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Varicella immunity in vaccinated healthcare workers☆

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ABSTRACT

Background: Nosocomial spread of varicella-zoster virus (VZV) infection can cause severe disease among vulnerable patient-populations and healthcare personnel (HCP). Limited data are available on duration of varicella vaccine-induced protection among adults and to what extent cell-mediated immunity (CMI) and antibody avidity contribute to protection.
Objective: Evaluate humoral and cell-mediated immune responses of HCP who received a 2-dose regimen of varicella vaccine, and observe the responses to a 3rd vaccine dose among HCP who were seronegative after vaccination.
Study design: A convenience sample of HCP with documented 2 doses of varicella vaccine was used to assess acquired VZV immune parameters (cytokine production, IgG avidity). HCP seronegative after 2 doses of vaccine were offered a third dose and evaluated further. Vaccine recipients' immune responses were compared with responses from persons with history of wild-type VZV infection.
Results: The convenience sample consisted of 101 HCP with documented 2 doses of varicella vaccine; 12 (11.9%) were seronegative post-vaccination. 11.5% of 61 seropositive 2-dose recipients produced low avidity antibody, suggesting suboptimal response to vaccine. Seven 2-dose vaccinees who were VZV seronegative seroconverted after a third dose; however, 3/7 (42.9%) produced low avidity IgG. 142 persons with a history of varicella were all VZV IgG seropositive, and all had moderate to high avidity IgG.
Conclusions: Measurements of serum IgG titers alone may not accurately reflect vaccine protection. Varicella vaccination of HCP remains important but further studies are needed to evaluate CMI and antibody avidity responses in HCP vaccinated with two doses of varicella vaccine.

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1. Background

Nosocomial exposure and transmission of varicella zoster virus (VZV) are well documented.[1–7] Control and prevention of VZV infection within healthcare settings is crucial as severe disease among vulnerable patient populations (i.e., immunosuppressed persons, pregnant women, infants and susceptible adults) can occur.[8–12]

In 1996, two doses of varicella vaccine administered 4–8 weeks apart were recommended for susceptible adults, including healthcare personnel (HCP),[13] with ~99% of adults and adolescents seroconverting following the second dose of vaccine.[14] However, seroconversion to vaccination may not ideally correlate with protection as vaccine-induced antibody titers can decline or disappear over time.[15–17] In one study, lower post-vaccination VZV antibody titers measured using the fluorescent antibody to membrane antigen (FAMA) assay were associated with an increased risk of breakthrough varicella.[18] The clinical presentation of varicella in vaccinated persons (breakthrough varicella) is uncharacteristic and often described as mild; however it is contagious[19] and may cause severe disease among vulnerable populations. The modified clinical presentation of breakthrough varicella suggests that cell-mediated immunity (CMI) provides some degree of protection from VZV infection in the absence of detectable antibodies.[18]

Limited data are available on 2-dose varicella vaccine-induced protection among adults and to what extent CMI and antibody avidity contribute to protection. We assessed VZV antibody titers among vaccinated HCP and examined the role of CMI by measuring cytokine production and immunoglobulin G (IgG) antibody avidity.

☆ *Disclaimer*: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention, US Department of Health and Human Services.

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2. Study design

2.1. Study populations

A convenience sample of 101 HCP (≥18 years of age) with documented history of two doses of varicella vaccine was enrolled prospectively (November 2005–May 2007) from a large urban medical center. Consenting participants completed a questionnaire to collect demographic, epidemiologic, and clinical information. Dates for each dose of varicella vaccine received were collected from medical records. Blood was collected at enrollment and tested for VZV-specific IgG using two different methods: purified glycoprotein ELISA (gpELISA),[20–22] and a CDC-developed whole-cell ELISA (wc-ELISA).[2] Participants seronegative by gpELISA and with no medical contraindications were offered a third dose of the varicella vaccine (Varivax, Merck & Co., Whitehouse Station, NJ). Additional blood specimens were scheduled to be collected within 4–6 weeks after receiving the third dose to assess seroconversion.

Anonymized blood specimens (whole and serum) obtained from 143 adult volunteers for a blood donation program established between the CDC and the Emory University Hospital were used as a comparison group. Among these volunteers one person had received two doses of varicella vaccine and was VZV seronegative. This volunteer provided sequential blood samples, before and at multiple time points after receipt of a third dose, which facilitated a prospective assessment of vaccine-induced antibody levels and CMI response over time. The remaining 142 volunteers with specimens available in the blood donation program had wild-type (wt) VZV infection. Participants provided consent for use of contributed blood for research purposes, and the contract was reviewed and approved by the IRBs of CDC and Emory University. This study was approved by the Institutional Review Boards of the CDC and the University of Pennsylvania.

2.2. Laboratory methods

2.2.1. Serological methods

All sera were tested using the CDC whole cell VZV IgG ELISA,[2] which performs comparably to other commercially available wcELISA assays. Sera were also tested by gpELISA,[22] using antigens obtained from Merck and Co (Valley Forge, PA). Whole cell ELISA results with an adjusted optical density (OD) < 0.265 were negative, and those with OD ≥ 0.265 were positive. For gpELISA, an OD < 0.200 was negative and ≥0.200 positive. Since no common IgG standard for gpELISA is available, we used adjusted OD values rather than geometric mean titer to define the cut-off for this method. The cut-off for gpELISA was determined by receiver operating characteristic (ROC) analysis[23] using a panel of sera from children 12 to 18 months of age that were definable as true negative (pre-vaccination) and true positive (post-vaccination) samples. ROC analysis also established the cut-off for wcELISA using gpELISA as the gold standard. All serum samples with VZV IgG antibody titers were also evaluated for IgG antibody avidity. The wcELISA method was used to perform avidity assays as described previously.[24]

2.2.2. Cell-mediated immunity testing

Luminex100 (BioRad, Hercules, CA) cytokine profiles were performed on VZV-stimulated peripheral blood lymphocytes (PBL) before and at two time points after receiving a third dose of varicella vaccine (7 days and 44 months after receipt of 3rd dose). PBL were separated on Lymphocyte Separation Medium (MP Biomedicals, Solon, OH), and washed 3 times with sterile phosphate-buffered saline, pH 7.2. Cells were re-suspended in complete RPMI1640 medium (Invitrogen) supplemented with 20% heat-inactivated fetal calf serum, L-glutamine, and penicillin/streptomycin (Invitrogen, Carlsbad, CA), viable cells were counted, suspended at 2×10^6 cells/ml in complete medium, and aliquoted to wells in 24-well sterile tissue culture plates (Fisher Scientific, Pittsburgh, PA). Cells were stimulated with various antigens, mitogens, or mock antigen and incubated for 5 days at 37 °C in a humidified 5% CO_2 incubator. Test antigens included VZV strain Webster (1×10^3 pfu/well), HSV-1 strain F (1×10^3 pfu/well), Concanavalin A (75 μg/ml; Sigma–Aldrich Chemical, St. Louis, MO), and mock antigen (uninfected HLF cell lysate; 100 μl/well). Multiplex cytokine analysis was performed on 100 μl of culture fluid. Supernatant fluids were evaluated for 9 cytokines and chemokines, and for granzyme B, a marker for cytolytic activity.

2.2.3. Cytokine bead reagents

Cytokine bead reagents were generated by conjugating various monoclonal capture antibodies to Luminex fluorescent beads according to the manufacturer's instructions (BioRad). Bead reagent sets were validated and optimized against known concentrations of the target cytokines. The reagents used for each cytokine are available on-line (Appendix 1).

Supplementary material related to this article found, in the online version, at http://dx.doi.org/10.1016/j.jcv.2013.01.015.

2.3. Data analysis

Univariate analysis was performed to examine differences between gpELISA seropositive and seronegative two-dose vaccinated HCP, taking into account age, sex, race, country of origin, time since vaccination, and whether HCP had direct contact with patients (used as a surrogate for exposure to VZV). Fisher's exact test was used to compare proportions, and Wilkoxon Rank Sum test was used to compare differences between medians. Seroprevalence rates were calculated based on gpELISA and wcELISA results. Ninety-five percent confidence intervals (95% CI) for single proportions were calculated using continuity-corrected score methods.[25] Results obtained by wcELISA were compared with those obtained by gpELISA to estimate sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV). A one-way analysis of variance (ANOVA) was performed to compare antibody avidity results by vaccination status. All analyses were performed using SAS, version 9.2.

3. Results

3.1. Serology

Among the 101 HCP who received two doses of varicella vaccine, 12 (11.9%; 95% CI 6.6, 20.2) were seronegative by gpELISA and 42 (41.6%; 95% CI 31.9, 51.8) by wcELISA. Recently vaccinated HCP (≤5 years ago) were almost twice as likely to be VZV seropositive by gpELISA as persons vaccinated >5 years ago. HCP with direct patient contact were more than three times likelier to be VZV seropositive by gpELISA. However, no statistically significant associations between serologic results and characteristics potentially affecting duration of immunity were identified (Table 1).

Sensitivity for wcELISA was 66.3% (95% CI 55.4, 75.8), negative predictive value (NPV) was 28.6% (95% CI 16.2, 44.8), and specificity and positive predictive value (PPV) were 100%. As such, 30 (33.7%) specimens that were seropositive by gpELISA were falsely negative by wcELISA.

Six HCP that were seronegative after the second dose of varicella vaccine received a third dose and all had serology results performed pre- and post-third dose. Additionally, one volunteer from the blood donation program who was also a two-dose seronegative vaccine had sera available for laboratory analysis post-receipt of the third dose of varicella vaccine. All seven subjects produced IgG