Varicella-Zoster Virus–Specific Immune Responses in Elderly Recipients of a Herpes Zoster Vaccine

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Background. A double-blind, placebo-controlled trial that involved 38,546 subjects ⩾60 years old demonstrated efficacy of a high-potency live-attenuated Oka/Merck varicella-zoster virus (VZV) vaccine. The trial included an immunology substudy to determine the relationship of VZV-specific immune responses to vaccination and clinical outcome.

Methods. The immunology substudy enrolled 1395 subjects at 2 sites where blood samples obtained prior to vaccination, at 6 weeks after vaccination, and at 1, 2, and 3 years thereafter were tested for VZV-specific cell-mediated immunity (VZV-CMI) by γ-interferon ELISPOT and responder cell frequency assays and for VZV antibody by glycoprotein ELISA.

Results. VZV-CMI and VZV antibodies were significantly increased in vaccine recipients at 6 weeks after vaccination. The vaccine-induced increases in VZV-CMI persisted during the 3 years of follow-up, although their magnitude decreased over time. The magnitude of these VZV-specific immune responses was greater in subjects 60–69 years old than in subjects ⩾70 years old.

Conclusions. The zoster vaccine induced a significant increase in VZV-CMI and VZV antibody. The magnitude and duration of the boost in VZV-CMI in vaccine recipients and the relationship of this boost to age paralleled the clinical effects of the vaccine observed during the efficacy trial. These findings support the hypothesis that boosting VZV-CMI protects older adults against herpes zoster and postherpetic neuralgia.

Herpes zoster (HZ) is an often painful neurocutaneous syndrome resulting from reactivation of varicella-zoster virus (VZV) that has remained latent in sensory ganglia after primary VZV infection (varicella) [1–3]. The frequency and severity of HZ and its most common debilitating complication, postherpetic neuralgia (PHN), increase with age [4–9]. This age-related increase in disease correlates closely with the decline in VZV-specific T cell mediated immunity (VZV-CMI) that accompanies aging [10–14]. It is very unlikely that antibodies to VZV play a role in this relationship, because they do not decline with aging [13, 14]. Furthermore, HZ frequently occurs in circumstances when VZV-CMI is depressed while levels of VZV antibody are main-

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tained by intravenous γ-globulin, such as those following hematopoietic stem cell transplantation [15–17].

On the basis of these observations, it was hypothesized that HZ might be prevented or attenuated (i.e., less pain and PHN) in elderly individuals if their waning VZV-CMI could be boosted with a VZV vaccine [18–20]. Pilot studies indicated that VZV-CMI could be boosted in subjects ⩾60 years old with live attenuated Oka strain VZV vaccines [13, 14, 21, 22]. Subsequent trials demonstrated the safety and immunogenicity of a high-potency Oka/Merck VZV vaccine in elderly subjects, including persons with diabetes and chronic lung disease, and established the optimal vaccine formulation and potency (M.J. Levin et al., unpublished data).

A double-blind, placebo-controlled trial (Veterans Affairs Cooperative Study 403: "The Shingles Prevention Study") that involved 38,546 subjects ⩾60 years of age demonstrated that a high potency live attenuated Oka/Merck VZV vaccine (hereafter, "zoster vaccine") significantly reduced the burden of illness due to HZ, understood in terms of a severity-by-duration measure of HZ pain and discomfort (i.e., the vaccine decreased the incidence of HZ and decreased the average severity of HZ in vaccinees who developed HZ), and substantially reduced the incidence of PHN in vaccine recipients [9]. The trial included an immunology substudy in which a subset of subjects had immunologic assessments performed before and after vaccination. We describe here the magnitude and kinetics of VZV-specific immune responses to zoster vaccine measured during the immunology substudy and their possible association with the occurrence of HZ.

METHODS

Study design. Subjects at each of 22 study sites were randomized into a double-blind, placebo-controlled trial and stratified by age (60–69; ⩾70 years) to receive a single 0.5-mL subcutaneous injection of zoster vaccine or placebo [9]. Consenting subjects at the Denver site ($N = 709$) and San Diego site ($N = 688$) were enrolled concurrently into the shingles prevention study and the immunology substudy. The diagnosis in suspected cases of HZ was determined by use of a hierarchical algorithm that incorporated the results of a polymerase chain reaction (PCR) assay of lesion specimens performed at a central laboratory, local virus culture, and the clinical diagnosis of a clinical evaluation committee. Primacy was assigned to the PCR assay. Both sites established laboratories for processing, storing, and assaying blood specimens for VZV-specific immune responses without overnight shipment. The immunology substudy subjects were tested for VZV-CMI and antibodies to VZV glycoproteins prior to vaccination (baseline), 6 weeks later, and annually for 3 years. Data from subjects who developed HZ were thereafter censored from analyses of immune responses.

Immunological assessments

Responder cell frequency (RCF) assay. VZV-CMI was measured by an RCF assay in which $CD4^+$ memory T cells were enumerated by adding a limiting dilution step to a lymphoproliferation assay [23, 24], using cell-free VZV and control antigens [25]. The RCF was calculated by importing the counts per minute of H^3-thymidine incorporation into an Excel spreadsheet that calculated the RCF by use of a prespecified maximum likelihood estimate modification of the method described by Henry et al. [26] with binary data (i.e., positive or negative) from samples at different peripheral blood mononuclear cell (PBMC) concentrations. The program calculated the median control plus 3 median control absolute deviations and 3 times the median control at each PBMC concentration. Two criteria were used to determine the number of responder wells (i.e., wells containing 1 or more responding cells). VZV-stimulated wells with >100 cpm were considered valid responders if their count-per-minute values were greater than the median control counts per minute plus 3 median control absolute deviations for that PBMC concentration. VZV-stimulated wells with <100 cpm were considered valid responders if their count-per-minute values were greater than 3 times the median control counts per minute for that PBMC concentration. Data from the PBMC concentration with the highest proportion of valid responding wells and from all lower PBMC concentrations were used to calculate the RCF, employing the maximum likelihood estimate of the number of PBMC per well needed to identify 1 VZV-specific proliferating lymphocyte. The resulting probability was multiplied by 100,000 to express the RCF as the number of VZV-specific responding cells per 10^5 PBMCs [27]. A positive response was defined as ⩾1 responding cell/10^5 PBMCs.

ELISPOT assay. An interferon-γ (IFN-γ) ELISPOT assay of VZV-CMI responses was performed on PBMCs collected and frozen at immunology substudy sites [28]. Spots were enumerated with an ImmunoSpot reader, and reported as the net number of VZV-specific IFN-γ spot-forming cells (sfc) per 10^6 PBMCs (response to VZV antigen minus response to control antigen). Assays with <10 sfc/10^6 PBMCs were considered negative. Assays were performed on aliquots of the same PBMC preparations tested in the RCF assay. They were shipped on dry ice to Merck Research Laboratories, West Point, Pennsylvania, where ELISPOT assays were performed. Results of assays that used PBMCs processed >24 h after the blood specimen was obtained or with phytohemagglutinin responses < 500 sfc were not analyzed.

Glycoprotein ELISA (gpELISA). VZV-specific antibodies were measured at Merck Research Laboratories by use of a previously validated quantitative ELISA method [29] that detected antibodies to VZV glycoproteins purified from VZV-infected human fibroblasts. Control antigen was prepared from uninfected cells. The negative control was a 1:50 dilution of serum from a volunteer with no history of varicella and no detectable

antibody to VZV. A VZV antibody-positive serum sample from an individual who had previously had varicella was used to generate a standard curve. The cutoff to define VZV seropositivity was 1.25 gpELISA units/mL after correcting for the 1:50 dilution.

All immunologic assays were performed and results were calculated without knowledge of treatment assignments.

Statistical analyses. VZV-specific immune responses to zoster vaccine were summarized for each treatment group as geometric means and percentage increase in geometric mean in vaccine recipients relative to that in placebo recipients at various times after vaccination. The 95% confidence intervals (CIs) for the geometric means were calculated. Comparisons of these responses between the vaccine and placebo groups and between other subgroups were performed using an analysis of covariance model (ANCOVA) that included the log transformed VZV-specific response as the response variable and treatment or subgroup as independent variables; it also adjusted for age, sex, time point(s) after vaccination, and immune responses at baseline. The effects of age, sex, and baseline immune response prior to vaccination were also evaluated by use of an ANCOVA model. The effect of age was evaluated as a continuous variable in the 2 prespecified age strata. At immunology substudy sites, PBMCs were prepared and the RCF assay was performed with the same operating procedures, VZV and control antigens, and reagents. Nevertheless, the complicated nature of the laboratory procedures may have resulted in differences in the processing PBMCs and in the performance of RCF assays in the 2 laboratories. Consequently, the ANCOVA models also included immunology substudy study site as a covariate. Spearman rank correlation coefficients were used to evaluate associations among RCF, ELISPOT, and gpELISA results by treatment group, at baseline and at each time after vaccination.

Exploratory analyses were performed to evaluate the correlation of immune responses with protection against HZ. An ANCOVA model was used to compare the immune responses of subjects who did or did not develop HZ at baseline and 6 weeks after vaccination. The analysis included the log-transformed VZV-specific response as the response variable, and HZ status as the independent variable, adjusting for treatment arm, site, age, and sex. Interaction between treatment and HZ status was tested at the 0.10 level. If no interaction was detected, the overall HZ status was tested without the interaction adjustment. Cox regression analyses were performed to assess the association between VZV-specific immune responses and the risk of HZ. The immune responses of subjects who developed HZ were compared with the immune responses of matched control subjects by use of the last valid immune assays performed prior to the onset of HZ. The control subjects were individuals who did not develop HZ (there were 58–72 control subjects in the vaccine arm and 169–186 in the placebo arm, depending on assay data available); they were matched with subjects who did develop HZ for age, sex, study site, treatment arm and blood specimen interval since study entry.

Table 1. Demographic and clinical characteristics of subjects at baseline.

Variable	Zoster vaccine ($N = 691$)	Placebo ($N = 704$)
Study site, no. (%)		
Denver	348 (50.4)	361 (51.3)
San Diego	343 (49.6)	343 (48.7)
Sex, no. (%)[a]		
Male	348 (50.4)	426 (60.5)
Female	343 (49.6)	278 (39.5)
Age		
60–69 years, no. (%)	394 (57)	429 (60.9)
⩾70 years, no. (%)	297 (43)	275 (39.2)
Mean ± SD, years	68.8 ± 6.2	68.2 ± 6.1
Range, years	60–93	60–88
Race, no. (%)		
White	676 (97.8)	679 (96.4)
Other[b]	15 (2.2)	25 (3.6)
Health status by EuroQoL [30]		
Mean ± SD	89.7 ± 8.5	88.4 ± 9.5
Median (range)	90.0 (50–100)	90.0 (40–100)

[a] $P < .001$ for testing vaccine vs. placebo in sex.
[b] The "other" category includes 7 black subjects, 17 Hispanic subjects, and 16 subjects of other racial origin.

RESULTS

Baseline assessment of immune responses. The immunology substudy included 1395 subjects at baseline. Participants in the vaccine and placebo treatment arms were balanced with respect to age, race, and health status. However, the male-to-female ratio in each treatment arm was significantly different (50:50 in the vaccine arm vs. 60:40 in the placebo arm) (table 1). At baseline 1395 blood specimens were collected, and 1388 specimens (99% of the original sample size) were collected at week 6 after vaccination. A total of 1351 (97%), 1324 (95%), and 1267 (91%) specimens were collected at years 1, 2, and 3 after vaccination, respectively.

The decline in the level of VZV-CMI with increasing age, which begins early in adulthood [10–14], is demonstrated in figure 1 to continue with increasing age. A regression analysis confirmed that VZV-CMI responses at baseline decreased with age ($P < .001$ for both VZV-CMI assays). VZV-specific RCF and ELISPOT levels at baseline were lower in subjects ⩾70 years than in those 60–69 years of age ($P < .001$). Figure 1 also confirms that VZV antibody does not decline with age; a regression analysis found no effect of age on the gpELISA titers ($P = .75$). The baseline ELISPOT results were higher for women (mean age, 68.3 years) than for men (mean age, 68.7 years), with a mean

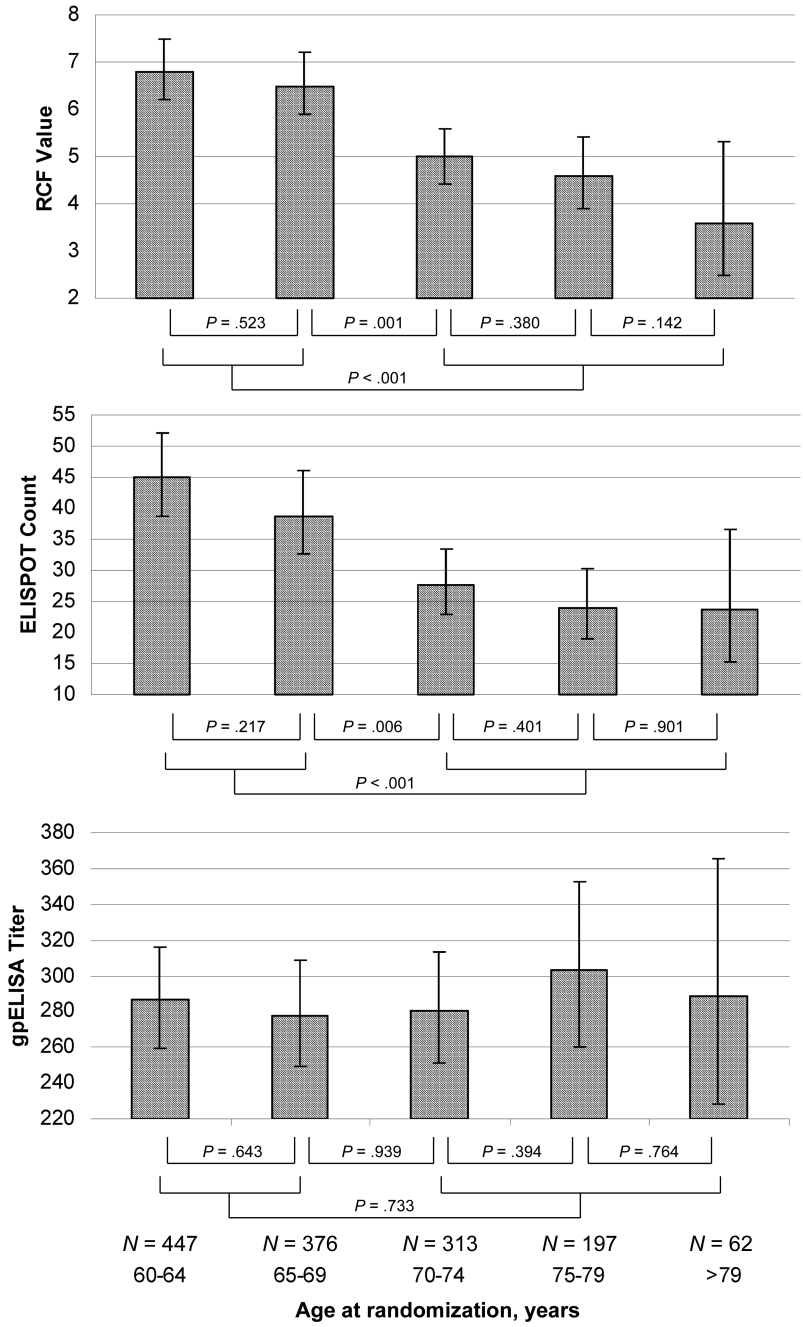

Figure 1. Varicella-zoster virus–specific immune responses at baseline (i.e., prior to vaccination), according to age group. Responder cell frequency (RCF) value, no. of responding cells per 10^5 peripheral blood mononuclear cells (PBMCs); ELISPOT counts, no. of spot-forming cells per 10^6 PBMCs; glycoprotein ELISA (gpELISA) titer, gpELISA units/mL. *Error bars,* 95% confidence intervals for the geometric mean. *N,* no. of subjects who had blood samples collected in the age group. *P* values for differences between age groups are shown below the graphs.

of 40.0 vs. 31.7 sfc/10^6 PBMCs ($P = .009$). However, neither the RCF nor the gpELISA titers were influenced by sex ($P = .65$ and .26, respectively). Results from the 2 study sites differed with respect to the group means for each assay (compared to the San Diego site, results from the Denver site were as follows: ELISPOT count, 16% lower; RCF value, 24% higher; gpELISA titer, 16% lower; $P < .001$ for all 3 assays), but the relative effect of each covariable (e.g., age or sex) on the geometric means was similar for the 2 sites.

We examined the effect of age on VZV-specific immunity as measured by all 3 assays, comparing the Akaike information criteria, a linear model, to a quadratic model, and found that the linear model best fit the data from all 3 assays. The estimated annual decline in the level of VZV-CMI per year of increase in

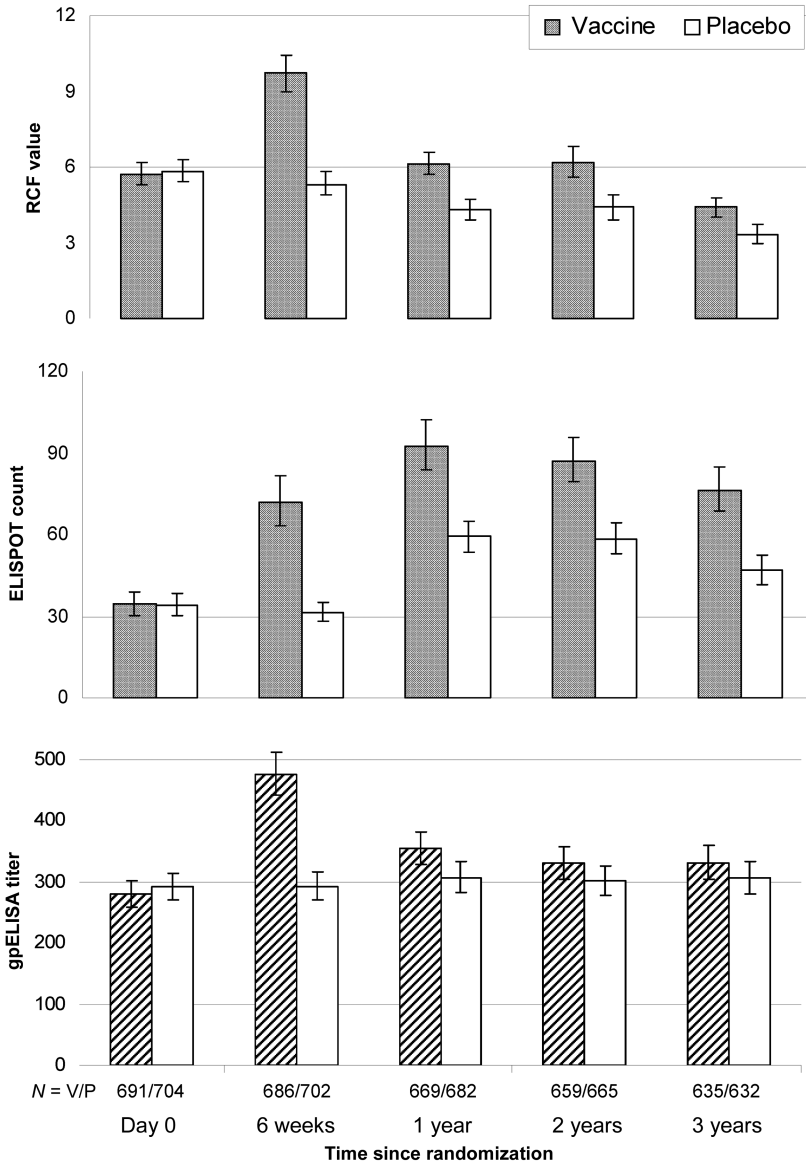

Figure 2. Varicella-zoster virus–specific immune responses, according to time since randomization. Responder cell frequency (RCF) value, no. of responding cells per 10^5 peripheral blood mononuclear cells (PBMCs); ELISPOT count, no. of spot-forming cells per million PBMCs; glycoprotein ELISA (gpELISA) titer, gpELISA units/mL. *Error bars,* 95% confidence intervals for the geometric mean. *N,* no. of subjects who had blood samples obtained within the time interval; *V,* no. of subjects in the vaccine group for each time interval; *P,* no. of subjects in the placebo group for each time interval. Data from subjects who developed herpes zoster were censored from subsequent time point analyses. The immune response at each time is the observed geometric mean of the responses to each assay for each treatment group. A total of 409 ELISPOT assays were excluded (6.1%); 154 were from baseline; 133, 52, 30, and 40 are from week 6, year 1, year 2, and year 3, respectively.

age was 2.7% for RCF and 3.9% for ELISPOT. The age-related decline in gpELISA levels was negligible.

At baseline, the RCF result was negative for 79 subjects (5.9%), and the ELISPOT result was negative for 228 subjects (18.5%); both assays were negative for 29 subjects (17 vaccine recipients and 12 placebo recipients [2.4%]). All subjects with sufficient serum for testing (1369) had VZV antibody.

The results of the RCF and ELISPOT assays were correlated with each other at baseline and all time points after vaccination for both the vaccine and placebo groups (Spearman rank correlations, 0.38–0.61). However, the RCF and ELISPOT results at baseline and after vaccination did not correlate with the gpELISA results (Spearman rank correlations, −0.05 to 0.13).

Assessment of immune responses. At 6 weeks after vaccination, immune responses in vaccine recipients as measured by all 3 assays were significantly increased, compared with responses in placebo recipients (figure 2; table 2). Longitudinal analysis of the ELISPOT assay was complicated by the fact that only the baseline and week 6 samples for each subject were tested together in the same ELISPOT assay, whereas subsequent samples were

Table 2. Varicella-zoster virus–specific immune responses at 6 weeks after vaccination in relation to baseline response.

	Zoster vaccine		Placebo	
Baseline Response	Subjects contributing to immunogenicity analysis, no.	6-week response (95% CI)	Subjects contributing to immunogenicity analysis, no.	6-week response (95% CI)
RCF value[a]		Geometric mean value		Geometric mean value
<1	37	2.3 (1.4–3.9)	40	1.6 (1.1–2.2)
⩾1 to <3.5	142	5.9 (5.2–6.7)	132	2.5 (2.1–3.0)
⩾3.5 to <7.0	151	8.7 (7.9–9.5)	161	4.3 (3.7–5.0)
⩾7.0 to <12.0	163	12.9 (11.8–14.1)	165	7.1 (6.3–8.0)
⩾12.0 to <64.0	143	18.7 (16.8–20.9)	162	11.7 (10.4–13.2)
⩾ 64	6	28.1 (13.8–57.4)	2	56.7
Overall	642	9.8 (9.2–10.5)	662	5.3 (4.9–5.7)
ELISPOT count[b]		Geometric mean count		Geometric mean count
⩽ 10	111	21.4 (15.3–29.8)	118	9.0 (6.8–12.0)
11– 25	101	51.3 (38.6–68.0)	93	14.3 (10.7–19.3)
26– 50	96	64.8 (49.1–85.6)	109	25.5 (20.9–31.1)
51– 100	117	84.6 (66.3–108.0)	134	49.9 (41.0–60.8)
101– 200	107	159.3 (128.9–196.9)	112	78.8 (64.2–96.9)
>200	50	237.7 (164.9–342.6)	45	198.5 (162.8–242.1)
Overall	582	70.1 (61.6–79.8)	611	31.7 (28.0–35.8)
gpELISA titer		Geometric mean titer		Geometric mean titer
⩾1.25 to <200	257	253.2 (228.5–280.5)	237	110.8 (101.5–120.8)
⩾ 200	398	703.9 (651.2–760.8)	436	495.4 (458.7–535.0)
Overall	655	471.3 (438.2–506.8)	673	292.3 (269.9–316.5)

NOTE. CI, confidence interval.

[a] Responder cell frequency (RCF) value, no. of responder cells per 10^5 peripheral blood mononuclear cells (PBMCs).

[b] ELISPOT count, no. of spot-forming cells per 10^6 PBMCs.

Glycoprotein ELISA (gpELISA titer), gpELISA units/mL.

tested in separate assays. The ELISPOT counts observed in placebo recipients at later time points were increased, relative to baseline values, whereas a slight age-related decrease would have been expected, indicating a change in the characteristics of the ELISPOT assay. Consequently, the vaccine-induced responses for each assay were expressed as the percentage increase over the responses of placebo recipients (figure 3). The vaccine-induced increase in VZV-CMI persisted for 3 years of follow-up, although the magnitude of these responses decreased over time, with the greatest decline observed between 6 weeks and 1 year after vaccination. From week 6 to 1 year after vaccination, the vaccine-induced boost declined from 85% to 42% as measured by RCF and from 120% to 60% as measured by ELISPOT, and it then remained relatively constant (figure 3). The boost in VZV antibody in vaccine recipients declined from 78% to 20% from week 6 to 1 year after vaccination, and it declined 7%–15% per year thereafter.

Effect of age on immune response. The VZV-CMI responses at 6 weeks after vaccination decreased with age (figure 4) and were significantly lower in subjects ⩾70 years old compared to subjects 60–69 years old ($P < .001$ for RCF; $P < .001$ for ELISPOT). RCF and ELISPOT responses at week 6 decreased 3.5% and 3.8%, respectively, per year of age when age was assessed as a continuous variable. At 1, 2, and 3 years after vaccination, the negative effect of age on VZV-CMI responses ranged from 1% to 4% per year of age. In contrast, there was no significant difference in gpELISA titer between the 2 prespecified age strata at week 6 or thereafter. When age was assessed as a continuous variable, a negative association between age and antibody response was observed, but this was only 1% per year of age at week 6 ($P = .034$) and negligible thereafter. When the >79 year age group ($n = 62$; no boost in gpELISA titer in vaccine recipients) was excluded from the week 6 analysis, the age effect on antibody response was not significant ($P = .11$).

At 6 weeks after vaccination, the RCF result from valid assays was negative for 46 subjects (3.4%; 11 [1.7%] vaccine recipients and 35 [5.1%] placebo recipients), and the ELISPOT assay result was negative for 188 subjects (15.1%; 67 [11.1%] vaccine recipients and 121 [18.9%] placebo recipients). Seventeen subjects (4 [0.7%] vaccine recipients and 13 [2.1%] placebo recipients) had negative results for both VZV-CMI assays at 6 weeks after vaccination.

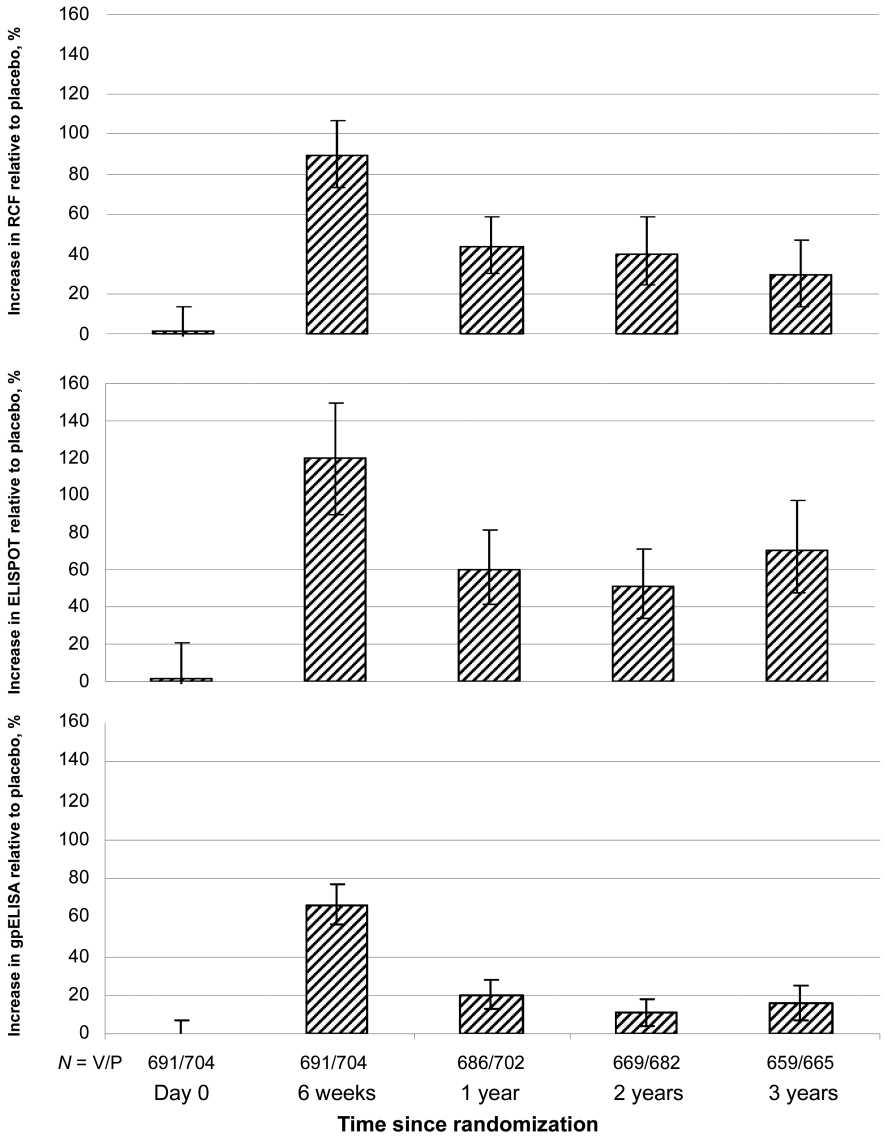

Figure 3. Estimated percentage increase in varicella-zoster virus–specific immune responses in vaccine recipients and placebo recipients, according to time after vaccination. The percentage increase in immune response in vaccine recipients, relative to placebo recipients, at each time is the estimated geometric mean percentage increase from the placebo recipients for each assay. The estimated increase is adjusted for age, sex, and study site. *Error bars,* 95% confidence intervals for geometric mean. *N,* no. of subjects who had blood samples obtained within the time interval; *V,* no. of subjects in the vaccine group for each time interval; *P,* no. of subjects in the placebo group for each time interval. Data from subjects who developed herpes zoster were censored from subsequent time points. None of the immune responses at baseline differed by treatment. At baseline, the responses of vaccine and placebo recipients did not differ significantly ($P = .489$ to .854). After baseline, all responses of the vaccine recipients differed significantly from those of the placebo recipients ($P \leq .001$ to .001).

A regression analysis of the vaccine-induced responses did not demonstrate the sex-related effect observed with the ELISPOT assay results at baseline. The levels of VZV-specific immunity achieved after vaccination were strongly correlated with the magnitude of the corresponding baseline levels ($P < .001$ for all 3 assays after correction for age, sex, and study site) (table 2).

Immune responses relative to the occurrence of HZ. The immunology substudy permitted comparison of immune responses in subjects who developed HZ with responses in those who did not develop HZ. Table 3 shows the immune measurements in the 7–9 (depending on sample availability) vaccine recipients who developed HZ and the 21–23 placebo recipients who developed HZ, as well as measurements for the remaining immunology substudy subjects who did not develop HZ. Because the relative differences at baseline were similar in vaccine recipients and placebo recipients and there was no significant quantitative interaction between treatment arm and HZ status, the treatment groups were combined for the analysis of HZ status. The average VZV-specific immune responses at baseline

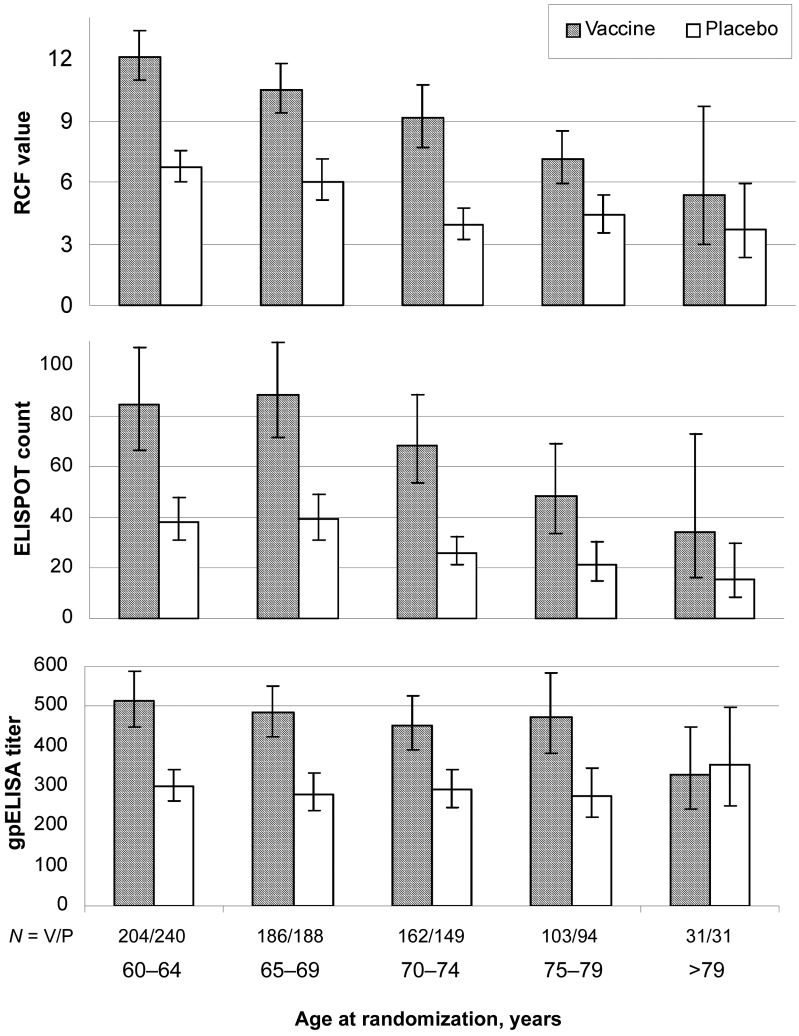

Figure 4. Varicella-zoster virus–specific immune responses at 6 weeks after vaccination, according to age group. Responder cell frequency (RCF) value, no. of responding cells per 10^5 peripheral blood mononuclear cells (PBMCs); ELISPOT counts, no. of spot-forming cells per million PBMCs; glycoprotein ELISA (gpELISA) titer, gpELISA units/mL. *Error bars,* 95% confidence intervals for geometric mean. *N,* no. of subjects who had blood samples obtained within the time interval; *V,* no. of subjects in the vaccine group for each age range; *P,* no. of subjects in the placebo group for each age range. Among vaccinees there was a significant linear age effect (age slope) for the week 6 responses as measured by RCF and ELISPOT ($P < .001$). There was a lesser association between age and gpELISA response (slope was 1% per year of age; $P = .034$). When the >79 age group was excluded ($n = 62$; no boost in gpELISA titer in vaccine recipients), the slope was not significant ($P = .11$)

were lower in subjects who developed HZ, in both vaccine and placebo groups, compared with subjects who did not develop HZ ($P < .001$ to .058) (table 3). The responses 6 weeks after vaccination as measured in all 3 assays were also lower in subjects who developed HZ than in those who did not, but this effect varied in significance, depending on the assay ($P = .006$ to .071). Furthermore, Cox regression analyses showed a significant inverse relationship between the immune responses 6 weeks after vaccination and risk of HZ, for all 3 assays ($P < .001$ to 0.017). An additional analysis compared the results of the last immune assays performed prior to the onset of HZ to the immune responses of comparator control subjects who did not develop HZ and who were matched with HZ cases for age, sex, study site, treatment arm and interval since study entry. This analysis showed significantly lower VZV-specific responses in subjects who developed HZ, as measured by all 3 assays ($P < .001$ to .030).

Plots of the cumulative occurrence of HZ by VZV-specific immune responses measured with each of the 3 assays failed to identify a specific level for any immune response that was predictive of protection against HZ (data not shown). Similar results were obtained when the severity of individual cases of HZ or the time interval between enrollment and development of HZ were plotted against immune responses (data not shown). Thus, although the magnitude of the CMI response to VZV was inversely correlated with the likelihood of developing HZ, we were unable to identify a surrogate marker or threshold level of protection.

Table 3. Comparison of varicella-zoster virus (VZV)-specific immune responses in immunology substudy subjects who developed herpes zoster (HZ) and those who did not.

Time, immune assay	Clinical endpoint	Vaccine		Placebo		P[a]
		Subjects, no.	Observed geometric mean (95% CI)	Subjects, no.	Observed geometric mean (95% CI)	
Day 0						
RCF	Developed HZ	9	2.3 (0.5–11.5)	22	2.6 (1.7–4.1)	<.001
	No HZ	655	5.8 (5.4–6.3)	655	5.9 (5.5–6.4)	
ELISPOT	Developed HZ	7	14.6 (2.2–97.5)	21	15.9 (6.8–36.9)	.003
	No HZ	600	34.8 (30.6–39.5)	608	35.1 (31.0–39.7)	
gpELISA	Developed HZ	9	244.6 (136.2–439.1)	23	196.4 (146.7–262.8)	.058
	No HZ	669	279.3 (258.2–302.1)	667	295.3 (273.2–319.3)	
Week 6[b]						
RCF	Developed HZ	9	7.0 (4.2–11.6)	22	3.8 (2.4–5.9)	.071
	No HZ	659	9.7 (9.1–10.5)	665	5.4 (5.0–5.9)	
ELISPOT	Developed HZ	7	39.4 (7.9–196.6)	21	17.4 (8.8–34.4)	.049
	No HZ	599	72.5 (63.9–82.3)	621	32.2 (28.5–36.4)	
gpELISA	Developed HZ	9	271.9 (161.9–456.7)	23	181.6 (133.5–246.9)	.006
	No HZ	658	478.4 (444.6–514.7)	661	296.2 (273.3–321.1)	
Before rash onset[c–e]						
RCF	Prerash- HZ	9	3.8 (2.1–6.9)	23	2.4 (1.3–4.4)	.006
	Matched control	58	6.5 (5.3–8.0)	169	5.6 (4.8–6.5)	
ELISPOT	Prerash- HZ	9	28.6 (7.1–114.2)	23	28.8 (17.2–48.2)	<.001
	Matched control	72	99.8 (80.8–123.2)	174	56.7 (47.3–68.0)	
gpELISA	Prerash- HZ	9	252.4 (126.1–504.9)	23	181.3 (122.3–268.8)	.030
	Matched control[d]	72	331.9 (267.8–411.3)	186	346.6 (301.3–398.6)	

NOTE. CI, confidence interval; gpELISA, glycoprotein ELISA; RCF, responder cell frequency.

[a] P values are from the analysis of covariance effect for the comparison of HZ case subjects and subjects who did not develop HZ (or control subjects), adjusted for the covariates: treatment, age, sex, and study site.

[b] The P value of testing interaction between treatment and HZ status at week 6 is .900 for RCF, .692 for ELISPOT, and .934 for gpELISA. The P value for the effect of week 6 varicella-zoster virus immune responses on risk of HZ from the Cox regression model is <.001 for RCF, .017 for ELISPOT, and <.001 for gpELISA.

[c] Indicates most recent immune assay result available before onset of HZ.

[d] The comparator control group consisted of immunology substudy subjects who did not develop HZ and were matched for age, sex, study site, treatment arm and interval since study entry with subjects who did develop HZ.

[e] The P value of testing interaction between treatment and HZ status before rash onset is .433 for RCF, .285 for ELISPOT, and .337 for gpELISA.

DISCUSSION

Immunological assays performed at baseline during a placebo-controlled trial of zoster vaccine confirmed the progressive decline in VZV-CMI that occurs with aging and the absence of an age effect on levels of antibody to VZV. VZV-CMI responses were 2.7%–3.9% lower (depending on the assay) for each year of increase in age. This finding reflects the normal decline in VZV-CMI that occurs after a varicella-induced stimulus earlier in life, and provides a biological basis for the continuous increase in the incidence and severity of HZ observed as a function of aging. This trend likely reflects the importance of VZV-CMI in limiting the reactivation and/or subsequent replication of latent VZV in sensory ganglia, which is the proximate cause of HZ and PHN. The observations that VZV-specific antibody does not decline with increasing age and does not correlate with VZV-CMI provide additional evidence that humoral immune responses are not protective against HZ, although they probably play a major role in preventing second episodes of varicella.

Zoster vaccine induced a significant increase in VZV-CMI and VZV antibody. However, the Spearman rank correlation between VZV-CMI and VZV antibody was low, which provides additional evidence that these classes of immune response are independent. These VZV-CMI responses are consistent with the results observed in pilot studies utilizing less potent vaccines that demonstrated that VZV-CMI responses could be boosted in elderly subjects [14, 21, 22]. The VZV-CMI responses to zoster vaccine correlate with the clinical efficacy observed in the shingles prevention study [9], confirming the hypothesis that boosting VZV-CMI would result in clinical efficacy. While VZV-specific antibody is not believed to be protective against HZ, antibody levels did rise in parallel with VZV-CMI following vac-

cination, reflecting the immunogenicity of this formulation of zoster vaccine.

The increased levels of VZV-CMI demonstrated at 6 weeks after vaccination declined markedly between 6 weeks and 1 year after vaccination, but then decreased by only 2%–4% in the second year, and were stable during the third year. VZV-CMI responses remained significantly higher in vaccine recipients than in placebo recipients throughout the 3-year immunology substudy follow-up period. This pattern parallels the greater efficacy seen in the first year after vaccination and the subsequent persistence of the efficacy of the zoster vaccine against HZ and PHN for at least 4 years [9, 31, 32].

Vaccinated subjects exhibited boosting of VZV-CMI and VZV antibody regardless of their age, but the negative correlation between the immune response to vaccine and increasing age (3%–4% decline per year) was consistently demonstrated only for the VZV-CMI responses. The effect of age on the immune response to vaccination was mirrored by the clinical responses to zoster vaccine observed in the shingles prevention study [9, 33]. Although the efficacy of zoster vaccine for incidence of PHN was undiminished in the older subjects (i.e., those $\geqslant$70 years old), the vaccine efficacy with respect to the incidence of HZ and the HZ burden of illness were less in older subjects, compared with younger subjects [32, 33]. Nevertheless, zoster vaccine reduced the severity of HZ and incidence of PHN well into the eighth decade of life [33], which correlates with the increased levels of VZV-CMI observed in zoster vaccine recipients in all age groups.

Vaccinees with higher baseline levels of VZV-CMI achieved greater absolute levels of VZV-CMI after vaccination. Perhaps higher baseline levels of VZV-specific immunity identify subjects with a greater capacity to develop VZV-CMI responses when exposed to antigenic stimulation. Alternatively, this relationship could result from lower baseline levels of VZV-CMI that identify individuals with a limited ability to respond to HZ vaccine due to smaller populations of VZV-specific memory T cells or larger populations of regulatory cells that impede expansion of vaccine-induced VZV-specific responses.

Analysis of the occurrence of HZ in the immunology substudy subjects (table 3) further demonstrated the inverse correlation between the level of VZV-CMI and the likelihood of developing HZ. However, we did not identify a level of VZV-CMI that predicted protection against HZ and could not delineate a surrogate marker of protection. This failure may reflect the small number of HZ cases observed among the immunology substudy subjects. Moreover, because of the unpredictability of the occurrence of HZ, we rarely had measurements of VZV-specific immune responses that had been obtained close to the onset of HZ. It is possible that alternative VZV-specific immune responses are better predictors of clinical outcome than those measured by the RCF and ELISPOT assays, and it is also likely that additional factors are involved in VZV reactivation, such as local trauma [34] and physiologic stress that may affect the virus-neuron interaction. Others factors, such as emotional stress [35, 36] or intercurrent viral infection [37, 38], could induce transient decreases in protective VZV-CMI. Neither the durability of the vaccine-induced increase in VZV-CMI nor the duration of protection against HZ and PHN induced by the zoster vaccine have yet been determined, although the immunology substudy indicates that this increase in VZV-CMI persists for at least 3 years and the shingles prevention study indicates that the clinical efficacy of the vaccine persists for at least 4 years [9, 31].

VETERANS AFFAIRS COOPERATIVE STUDIES PROGRAM SHINGLES PREVENTION STUDY

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