpELISA for VZV-specific immunoglobulin G antibody (performed at PPD Vaccines and Biologics) detects antibodies to purified VZV glycoproteins from MRC-5 (normal human lung fibroblasts, Medical Research Council 5 cell line) cells infected with VZV (KMcc strain), using methods described elsewhere [11]. First, VZV glycoproteins or uninfected MRC-5 lysates were adsorbed to polystyrene microtiter wells. Experimental, control, and standard curve serum samples were incubated in coated tissue culture wells in duplicate at 23°C for 40–80 minutes until the difference in optical density (OD) between the VZV glycoprotein–containing (positive) wells and control (negative) wells was >0.700, as measured in the plate reader at 405 nm approximately every 5 minutes after an initial 40-minute incubation. Color development was stopped with 50 μL of 3N sodium hydroxide. Delta OD was calculated for each serum sample as the difference between the average OD of the 2 VZV antigen wells and that of the 2 MRC-5 control wells. Quantitation was performed by comparing sample delta OD with a standard curve, with results reported as concentrations of antibody in gpELISA units per milliliter.

Statistics
The immunogenicity objectives were (1) to determine whether administered ZV is immunogenic and (2) to assess the association between antibody response 6 weeks after vaccination and the risk of HZ. To show a significantly higher geometric mean titer (GMT) in VZV antibody titers at 6 weeks after vaccination in the ZV group compared with the placebo group, 2230 subjects for the 10% subcohort (1115 randomly selected in each group) would provide an overall power of approximately 98% at the .025 significance level (1 sided; noninferiority criterion, lower bound of 2-sided 95% confidence interval for GMT ratio [ZV/placebo] >1.4). This assumed that the true GMT ratio is 1.7 [12], the standard deviation of the natural-log-transformed titers is 1.1, and there would be a 10% nonevaluable rate for immunogenicity measurements. The immunogenicity summaries and analyses were based on a per-protocol approach. Subjects and observations with protocol deviations that might invalidate the evaluation of VZV-specific gpELISA antibody response were excluded from the immunogenicity analyses.

RESULTS
There were 22,439 subjects randomly assigned to receive ZV or placebo (Supplementary Figure 1 - CONSORT diagram). Serum samples were obtained in all subjects before and 6 weeks after vaccination; VZV antibody was measured in the 10% immunology subcohort and in patients with suspected HZ. The ZV and placebo recipients in the immunology subcohort were well matched by sex, age, race, and study completion. Most subjects (94%) were white, and 62% were female (Supplementary Table 1 - demographics); >94% of subjects completed the study.

At baseline (day 1), 5 subjects (3 ZV and 2 placebo recipients) did not have VZV-specific antibody measured by gpELISA. The 2 treatment arms were well matched before vaccination in the distribution of high and low antibody titers (P = .84; χ² test for homogeneity) (Table 1). Six weeks after vaccination the GMT was 660 gpELISA units/mL in ZV recipients, versus 293 gpELISA units/mL in placebo recipients, for an estimated GMT ratio (ZV/placebo) of 2.3 (95% confidence interval, 2.2–2.4; P < .001), which met the prespecified statistical criterion. Half of the ZV recipients had at least a doubling of VZV antibody titer. The geometric mean fold rise (GMFR) in titer in ZV recipients was 2.31, compared with no fold rise in placebo recipients (P < .025).

During the study, 30 ZV and 99 placebo recipients developed HZ; 6 ZV and 10 placebo recipients developed HZ before collection of their postvaccination blood sample and thus were excluded from the immunogenicity analyses (Table 2). For the subjects included in the immunogenicity analyses, HZ was
Table 1. VZV-Specific gpELISA Titers in ZV and Placebo Recipients

<table>
<thead>
<tr>
<th>Immunogenicity End Point</th>
<th>ZV Recipients (n = 1136)</th>
<th>Placebo Recipients (n = 1133)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer at day 1, gpELISA units/mL&lt;sup&gt;cd&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.25</td>
<td>3 (0.3)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>≥1.25 to &lt;100</td>
<td>161 (14.3)</td>
<td>157 (14.0)</td>
</tr>
<tr>
<td>&gt;100 to ≤300</td>
<td>469 (41.8)</td>
<td>453 (40.3)</td>
</tr>
<tr>
<td>&gt;300 to ≤500</td>
<td>174 (15.5)</td>
<td>192 (17.1)</td>
</tr>
<tr>
<td>&gt;500</td>
<td>316 (28.1)</td>
<td>320 (28.5)</td>
</tr>
<tr>
<td>GMT, gpELISA units/mL</td>
<td>283.6</td>
<td>292.8</td>
</tr>
<tr>
<td>Titer at week 6, gpELISA units/mL&lt;sup&gt;ef&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;1.25</td>
<td>0 (0.0)</td>
<td>2 (0.2)</td>
</tr>
<tr>
<td>≥1.25 to &lt;100</td>
<td>23 (2.1)</td>
<td>141 (13.0)</td>
</tr>
<tr>
<td>&gt;100 to ≤300</td>
<td>201 (18.5)</td>
<td>443 (40.8)</td>
</tr>
<tr>
<td>&gt;300 to ≤500</td>
<td>197 (18.1)</td>
<td>188 (17.3)</td>
</tr>
<tr>
<td>&gt;500</td>
<td>667 (61.3)</td>
<td>313 (28.8)</td>
</tr>
<tr>
<td>GMT, gpELISA units/mL</td>
<td>660.0</td>
<td>293.1</td>
</tr>
<tr>
<td>Fold rise from day 1&lt;sup&gt;g&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>541 (49.8)</td>
<td>36 (3.3)</td>
</tr>
<tr>
<td>≥3</td>
<td>330 (30.4)</td>
<td>4 (0.4)</td>
</tr>
<tr>
<td>≥4</td>
<td>221 (20.3)</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>≥5</td>
<td>166 (15.3)</td>
<td>3 (0.3)</td>
</tr>
<tr>
<td>GMFR&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.31</td>
<td>1.00</td>
</tr>
</tbody>
</table>

Abbreviations: GMFR, geometric mean fold rise; GMT, geometric mean titer; gpELISA, glycoprotein enzyme-linked immunosorbent assay; VZV, varicella-zoster virus; ZV, zoster vaccine.

<sup>a</sup> Immunogenicity subcohort population; does not include all subjects who developed suspected HZ.
<sup>b</sup> Values represent No. (%) of subjects except in rows for GMT and GMFR.
<sup>c</sup> Prevaccination: 1123 ZV and 1124 placebo subjects contributed to this analysis.
<sup>d</sup> P = .84 (χ² test for homogeneity in distributions of baseline titers between vaccine and placebo arms).
<sup>e</sup> Week 6: 1088 ZV and 1087 placebo recipients contributed to this analysis.
<sup>f</sup> P < .025 (χ² test for homogeneity in distributions of week 6 titers between vaccine and placebo arms).
<sup>g</sup> Fold rise: 1087 ZV and 1086 placebo subjects contributed to this analysis.

Identified by PCR in 19 of 24 ZV and 78 of 89 placebo recipients; for the rest, HZ cases were confirmed by the clinical evaluation committee assessment.

In each treatment arm after vaccination, the GMT for subjects who did not develop HZ was significantly higher than for subjects who developed HZ, although the GMT for the placebo...
DISCUSSION

This trial confirms that persons who indicate that they had prior varicella and/or had resided in the United States for ≥30 years have serologic evidence of prior varicella infection (only 5 of 2369 individuals lacked antibody at baseline by gpELISA). In the previous trial of subjects ≥60 years old, this was true of all 1395 samples tested with gpELISA [8]. The 3 subjects in the current trial who were seronegative at the time of ZV administration did not develop any serious adverse events or VZV-like rashes, thereby adding to the safety data available from seronegative ZV recipients [13].

In subjects 50–59 years old, ZV was immunogenic, as measured by a significant rise in VZV antibody titer. The postvaccination GMT was 660 gpELISA units/mL versus 293 gpELISA units/mL in the control group, and the 6-week GMFR was 2.3. This response was greater than that observed in the trial of older subjects [12], in whom the postvaccination GMT and GMFR were 478.4 and 1.7, respectively. These results indicate a more robust VZV antibody response to ZV in younger vaccinees (50–59-year-olds) and is consistent with greater efficacy for HZ prevention (69.8%) in 50–59-year-olds than in older subjects (64% and 38% for the 60–69- and ≥70-year age groups, respectively) in the SPS [1, 9].

The VZV antibody response 6 weeks after vaccination in this younger group was strongly inversely correlated (P < .001) with the likelihood of developing HZ, as demonstrated elsewhere in the ZV trial in older subjects, but neither trial established a titer of VZV antibody that would serve as a surrogate of protection [8]. The lack of a quantitative surrogate of protection is demonstrated in the current findings; VZV antibody titers measured in the placebo recipients who did not develop HZ were lower than those achieved by ZV recipients who did develop HZ. This confirms that VZV antibody should not be considered directly responsible for the efficacy of ZV against HZ; rather, VZV CMI is necessary and sufficient for preventing HZ. This essential role of VZV CMI has previously been established by (1) substantial clinical observations indicating that HZ occurs in immunocompromised patients with high levels of VZV antibody [4–6] and (2) the relationship between the increasing incidence of HZ with increasing age and the decline in VZV CMI [14], whereas there is no such relationship with VZV antibody [7]. In addition, the trial in older subjects did not demonstrate any correlation between VZV antibody and VZV CMI. This lack of correlation between these 2 classes of immune responses, which has been confirmed [15], may represent the detection of different VZV epitopes unique to each class of immune response.

The absence of paired VZV CMI and VZV antibody data is a limitation of our study. Another limitation is the lack of data on chronic pain, which may have been related to the magnitude of the immune response. Postherpetic neuralgia greatly affects quality of life and is the most common complication of HZ but the role of the immune response to HZ and the subsequent development of postherpetic neuralgia are poorly understood. In addition, the study was performed almost entirely in white subjects; immune response to HZ may differ by racial origin, just as the incidence of HZ is lower in blacks than in whites [16].

The practical implication of the study data is that although this specific antibody measure is predictive of a ZV response and is a suitable immunogenicity marker for comparative studies of ZV, it does not provide a precise threshold for protection. Given that protection from HZ depends on VZV-specific CMI, gpELISA may be inadequate for assessments among individuals with altered immune function, in whom there may be a lack of correlation between cellular and humoral responses. Also important when considering comparative immunogenicity studies is the relationship between gpELISA GMT and GMFR and clinical efficacy, which may be specific to ZV, a vaccine that contains the entire Oka strain virus. These immunogenicity measures may not be correlated with the efficacy of alternative HZ vaccines based on different formulations (such as subunit or recombinant vaccines) that may be developed in the future.

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

References


