

Live Attenuated Zoster Vaccine Boosts Varicella Zoster Virus (VZV)–Specific Humoral Responses Systemically and at the Cervicovaginal Mucosa of Kenyan VZV-Seropositive Women

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Background. Attenuated varicella zoster virus (VZV) is a promising vector for recombinant vaccines. Because human immunodeficiencyvirus (HIV) vaccines are believed to require mucosal immunogenicity, we characterized mucosal VZV-specific humoral immunity following VZV_{Oka} vaccination.

Methods. Adult Kenyan VZV-seropositive women (n = 44) received a single dose of the live zoster VZV_{Oka} vaccine. The anamnestic responses to the virus were followed longitudinally in both plasma and mucosal secretions using an in-house glycoprotein enzyme-linked immunosorbent assay and safety and reactogenicity monitored. VZV seroprevalence and baseline responses to the virus were also characterized in our cohorts (n = 288).

Results. Besides boosting anti-VZV antibody responses systemically, vaccination also boosted anti-VZV immunity in the cervicovaginal mucosa with a 2.9-fold rise in immunoglobulin G ($P < .0001$) and 1.6-fold rise in immunoglobulin A (IgA) ($P = .004$) from the time before immunization and 4 weeks postvaccination. Baseline analysis demonstrated high avidity antibodies at the gastrointestinal and genital mucosa of VZV-seropositive women. Measurement of VZV-specific IgA in saliva is a sensitive tool for detecting prior VZV infection.

Conclusions. VZV_{Oka} vaccine was safe and immunogenic in VZV-seropositive adult Kenyan women. We provided compelling evidence of VZV ability to induce genital mucosa immunity.

Clinical Trials Registration. NCT02514018.

Keywords. varicella zoster virus; zoster vaccine; mucosal immunity; vaccine vector; HIV/AIDS vaccine.

Varicella zoster virus (VZV) is a member of the Herpesviridae family and the causative agent of varicella (chickenpox) and herpes zoster (HZ; shingles). Exposure to VZV often results in productive infection in individuals without preexisting immunity to the virus. Unlike varicella, which occurs mainly during childhood [1], secondary reactivation disease with VZV (HZ) occurs mainly in older adults and immunocompromised individuals as a consequence of waning VZV-specific cellular

immunity [2, 3]. Live attenuated VZV vaccines available for the prevention of varicella and HZ have been shown to be safe and effective in preventing both diseases [4–9]. Most recently, a recombinant glycoprotein E–based HZ vaccine combined with a novel adjuvant has demonstrated promise in elderly patients [10], although this approach has not been used to prevent varicella. While recombinant protein vaccines typically require periodic booster doses as the only means to boost immunity, live attenuated vaccines such as the Oka strain VZV (VZV_{Oka}) can establish latency in human neural ganglia and the autonomic nerve ganglia found in the basal layer of the enteric mucosa similarly to the wild-type VZV [11, 12]. It has been proposed that periodic VZV subclinical reactivation boosts the humoral and cellular immune responses against the virus, thus providing long-lived protection.

The well-established record of safety and immunogenicity of attenuated, replication-competent VZV_{Oka} vaccine in preventing varicella and HZ, combined with intrinsic viral features, has raised interest in using VZV_{Oka} as a platform for the expression of foreign

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antigens in recombinant vaccines, including human immunodeficiency virus (HIV) [13, 14]. However, despite the extensive knowledge of VZV pathogenesis, there are still key questions to address before pursuing its use as a viral vector in vaccines. For instance, it is unknown whether VZV also induces immunity widely across the mucosa including the genital mucosa and rectum. These sites are the primary portal of entry for infection by several pathogens such as HIV and herpes simplex virus type 2.

Additionally, most of the epidemiological data on VZV infection and clinical trials on VZV vaccine efficacy have been derived from non-African countries [15]. Differences in parasitic, mycobacterial and other coinfections, and the immune microenvironment as well as specific and global nutritional deficiencies are more prevalent in parts of Africa, and are just now beginning to be understood in terms of their impact on a number of vaccine responses [16–18]. Finally, for reasons that are poorly understood, the seroprevalence of VZV is not ubiquitously near 100% among adults in the tropics as it is in the temperate parts of the world. In Africa, the geographic variation and changing seroprevalence of VZV is just now being appreciated as rural–urban migration accelerates.

Therefore, in this study we sought to characterize humoral immune responses against VZV in a cohort of VZV-seropositive Kenyan women ($n = 44$) prior to and after vaccination with a commercial zoster vaccine (Zostavax, MSD, Merck & Co). Because the low concentration of antibodies in mucosal secretions may hamper the ability to characterize mucosal humoral responses, we developed and validated a highly sensitive in-house quantitative VZV glycoprotein enzyme-linked immunosorbent assay (gpELISA) to measure VZV-specific immunoglobulin G (IgG) and immunoglobulin A (IgA) at mucosal sites. We performed a comprehensive analysis of the antibody response systemically (blood), in the female genital tract (FGT), in the rectum, and in oral mucosal secretions, which included the longitudinal quantification of VZV-specific IgG and IgA as well as the assessment of anti-VZV antibody avidity to measure affinity maturation. Clinical parameters relevant to the safety and reactogenicity of zoster vaccine were also closely monitored in this cohort.

MATERIALS AND METHODS

Study Design

The Kenyan AIDS Vaccine Initiative (KAVI)–VZV-001 study design, including eligibility criteria, study outcomes, immunization schedule, timeline, and sample collection are detailed in the study protocol by Perciani et al [14]. In brief, 44 healthy Kenyan women with a median age of 26 years (interquartile range, 21.5–30.5 years), seropositive for VZV, seronegative for HIV types 1 and 2, and at low risk for HIV infection were enrolled in the study. Upon enrollment, the subjects were randomly assigned (1:1) to an immediate group that received the vaccine right after baseline sample collection or into a delayed group that received the vaccine 12 weeks postenrollment, after

undergoing baseline sampling collection a total of 4 times prior to immunization [14]. All subjects were determined to be not pregnant prior to vaccination and agreed to use an effective contraceptive method during the study duration. Participants were followed for 36–48 weeks postimmunization depending on the group they were assigned to. The quantification of VZV-specific antibodies (Abs) prior to and after vaccination was performed in plasma, cervicovaginal secretions, saliva, and rectal secretions as previously described [14]. All participants included in this study provided written informed consent. KAVI-VZV-001 is registered with ClinicalTrials.gov (NCT02514018) and was approved by Kenyatta National Hospital/University of Nairobi (KNH/UON), Ethics and Research Committee (ERC), the University of Toronto Research Ethics Board (REB), and the Kenyan Pharmacy and Poisons Board.

Zoster Vaccine Administration

The commercial zoster vaccine live, Zostavax, containing live attenuated VZV_{Oka} (≥ 19400 plaque-forming units [PFU]) was stored and administered according to the manufacturer's directions. Study participants in the KAVI-VZV-001 trial received the vaccine by subcutaneous route in a single-dose regimen.

Safety and Tolerability

Participants were instructed to report any adverse experience (AE) over the course of the study to the clinic staff. A reactogenicity card, a thermometer, and a ruler were provided at the day of vaccination for the daily documentation of injection-site reactions and systemic AEs during a 14-day period following vaccination.

Clinical Specimens for Baseline Assessments

Plasma samples from women screened in the KAVI-VZV-001 trial ($n = 88$) and from the Pumwani Women's cohort ($n = 200$) were used for the estimation of VZV seroprevalence in adult Kenyan women. Stored samples of plasma and cervicovaginal secretions from the Pumwani Women's cohort ($n = 41$), plasma collected from women screened for the KAVI-VZV-001 trial ($n = 84$), and cervicovaginal secretions, rectal secretions, and saliva collected from women enrolled in the KAVI-VZV-001 trial or KAVI-VZV-001 negative control group ($n = 52$) were utilized for the development and validation of our in-house gpELISA. The use of stored samples from the Pumwani Women's cohort were approved by KNH/UON ERC, and the KAVI-VZV-001 negative control group study was approved by both KNH/UON ERC and the University of Toronto REB.

Screening With the Vitek Immuno Diagnostic Assay System

A qualitative assay, the VIDAS VZV-IgG assay (bioMérieux SA) was used for the initial screening of VZV serostatus as per the manufacturer's instructions. In brief, commercial VZV antigen immobilized on strips was incubated with 100 μ L of

human plasma followed by incubation with alkaline phosphatase-labeled mouse monoclonal antihuman IgG and a fluorescent substrate, 4-methylumbelliferone. The readout for this method is a fluorescence measurement detected using the Vitek Immuno Diagnostic Assay System (VIDAS) instrument and is interpreted as a ratio against the standards as per the manufacturer's instructions.

VZV-Specific Antibody Responses

The concentrations of anti-VZV IgG and IgA Abs were quantified using an in-house ELISA containing VZV purified glycoproteins as coating (gpELISA) (see [Supplementary Methods](#), [Supplementary Table 1](#), and [Supplementary Figures 1 and 2](#) for experimental details and reproducibility assessments). VZV-specific IgG and IgA avidity were measured using thiocyanate (SCN⁻) as a chaotropic agent in our in-house gpELISA and the avidity index calculated using the titration curve obtained for the sample with and without SCN⁻ as previously reported [19] (see [Supplementary Methods](#) for details).

Statistical Analysis

Nonparametric tests (Spearman's correlation [r_s (df)] and Wilcoxon signed-rank test) were used to compare the variables. Statistical analyses were performed using IBM SPSS Statistics (version 23). Graphs were generated using Prism 6 (GraphPad) software. Results were adjusted for multiple comparisons using the Holm step-down procedure. An adjusted *P* value of <.05 was considered to be statistically significant.

RESULTS

VZV Seroprevalence in Adult Kenyan Women

Plasma samples from a total of 288 adult Kenyan women (88 from the KAVI-VZV-001 cohort and 200 from the Pumwani Women's cohort) were tested for the presence of anti-VZV antibodies using the VIDAS VZV-IgG assay. Eighty-three percent of the subjects (230/288) tested positive for anti-VZV antibodies whereas 7% (19/288) were shown to be negative for anti-VZV antibodies. The other 30 individuals (10%) were reported as equivocal according to the VIDAS assay interpretation guidelines (indeterminate value that does not meet the threshold for a positive but is in excess in comparison with the negative standards).

Characterization of VZV-Specific Humoral Immunity Systemically and at Mucosal Sites

Anti-VZV Ab responses were measured using our in-house gpELISA in plasma, cervicovaginal secretions, rectal secretions, and saliva samples of participants enrolled in KAVI-VZV-001, all of whom were formerly determined to be VZV seropositive by VIDAS. We observed that at baseline (ie, prior to vaccination), 93% of plasma and 98% of cervicovaginal secretion samples (*n* = 44) from the participants enrolled in KAVI-VZV-001 showed VZV-specific IgG concentrations above the

limit of detection (LOD) determined for our in-house method. However, VZV-specific IgG responses in rectal secretions and saliva samples from these subjects were often under the LOD, with only 49% of rectal secretions and 44% of saliva samples in the detectable range ([Figure 1A](#)). In comparison, the concentration of anti-VZV IgA above the LOD in mucosal baseline samples of these subjects was 84% for cervicovaginal secretions, 100% for saliva, and 89% for rectal secretions ([Figure 1A](#)). Given that approximately half of the saliva and rectal secretion samples showed anti-VZV IgG levels under the LOD, we limited our subsequent humoral analysis to IgA in these sites while incorporating both IgG and IgA assessments for cervicovaginal secretion samples.

Because persistent viral replication is likely to promote affinity maturation of the Ab responses, we were also interested in determining the avidity index for the VZV-specific Abs measured. As shown in [Figure 1B](#), anti-VZV IgG in both cervicovaginal secretions and plasma and anti-VZV IgA in rectal secretions were of high avidity (>0.50), indicating that VZV-specific B-cell clones have matured, presumably by subclinical reactivation of VZV over time.

Safety and Tolerability of Zoster Vaccine Live in VZV-Seropositive Adult Kenyan Women

Participants enrolled at KAVI-VZV-001 received a single dose of the commercial live attenuated zoster vaccine, Zostavax, and were closely monitored for systemic and local reactions during the 14-day period that followed vaccination. The clinical AEs recorded by the participants in the provided reactogenicity card and by the clinic staff are summarized in [Table 1](#).

Seventy-four percent of the subjects reported at least 1 vaccine-related AE. Most of the AEs (77%) occurred within the first 5 days following vaccination and 90% of all events resolved within 4 days and were considered mild by the participants. Importantly, there was no serious AEs observed in the trial.

Systemic VZV-Specific IgG Prior to and After VZV Vaccination

After enrollment into the KAVI-VZV-001 study, participants were randomly assigned to receive the live attenuated zoster vaccine immediately after baseline collection (week 0) or to be followed for 12 weeks prior to vaccination in a delayed group ([Figure 2A](#)). Subjects enrolled into the first group (immediate group) were followed for 48 weeks postvaccination and subjects enrolled into the second group (delayed group) were followed for 36 weeks postvaccination ([Figure 2A](#)). The temporal kinetics of the humoral response induced systemically against VZV are shown in [Figure 2B](#). Anti-VZV IgG levels remained stable in the 12-week period that preceded vaccination ([Figure 2B](#)). After a single dose of the zoster vaccine live, VZV-specific IgG concentration increased significantly and remained significantly elevated up to week 48 postvaccination ([Figure 2B](#)

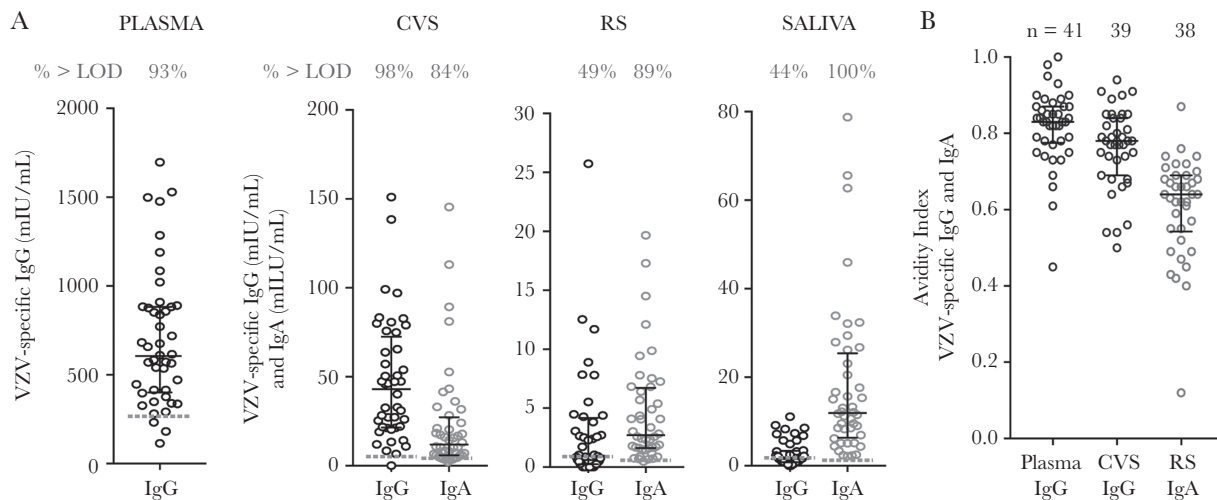


Figure 1. Assessment of mucosal varicella zoster virus (VZV)-specific antibody responses in a cohort of VZV-seropositive women. *A*, Concentration of VZV-specific immunoglobulin G (IgG) in plasma and VZV-specific IgG and immunoglobulin A (IgA) in cervicovaginal secretions (CVS), rectal secretions (RS), and saliva from 44 VZV-seropositive women. *B*, Avidity index of VZV-specific IgG in plasma and CVS and of IgA in RS prior to vaccination. Graphs show median and interquartile range. Dotted lines show limit of detection (LOD).

and 2C). The geometric mean of the VZV-specific IgG concentrations and fold changes from week 0 in plasma samples are shown in Table 2. At week 4 postvaccination there was a 4.3-fold rise (95% confidence interval [CI], 1.61- to 6.98-fold) in the antibody concentration (Table 2). This response gradually decreased, with a geometric mean of the fold rise equal to 3.07 (95% CI, 2.16–3.97) by week 8 postvaccination (Table 2) and 28% of the vaccinated individuals with ≥ 4 -fold rise.

Table 1. Adverse Experiences Following a Single Dose of Zoster Vaccine Live

Subjects	No.	(%)
Vaccinated	44	(100)
With reactogenicity data	43	(98)
Without reactogenicity data	1	(2)
≥ 1 AE	32	(74)
≥ 1 vaccine-related AE	32	(74)
Systemic AEs	31	(72)
Headache	22	(51)
Malaise/fatigue	13	(30)
Fever (temperature $\geq 37.7^\circ\text{C}$)	7	(16)
Muscle aches/pain	7	(16)
Joint pain	6	(14)
Body rash	3	(7)
Injection-site AEs	28	(65)
Tenderness/soreness	21	(49)
Hardening	7	(16)
Erythema	5	(12)
Rash	4	(9)
Serious AEs	0	(0)

Abbreviation: AE, adverse experience.

Mucosal VZV-Specific IgG and/or IgA Prior to and After VZV Vaccination

The live zoster vaccine significantly boosted both anti-VZV IgG and IgA levels in cervicovaginal secretions (Figure 3). The vaginal Ab responses rose in the first 4 weeks after vaccination and remained significantly elevated compared to baseline (week 0) up to week 12 postvaccination (Figure 3B). The geometric mean of the VZV-specific IgG concentrations and fold changes from week 0 in cervicovaginal secretions are shown in Table 3. At week 4 postvaccination there was a 2.9-fold rise (95% CI, .84- to 5.03-fold) in the anti-VZV IgG Ab titer (Table 3). VZV-specific IgA concentration in both rectal secretions and saliva tended to increase at week 12 postvaccination compared with week 0, although this difference was proven not to be significant (Figure 3A and 3B).

Correlations Between VZV-Specific Ab Responses at Different Sites

We next evaluated whether the concentration of anti-VZV IgG Abs in plasma could be used as a predictor of the concentration of anti-VZV Ab responses in the mucosa. As shown in Figure 4A, there was a significant positive correlation between the concentration of VZV-specific IgG Abs in plasma and VZV-specific IgG ($r_s(172) = 0.49$; $P < .0001$) and IgA Abs ($r_s(150) = 0.28$; $P = .0008$) in cervicovaginal secretions. Plasma anti-VZV IgG levels also positively correlated with anti-VZV IgA in saliva ($r_s(100) = 0.36$; $P = .0002$). At the mucosa, we observed a positive correlation between the concentration of anti-VZV IgA and IgG Abs in cervicovaginal secretions ($r_s(148) = 0.47$; $P < .0001$) and between the concentration of VZV-specific IgA in cervicovaginal secretions and in saliva ($r_s(98) = 0.39$; $P < .0001$) (Figure 4B). There was no correlation between the concentration of VZV-specific IgA measured

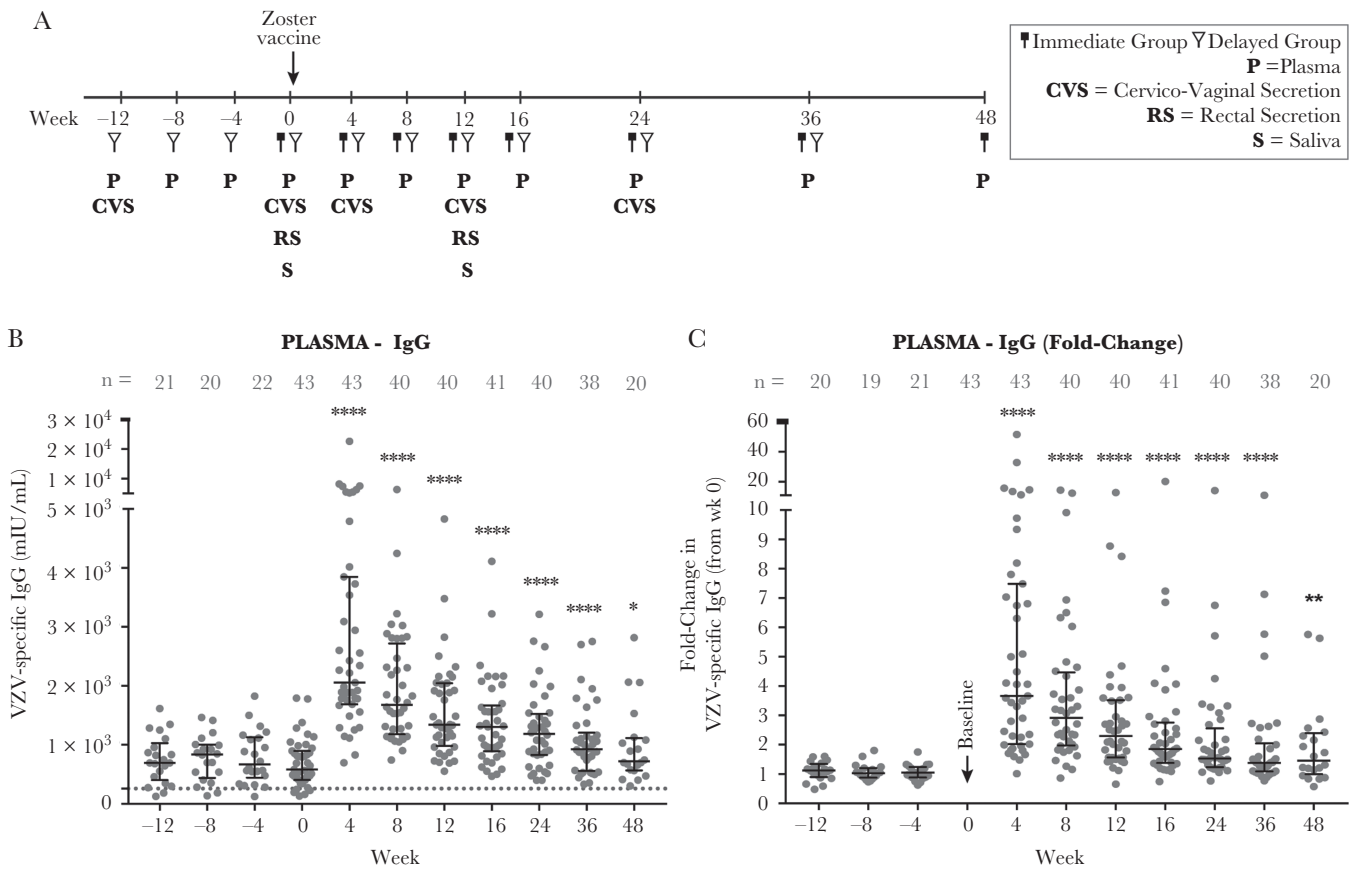


Figure 2. Systemic varicella zoster virus (VZV)-specific immunoglobulin G (IgG) prior to and after VZV vaccination in a cohort of VZV-seropositive women. *A*, Vaccination schedule and longitudinal sample collection time points used in this study. *B*, Concentration of VZV-specific IgG assessed longitudinally in plasma prior to and up to 48 weeks postvaccination. *C*, Fold change in VZV-specific IgG concentrations in relation to week 0 (baseline). Graphs show median and interquartile range. Time points were compared to week 0 using Wilcoxon signed-rank test, and adjusted for multiple comparisons using step-down procedure. * $P < .05$; ** $P < .01$; **** $P < .0001$. Dotted line shows limit of detection (LOD).

in rectal secretions and the anti-VZV Ab levels in plasma or cervicovaginal secretions (Figure 4A and B).

Table 2. Varicella Zoster Virus-Specific Immunoglobulin G Concentration and Fold Change From Week 0 Measured Longitudinally in Plasma Samples Using the Glycoprotein Enzyme-Linked Immunosorbent Assay

Week	IgG, mIU/mL		Fold Change (From Week 0)	
	GM (95% CI)	No.	GM (95% CI)	No.
-12	629 (455–802)	21	1.05 (.91–1.19)	20
-8	655 (487–822)	20	1.03 (.91–1.16)	19
-4	659 (474–845)	22	1.03 (.92–1.14)	21
0	621 (499–743)	43
4	2515 (1452–3578)	43	4.30 (1.61–6.98)	43
8	1790 (1461–2119)	40	3.07 (2.16–3.97)	40
12	1416 (1157–1675)	40	2.43 (1.71–3.15)	40
16	1227 (1000–1453)	41	2.12 (1.16–3.08)	41
24	1093 (888–1297)	40	1.88 (1.17–2.59)	40
36	929 (741–1117)	38	1.63 (.99–2.27)	38
48	829 (542–1117)	20	1.61 (.98–2.24)	20

Abbreviations: CI, confidence interval; GM, geometric mean; IgG, immunoglobulin G.

DISCUSSION

There is a wide variation in VZV seroepidemiology worldwide linked to the age, geographic region, and urbanization [1, 20] and limited VZV seroepidemiological data from African countries [15]. In our cohorts of adult Kenyan urban women, the VZV seroprevalence was 83% as assessed by the VIDAS VZV assay. This seroprevalence may be even greater (up to 93%) as the individuals tested equivocal by VIDAS can potentially carry protective levels of VZV antibodies as previously described [21, 22].

The lack of analytical tools sensitive enough to interrogate VZV humoral responses at the mucosa and the good correlation identified between protection against varicella and systemic VZV-specific IgG responses [23] have limited most of the analysis to blood to date. However sampling of mucosal secretions is less invasive, particularly saliva for seroepidemiological studies, so correlating antibodies against VZV at baseline is important. Here, we showed the validation of an in-house gpELISA for the quantification of VZV-specific IgA in cervicovaginal secretions, rectal secretions, and saliva

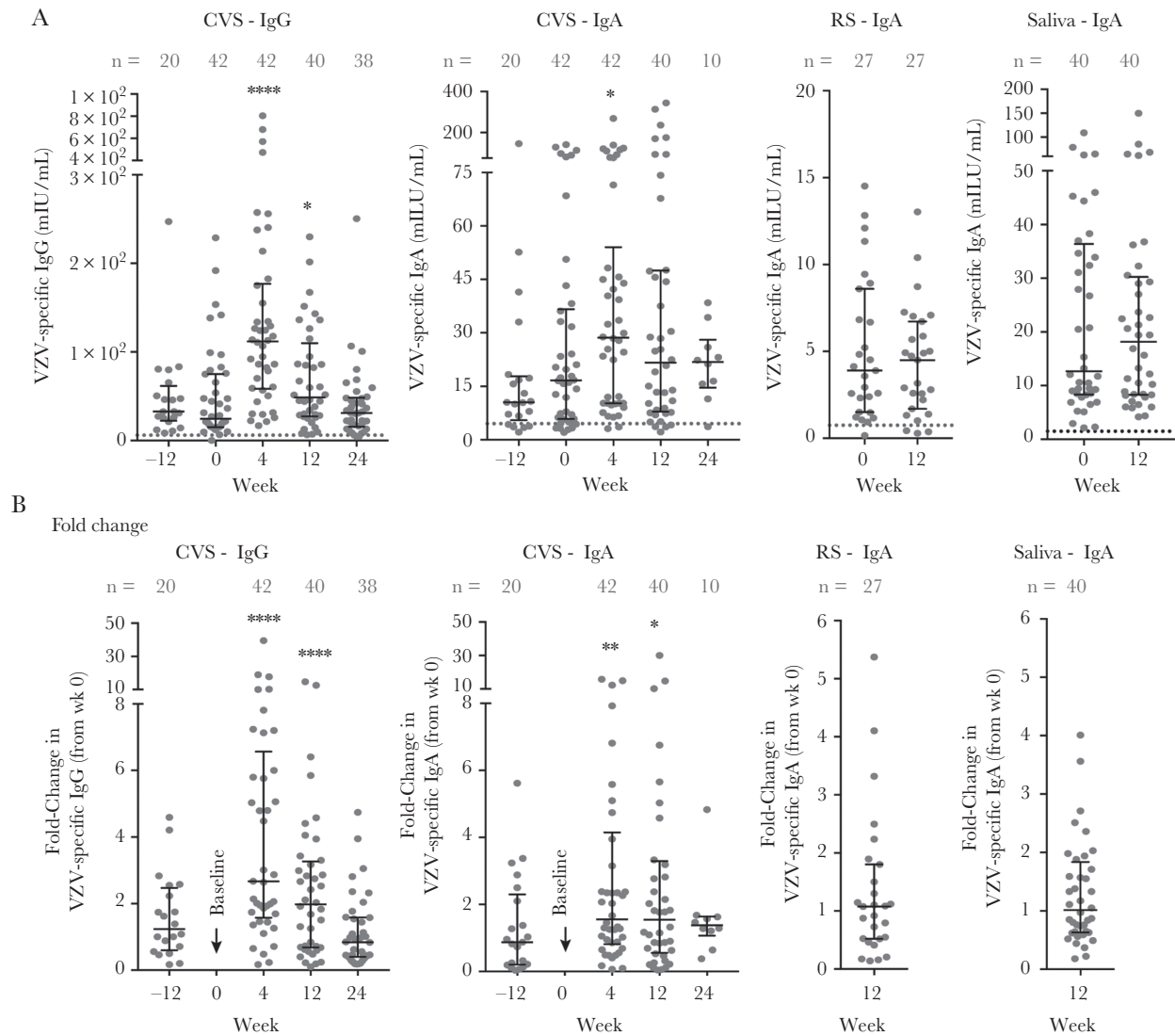


Figure 3. Mucosal varicella zoster virus (VZV)-specific immunoglobulin G (IgG) and/or immunoglobulin A (IgA) prior to and after VZV vaccination in a cohort of VZV-seropositive women. *A*, Concentration of VZV-specific IgG in cervicovaginal secretions (CVS) and of VZV-specific IgA in CVS, rectal secretion (RS), and saliva prior to and postvaccination. *B*, Fold-change in VZV-specific IgG concentration in CVS and of VZV-specific IgA concentration in CVS, RS, and saliva in relation to week 0. Graphs show median and interquartile range. Time points were compared to week 0 (baseline) using Wilcoxon signed-rank test, and adjusted for multiple comparisons using step-down procedure. * $P < .05$; ** $P < .01$; *** $P < .001$; **** $P < .0001$. Dotted lines indicate the respective limit of detection.

Table 3. Varicella Zoster Virus–Specific Immunoglobulin G Concentration and Fold Change From Week 0 Measured Longitudinally in Cervicovaginal Secretions Using the Glycoprotein Enzyme-Linked Immunosorbent Assay

Week	IgG, mIU/mL		Fold Change (From Week 0)	
	GM (95% CI)	No.	GM (95% CI)	No.
-12	35.2 (12.4–58.0)	20	1.15 (.60–1.70)	20
0	33.4 (17.2–49.6)	41
4	103.1 (50.8–155.4)	42	2.94 (.84–5.03)	42
12	49.2 (26.7–71.6)	40	1.53 (.58–2.47)	40
24	27.7 (14.0–41.4)	38	0.78 (.44–1.13)	38

Abbreviations: CI, confidence interval; GM, geometric mean; IgG, immunoglobulin G.

as well as VZV-specific IgG in cervicovaginal secretions and blood. Saliva anti-VZV IgA was detected positive in all enrollees at baseline. Using this method, we demonstrated that VZV-seropositive individuals sustain a humoral immunity directed against VZV in the gastrointestinal and genital mucosa. This immunity is marked by the presence of high-avidity antibodies, suggesting that VZV-specific memory mucosal B cells can undergo affinity maturation, potentially driven by asymptomatic VZV shedding. In this study we also show for the first time the safety and immunogenicity of zoster vaccine, live attenuated VZV_{Oka} (≥ 19400 PFUs), in a cohort of VZV-seropositive adult Kenyan women (median age, 26 years). Seventy-four percent of the subjects vaccinated at KAVI-VZV-001 reported at

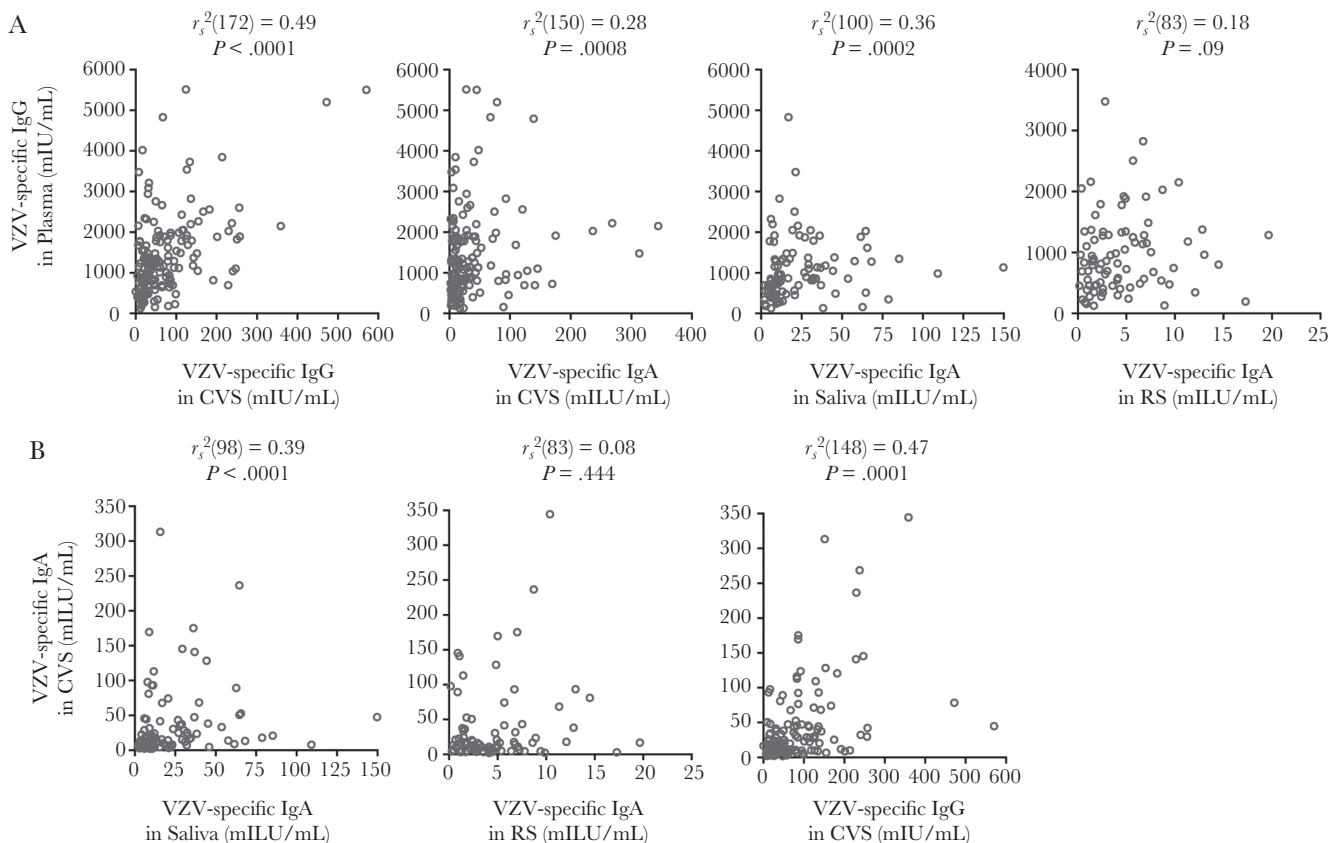


Figure 4. Correlations between the concentrations of varicella zoster virus (VZV)-specific immunoglobulin G (IgG) in plasma and VZV-specific IgG and immunoglobulin A (IgA) in mucosal secretions. *A*, Correlations between VZV-specific IgG levels in plasma and cervicovaginal secretion (CVS), and between VZV-specific IgG levels in plasma and VZV-specific IgA in CVS, saliva, and rectal secretions (RS). *B*, Correlation between the concentration of anti-VZV IgA in CVS and in the other mucosal secretions (saliva and RS) and between anti-VZV IgA and IgG in CVS. Graphs show the Spearman's correlation (r_s), degree of freedom (df), and *P* value.

least 1 vaccine-related AE and 65% reported at least 1 injection site AE. The greater majority of the AEs observed at KAVI-VZV-001 were reported to be mild, occurred within the first 5 days postvaccination, and resolved spontaneously with no report of any serious AEs. These results are similar to the ones obtained with a formulation of live attenuated VZV_{Oka} containing approximately 50 000 PFU previously tested in subjects at the same age group (mean age, 22.4 years) [24].

We next determined the vaccine immunogenicity by quantifying the VZV-specific plasma IgG during the anamnestic response. We observed a significant increase in plasma anti-VZV IgG concentration 4 weeks postvaccination that remained significantly elevated up to 48 weeks postvaccination when compared to the response prior to vaccination. At week 4 there was a 4.3-fold rise (95% CI, 1.61- to 6.98-fold) in anti-VZV IgG concentration when compared to baseline (week 0), and 49% of the vaccinated subjects showed ≥ 4 -fold rise. This level of response is comparable to that observed in a phase 3 efficacy and safety trial for this vaccine in elderly individuals [25] and in a multicenter clinical trial with a high titered formulation (~ 50 000 PFU/dose) in a cohort of young adults (mean age, 22.4 years) [24].

Live attenuated VZV carries several features that make it a promising vector in recombinant vaccines, including HIV [13]. Because the ability to induce mucosal immune responses is desired in new vaccine candidates, we next evaluated the effect of zoster live immunization on the VZV-specific antibody responses in mucosal sites.

We showed that subcutaneous immunization with a single dose of live attenuated VZV_{Oka} was able to boost anti-VZV IgG and IgA Abs in the FGT with a significant rise on Ab concentrations by week 4 postvaccination. Saliva at baseline is highly sensitive for the detection of anti-VZV IgA; however, analysis of the impact of vaccination on the levels of VZV-specific IgA Abs in rectal secretions and saliva collected at week 12 postvaccination did not reveal a significant increase at these sites. Collection of rectal secretions and saliva more shortly after vaccination, as was done at the FGT, may have revealed an impact of zoster live vaccination as was shown at the FGT.

Ultimately we examined whether the VZV-specific responses were synchronized across tissues. We observed that anti-VZV IgG Ab levels in plasma and cervicovaginal secretions are positively correlated ($r_s(172) = 0.49$; $P < .0001$) and that plasma anti-VZV IgG modestly correlated with anti-VZV IgA both in

cervicovaginal secretions and saliva ($r_s(150) = 0.28$ [$P = .0008$] and $r_s(100) = 0.36$ [$P = .0002$], respectively). These findings may suggest that a fraction of the mucosal anti-VZV Abs derives from the circulation. We also observed a modest positive correlation between VZV-specific IgA in cervicovaginal secretions and saliva ($r_s(98) = 0.39$; $P < .0001$), a correlation that may be worth further exploring due to easier implementation and higher acceptability of collecting saliva over cervicovaginal secretions in large vaccine trials.

The presence of antibodies in the FGT functions as a first line of defense against mucosally transmitted pathogens. Recent nonhuman primate studies highlighted the importance of specific vaginal antibodies in inducing protection against HIV [26–29]. Here, we demonstrated that VZV induces persistent immunity at the female genital mucosa and that this immunity can be boosted upon vaccination, an aspect that could be advantageous when exploring VZV as a vector in an HIV vaccine.

Our study had some limitations. Saliva and rectal secretion were collected only 12 weeks postvaccination, impairing our ability to potentially detect an Ab boost on the gastrointestinal mucosa early after vaccination. Our cohort comprised VZV-seropositive women; the inclusion of men and VZV-seronegative individuals would have provided valuable information about the impact of VZV vaccination on the male genital mucosa as well as about the differences between the kinetics of VZV humoral responses in individuals with and without preimmunity to the virus, something that could not be done here due to logistical challenges.

Altogether, our results add to the proven safety and immunogenicity record of VZV live vaccines by expanding its use for the first time in a cohort of Kenyan women and provide evidence that VZV can elicit the production of Abs at mucosal frontlines, which can be exploited for the development of an effective VZV-vectored vaccine for generation of high avidity and durable antibodies.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Author contributions. K. S. M., C. T. P., B. F., M. O., O. A., and W. J. conceived and designed the study. KAVI-ICR Team, UNITID, C. T. P., M. S., S. H., and L. M. collected or generated the study data. C. T. P., K. S. M., B. F., M. O., M. S., and S. H. were involved in the analysis or interpretation of the data. All authors contributed to the writing/reviewing of the manuscript and approved the final version for submission.

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