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SUPPLEMENT ARTICLE

Comparative Immune Responses to Licensed Herpes Zoster Vaccines

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Background. Live attenuated (ZV) and recombinant adjuvanted (HZ/su) zoster vaccines differ with respect to efficacy, effect of age, and persistence of protection. We compared cell-mediated immunity (CMI responses to ZV and HZ/su.

Methods. This was a randomized, double-blind, placebo-controlled trial stratified by age (50–59 and 70–85 years) and by HZ vaccination status (received ZV ≥5 years before entry or not). Varicella zoster virus (VZV)– and glycoprotein E (gE)–specific CMI were analyzed by interleukin 2 (IL-2) and interferon gamma (IFN-γ) FluoroSpot and flow cytometry at study days 0, 30, 90, and 365.

Results. Responses to ZV peaked on day 30 and to HZ/su (administered in 2 doses separated by 60 days) peaked on day 90. Age and vaccination status did not affect peak responses, but higher baseline CMI correlated with higher peak responses. HZ/su generated significantly higher VZV-specific IL-2$^+$ and gE-specific IL-2$^+$, IFN-γ$^+$, and IL-2$^+$/IFN-γ$^+$ peak and 1-year baseline-adjusted responses compared with ZV. VZV-specific IFN-γ$^+$ and IL-2$^+$/IFN-γ$^+$ did not differ between vaccines. HZ/su generated higher memory and effector-memory CD4$^+$ peak responses and ZV generated higher effector CD4$^+$ responses.

Conclusions. The higher IL-2 and other memory responses generated by HZ/su compared with ZV may contribute to its superior efficacy.

Clinical Trials Registration. NCT02114333.

Keywords. herpes zoster; live-attenuated zoster vaccine; recombinant zoster vaccine.

Herpes zoster (HZ) occurs when varicella zoster virus (VZV) latent in sensory ganglia reactivates and replicates to cause dermatomal pain and a vesicular rash [1, 2]. These events follow when some essential component(s) of VZV-specific cell-mediated immunity (CMI) falls below a critical level, which typically happens when VZV-specific CMI is compromised by disease, medical treatment, or aging [3–7]. The live attenuated zoster vaccine (ZV) boosts VZV-specific CMI in elderly vaccinees, thereby explaining the efficacy of the vaccine [8, 9]. However, efficacy against HZ is limited to 51% in vaccinees ≥60 years of age, and is lower as the age at the time of vaccination increases [9, 10]. Moreover, the protection provided by ZV declines significantly at 6–8 years after vaccination [11]. The magnitude and duration of protection have been confirmed by effectiveness studies [12, 13].

An alternative approach to prevent HZ utilizes the recently approved recombinant subunit glycoprotein E (gE) vaccine (HZ/su), which contains the $AS01_B$ adjuvant consisting of MPL (lipid A of bacterial lipopolysaccharide, a Toll-like receptor 4 agonist) and QS21 (a triterpene plant-derived saponin) packaged into liposomes [14]. HZ/su provides 97% protection against HZ in vaccinees aged ≥50 years, including 87% efficacy in those ≥80 years of age, indicating that the efficacy of HZ/su is minimally affected by the age of the vaccinee [15, 16]. Moreover, this strong protective effect persisted for the 3.8 years of follow-up reported. HZ/su-induced immune responses remained robust for the duration of the pivotal trials and are readily detected at 6–9 years after vaccination in long-term follow-up studies [17, 18].

The current report compares the immune responses elicited by ZV or HZ/su in participants aged 50–59 and 70–85 years who had never received a HZ vaccine, and an additional cohort of participants aged 70–85 years who had received ZV ≥5 years prior to enrollment. The primary objectives were to determine CMI responses that best differentiated the 2 vaccines and to compare the responses elicited by HZ/su in participants who had received ZV ≥5 years previously with responses of individuals receiving HZ/su for the first time.

PARTICIPANTS AND METHODS

Study Design

This study (ClinicalTrials.gov identifier NCT02114333), approved by the Colorado Multiple Institutions Review Board,

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enrolled 160 participants in good health except for treated chronic illnesses typical of the age of the vaccinees. All had prior varicella or resided in the United States at least 30 years; none had prior HZ. Exclusions from the study were immunosuppression and recent blood products or other vaccines. Arms A and B (Figure 1), which contained 90 total participants who had not previously had ZV, were randomly assigned to receive either ZV followed by placebo or 2 doses of HZ/su at days 0 and 60. Arms A and B were further stratified by age (50–59 years [n = 22] or 70–85 years [n = 23]). Arms C and D contained an additional 70 participants who were 70–85 years of age and had received ZV ≥5 years previously. They were randomly assigned to receive either an additional dose of ZV followed by placebo (arm C) or 2 doses of HZ/su (arm D). All vaccinations were blinded from the participant. Blood was obtained for immunologic assessment on days 0, 30, 90, and 356 from all participants. Additional blood was drawn from participants in arm A on day 7 and from participants in arm B on days 7 and 67. Peripheral blood mononuclear cells (PBMCs), plasma, and serum were cryopreserved within 4 hours of acquisition [19, 20].

FluoroSpot Assays

PBMCs were separated from heparinized blood on Ficoll-Hypaque gradients (Sigma) and frozen as previously described [20]. Cryopreserved PBMCs were thawed and rested overnight at 37°C and 5% carbon dioxide at 10^6 PBMCs/mL in growth medium consisting of RPMI 1640 (Mediatech) with L-glutamine (Gemini BioProducts), 10% human AB serum (Gemini Bio-Products), 2% HEPES (Mediatech), and 1% penicillin-streptomycin (Gemini Bio-Products). PBMCs were stimulated for 48 hours in 96-well dual-color interferon gamma (IFN-γ) and interleukin 2 (IL-2) FluoroSpot plates (Mabtech) with preoptimized amounts of gE peptide pools (15 mer overlapping by 11 mer; gift from GlaxoSmithKline) or inactivated VZV antigen [21] in duplicate wells at 250 000 cells/well. Medium-stimulated and phytohemagglutinin controls were included. Thereafter, assays were performed as per the manufacturer's instructions. Results were reported as the mean number of spot-forming cells (SFCs) per 10^6 PBMCs in VZV- and gE-stimulated wells after subtraction of the SFCs in mock wells. An assay control of PBMCs from a single leukopack with known performance characteristics was included in each run for validation.

Flow Cytometric Enumeration of VZV- and gE-Specific T-Cell Subsets

Thawed PBMCs were cultured as above at 2.5×10^6 cells/mL in growth medium in the presence of infectious VZV vaccine Oka (60 000 plaque-forming units/mL; GenBank accession number AB097932.1), gE peptide pools as above (2.5 μg/mL), or mock stimulation. CD28 (Mabtech) and CD49D (BD) monoclonal antibodies were added at 1 μg/mL. Glycoprotein E–stimulated and mock-stimulated PBMCs were incubated for 18 hours, while wells with infectious VZV were incubated for 42 hours. At the end of the incubation, PBMCs were washed and incubated with zombie yellow viability stain (Biolegend). PBMCs were then washed in 1% bovine serum albumin (Sigma) in phosphate-buffered saline (PBS; Mediatech) (stain buffer), and stained with antibodies against the following markers: CD3 (Ax700; clone UCHT1; Becton-Dickinson [BD]), CD4 (PC5.5; clone 13B8.2; Beckman Coulter), CD45RO (PE-CF594; clone UCHL1; BD), CCR7 (APC; clone 3D12; BD) and CD27 (PE-Cy7; clone M-T271; BD). Unbound antibodies were removed by washing with staining buffer and fixed in 2% paraformaldehyde (Electron Microscopy Sciences) in PBS. Two hundred thousand events or more were acquired with the Gallios (Beckman Coulter) instrument and analyzed using FlowJo (Tree Star) software.

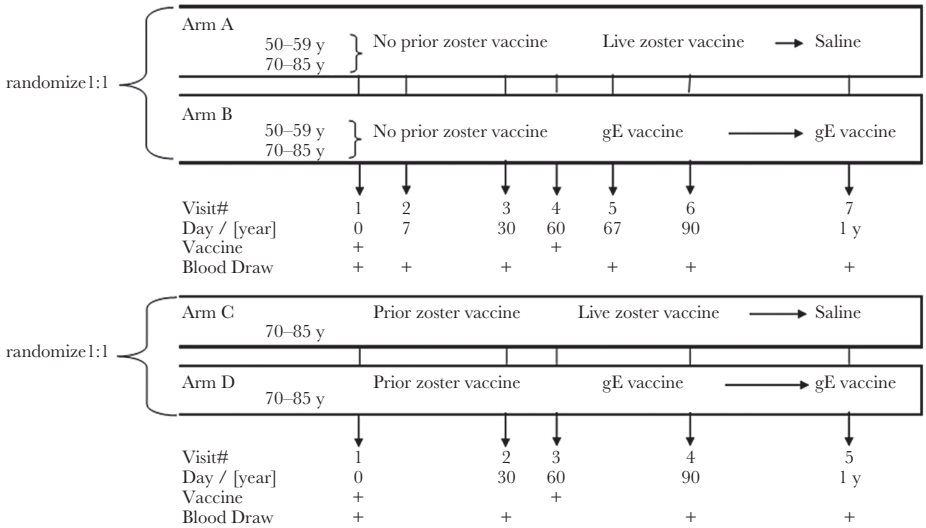

Figure 1. Schematic representation of the study design. Abbreviation: gE, glycoprotein E.

Statistical Analysis

Frequencies (%) or means and standard deviations were calculated for baseline patient demographics. To evaluate associations between peak and 1-year FluoroSpot responses and vaccine, linear regression models adjusting for baseline values were constructed. Age, gender, and booster status were evaluated as covariates and excluded from the models if not significant ($P < .05$). Glycoprotein E– and VZV-specific $CD4^+$ and $CD8^+$ T-cell differentiation profiles were compared at peak response between gE and ZV stimulation using a Tobit regression model (R function vglm from package VGAM) [22] to account for the lower detection limit in the flow cytometry data. T-cell differentiation profiles were log-transformed and models were adjusted for baseline response, with the threshold set at 0.005 for CD4 and 0.01 for CD8, reflecting their detection thresholds. To account for multiple comparisons, false discovery rate (FDR) corrections were implemented for each outcome, within cell type (CD4 and CD8) and for each stimulant (VZV and gE); unadjusted and adjusted *P* values are reported.

RESULTS

Demographic Characteristics

The study enrolled 160 participants, including 79 in each vaccine group who completed all visits (Table 1). The mean age was 70 years; 86 (54%) were women, 152 (97%) were white, and 155 (98%) were non-Hispanic. The demographic characteristics were similar between the 2 vaccine groups in each of the 3 subgroups: first time immunized among 50- to 59-year-olds (young primary); first time immunized among 70- to 85-year-olds (older primary); and 70- to 85-year-olds who received ZV ≥5 years before enrollment (older boosted).

Kinetics of Th1 Responses to the HZ Vaccines

VZV- and gE-specific $IL\text{-}2^+$, $IFN\text{-}\gamma^+$, and $IL\text{-}2^+IFN\text{-}\gamma^+$ double-positive (DP) Th1 responses were measured before vaccination, 30 days after ZV or after the first HZ/su dose, 30 days after the second HZ/su dose, and at 1 year after each vaccine (Figure 2). At baseline, participants had robust VZV Th1 CMI (ie, VZV IL-2: geometric mean, 108 [95% confidence interval {CI}, 81–140] SFC/10^6 PBMCs), but very low or undetectable gE Th1 CMI (gE IL-2: geometric mean, 7 [95% CI, 4–9] SFC/10^6 PBMCs). ZV recipients reached peak responses at 30 days after immunization, with 253 (95% CI, 213–301) VZV IL-2 SFC/10^6 PBMCs and 12 (95% CI, 7–19) gE IL-2 SFC/10^6 PBMCs. HZ/su recipients reached peak responses at 90 days after the first dose (ie, 30 days after the second dose of vaccine), with 335 (95% CI, 282–399) VZV IL-2 and 361 (95% CI, 298–439) gE IL-2 SFC/10^6 PBMCs. It is important to note that after the first dose of HZ/su, responses were much lower compared with peak (150 [95% CI, 114–196] VZV IL-2 and 72 [95% CI, 61–116] gE IL-2 SFC/10^6 PBMCs). In fact, VZV IL-2 responses after the first dose of HZ/su were lower than those of ZV recipients, underscoring the importance of the second dose for maximal immunogenicity of HZ/su.

Table 1. Demographic Characteristics of Study Participants

Characteristic		HZ/su	ZV
Age, y, mean (SD)		70.0 (9.7)	69.5 (9.7)
Sex	Male	38 (48)	34 (43)
	Female	41 (52)	45 (57)
Race	White	77 (97.5)	75 (95)
	Nonwhite	2 (2.5)	4 (5)
Ethnicity	Hispanic	1 (1)	2 (2.5)
	Non-Hispanic	78 (99)	77 (97.5)

Data are presented as No. (%) unless otherwise indicated. Seventy-nine of 80 in each vaccine group completed all study visits.

Abbreviations: HZ/su, recombinant subunit glycoprotein E zoster vaccine; SD, standard deviation; ZV, live attenuated zoster vaccine.

Effect of Age and Prior ZV Administration on Responses to ZV and HZ/su

Th1 response differences by age were not significant at any time point in ZV or HZ/su recipients (Figure 2). Likewise, responses in boosted older adults were not significantly different from primary groups in either vaccine group.

Comparison of VZV and gE Th1 Responses Between Vaccine Groups

The primary comparison was the peak response, 30 days after ZV and after the second dose of HZ/su. Because age, gender, or prior administration of ZV did not have a significant effect on the peak responses, the analysis was not adjusted for these variables. However, baseline VZV and gE Th1 significantly explained the peak responses in univariate analyses ($P < .001$). After adjusting for baseline values, VZV IL-2 peak responses were higher in HZ/su compared with ZV recipients, but there were no significant differences in VZV IFN-γ or VZV DP responses, indicating that the type of vaccine had a significant effect only on VZV IL-2 among all VZV Th1 peak responses tested (Table 2). Baseline-adjusted gE Th1 peak responses were significantly higher in HZ/su compared with ZV recipients, indicating that the type of vaccine significantly explained all gE Th1 (Table 2). At 1 year, baseline-adjusted gE IL-2, IFN-γ, and DP responses, and VZV IL-2 responses remained higher in HZ/su compared with ZV recipients, whereas VZV IFN-γ and DP responses showed no difference (Table 3).

T-Cell Differentiation in Response to HZ/su and ZV

In a subset of 60 participants equally distributed between the 2 vaccines and across the 3 age/treatment groups, we analyzed gE- and VZV-$CD4^+$ and -$CD8^+$ T-cell differentiation profiles by flow cytometry at peak response. After ex vivo restimulation with either gE peptide pools, replication-competent VZV, or mock stimulation, we identified $CD4^+$ and $CD8^+$ central memory (Tcm; $CCR7^+CD27^+CD45RO^+$), effector memory (Tem; $CCR7^-CD27^+CD45RO^+$), differentiated effectors (Teff;

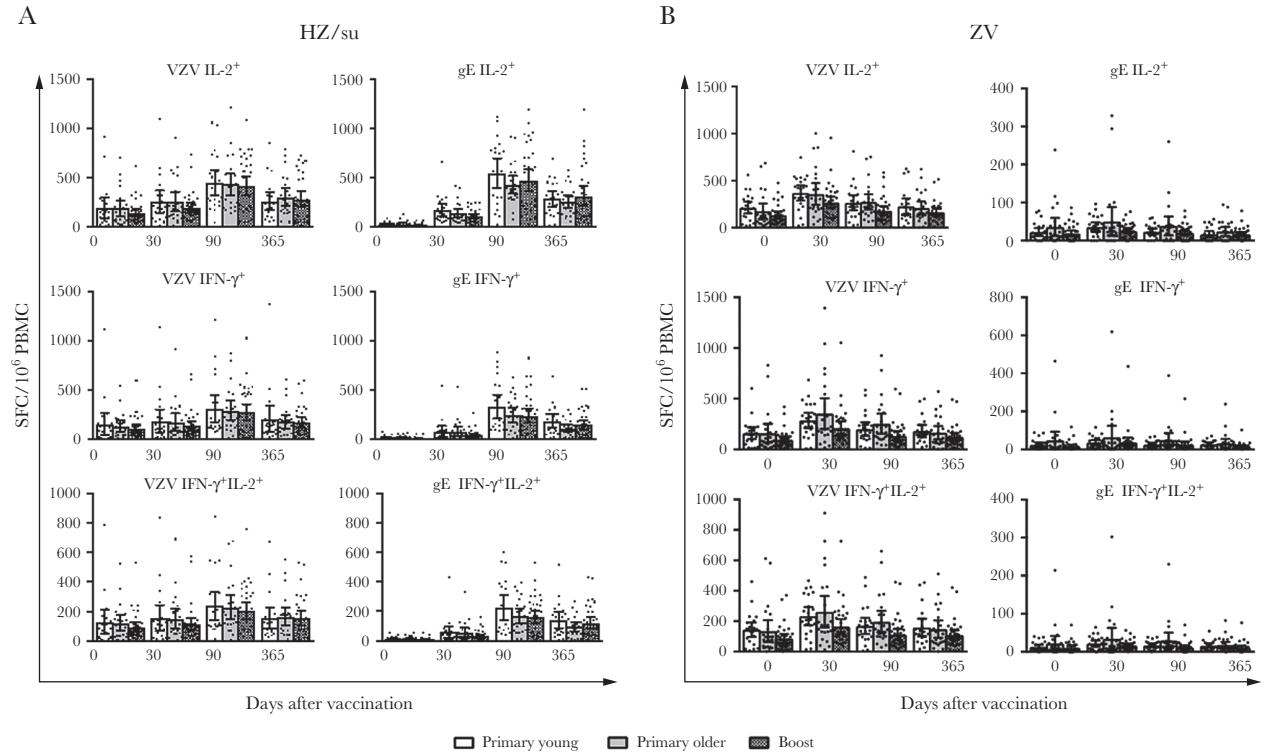

Figure 2. FluoroSpot responses to recombinant subunit glycoprotein E zoster vaccine (*A*) and live attenuated zoster vaccine (*B*) by age and treatment group. Data were derived from 158 participants. Bars represent geometric mean spot-forming cells per 10^6 peripheral blood mononuclear cells and 95% confidence interval at the time points indicated at the bottom of each graph. Analytes and stimulants are indicated in the title of each graph. Abbreviations: HZ/su, recombinant subunit glycoprotein E zoster vaccine; gE, glycoprotein E; IFN-γ, interferon gamma; IL-2, interleukin 2; PBMCs, peripheral blood mononuclear cells; SFC, spot-forming cell; VZV, varicella zoster virus; ZV, live attenuated zoster vaccine.

$CCR7^-CD27^-CD45RO^+$), intermediate effectors (Tei; $CCR7^-CD27^+CD45RO^-$) and terminally differentiated effectors (Ted; $CCR7^-CD27^-CD45RO^-$ and confirmed their specificity to the stimulating antigen by IFN-γ production (Figure 3). It is important to note that both gE peptide pools and replication-competent VZV allow T-cell epitope presentation in the context of major histocompatibility complex classes I and II. The comparison of the baseline-adjusted peak responses showed that HZ/su generated significantly higher gE-specific $CD4^+$ Tcm and Tem and lower $CD4^+$ Teff compared to the VZV-specific responses generated by ZV (FDR $P < .05$; Table 4).

DISCUSSION

The primary objective of this study was to identify immune responses that may explain the superior protection against HZ conferred by HZ/su compared with ZV. Immune responses that clearly distinguished the 2 vaccines were the higher gE- and VZV-specific memory Th1 responses generated by HZ/su,

Table 2. Comparison of Varicella Zoster Vaccine–Specific Peak and 1-Year Responses to Live Attenuated and Recombinant Subunit Glycoprotein E Zoster Vaccines

Variable	Estimated Mean Fold-Difference Between Vaccine Groups	(95% CI)	FDR-Adjusted *P* Value
Peak response (30 days after the last dose of vaccine)			
$IL\text{-}2^+$	0.74	(.60–.91)	.01
$IFN\text{-}\gamma^+$	0.91	(.73–1.14)	1
$IL\text{-}2^+IFN\text{-}\gamma^+$	0.91	(.73–1.14)	1
1-year response			
$IL\text{-}2^+$	0.71	(.56–.89)	.009
$IFN\text{-}\gamma^+$	0.87	(.70–1.07)	.53
$IL\text{-}2^+IFN\text{-}\gamma^+$	0.82	(.67–1.01)	.19

Abbreviations: CI, confidence interval; FDR, false discovery rate; IFN-γ, interferon gamma; IL-2, interleukin 2.

Table 3. Comparison of Glycoprotein E–Specific Peak and 1-Year Responses to Live Attenuated and Recombinant Subunit Glycoprotein E Zoster Vaccines

Variable	Effect Estimate	(95% CI)	FDR-Adjusted *P* Value
Peak response (30 days after the last dose of vaccine)			
IL-2$^+$	0.08	(.06–.11)	**<.0001**
IFN-γ$^+$	0.13	(.10–.18)	**<.0001**
IL-2$^+$IFN-γ$^+$	0.13	(.10–.19)	**<.0001**
Persistent response (1 year)			
IL-2$^+$	0.08	(.06–.12)	**<.0001**
IFN-γ$^+$	0.14	(.10–.14)	**<.0001**
IL-2$^+$IFN-γ$^+$	0.11	(.08–.16)	**<.0001**

The effect estimate indicates the magnitude of the difference between vaccines on linear scale. Positive effect estimates indicate higher responses in the recombinant compared with live attenuated zoster vaccine recipients. The absolute values of the effect estimates represent the magnitude of the differences. *P* values were adjusted for multiple comparisons using the FDR correction. Bold font highlights significant differences.

Abbreviations: CI, confidence interval; FDR, false discovery rate; IFN-γ, interferon gamma; IL-2, interleukin 2.

including peak CD4$^+$ Tcm% and Tem% and gE and VZV IL-2 SFCs. It is likely that the predominance of memory responses in HZ/su recipients explains the sustained protection against HZ of ≥87% up to 4 years after HZ/su administration compared with approximately 40% protection by ZV after a similar interval [15, 16, 23, 24].

The very low or absent gE Th1 responses before HZ/su administration, even in those who had received ZV ≥5 years before entering the study suggests that T-cell responses to gE are not dominant after wild or attenuated VZV infection and that some individuals do not mount responses to gE or lose these responses over time. In fact, after the first dose of HZ/su, responses to gE

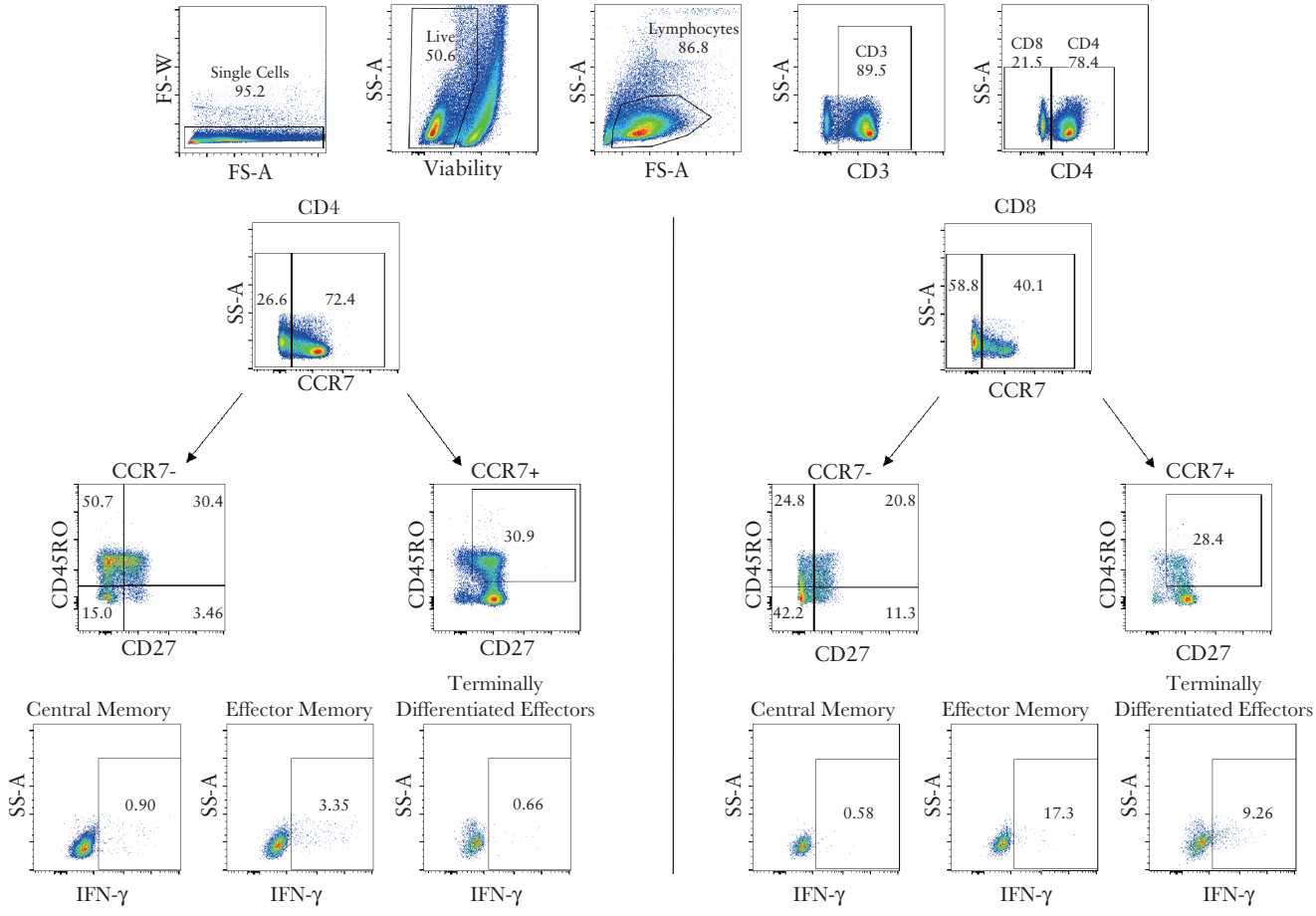

Figure 3. T-cell differentiation profiles in response to live attenuated and recombinant subunit glycoprotein E zoster vaccines. Gating strategy. Abbreviations: FS-W, forward scatter width; IFN-γ, interferon gamma; SS-A, side scatter area.

Table 4. Comparative Analysis of Peak Response Glycoprotein E– and Varicella Zoster Virus–Specific T-Cell Differentiation in Recipients of Recombinant Subunit Glycoprotein E Zoster Vaccine (n = 30) and Live Attenuated Zoster Vaccine (n = 30), Respectively

T-Cell Subset	Effect Estimate	(95% CI)	*P* Value	FDR *P* Value
Effector $CD4^+$	2.07	(1.56–2.76)	<.0001	<.0001
Effector memory $CD4^+$	0.55	(.40–.74)	.0001	.007
Central memory $CD4^+$	0.76	(.63–.92)	.005	.02

The effect estimates indicate the magnitude of the difference between the 2 vaccines on log scale. Effect estimates <1 indicate higher responses after recombinant zoster vaccine and estimates >1 indicate higher responses after live attenuated zoster vaccine.

Abbreviations: CI, confidence interval; FDR, false discovery rate.

were very low, and responses to VZV were lower than those of ZV recipients. This finding is in agreement with previously published data showing gE-specific $CD4^+$ Th1 responses by flow cytometry in only 20% of vaccinees after the first dose of HZ/su [25]. Sei et al [26] also showed that other VZV gene products, including *IE63*, *IE62*, *gB*, and *ORF9*, were targeted more frequently than gE by $CD4^+$ and $CD8^+$ T cells in response to ZV administration. Taken together, these observations underscore 2 important points: (1) The second dose of HZ/su is essential for the immunogenicity and, consequently, efficacy of this vaccine; and (2) many gE Th1 responder T cells arise from naive cells. It is not known if drawing responses from the naive T cell pool may be advantageous for the host because these cells have undergone less cycles of replication than memory cells and/or are less exhausted and, thereby, may generate longer lasting memory or more efficient killing. Akondy et al showed that ZV also draws Th1 responders from the naive T-cell pool, but those responders died quickly and did not contribute to persistent immunity [27]. The role of de novo responses to HZ/su in its efficacy warrants further investigation, because this factor may have important implications for the design of other vaccines for older adults.

This study was the first to compare immune responses to HZ/su between older adults who previously received ZV and those who had not [28]. The FluoroSpot responses of individuals immunized with HZ/su were similar regardless of prior ZV administration, which was confirmed by a recent publication [29].

The results obtained with HZ/su may also provide insight into the immunologic mechanism(s) responsible for preventing HZ. Latent VZV in human ganglia is present only in sensory neurons [30]. Current models suggest that latency is maintained either by (1) unique VZV T cells that synapse with latently infected neurons to provide signals required to maintain latency; or (2) sporadic reactivation of latent VZV, for which there is growing evidence, aborted by VZV CMI before replication proceeds to clinical disease (ie, subclinical reactivation) [31–33]. Since HZ/su stimulates only gE CMI, and since latently infected neurons make a limited number of VZV transcripts and proteins that do not include gE (when measured in ganglia collected <9 hours after the death of the host) [34–36], this suggests that latency is maintained by surveillance for and rapid resolution of sporadic VZV reactivation. This hypothesis is yet to be proven.

The high efficacy of HZ/su, demonstrated in clinical studies that enrolled 29 311 individuals ≥50 years of age, is exceptional among vaccines given to older adults and among investigational vaccines against herpesviruses. Robust and persistent memory responses distinguish HZ/su from ZV. The $AS01_B$ adjuvant is critical to this difference. However, more studies are needed to define additional factors related to gE by comparison to whole virus VZV.

Notes

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