Serologic Analysis of the IgG Antibody Response in Children With Varicella Zoster Virus Wild-type Infection and Vaccination

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Introduction: In contrast to varicella zoster virus (VZV) primary infection, VZV vaccination does not seem to provide lifelong immunity against varicella. Because more people get vaccinated every year, the development of sensitive serological test systems for the detection of protective anti-VZV IgG will become important in the future.

Methods: We have previously developed a novel VZV line assay based on 5 different recombinant VZV antigens. In this study, we compared this novel assay with a commercially available glycoprotein enzyme immunoassay (RIDASCREEN VZV IgG) in detecting anti-VZV IgG of children with previous varicella infection and VZV vaccination.

Results: One hundred twenty-five children were included in this study, 72 with a history of varicella infection and 53 with VZV vaccination. Both assays detected anti-VZV IgG antibodies in both study groups with similar sensitivities. The VZV line assay revealed striking differences in the anti-VZV IgG composition against the VZV open reading frames, 4, 14 and 49, between both study groups, indicating that wild-type varicella infection causes a more diverse immune response against VZV than does vaccination. The exploitation of these results enabled the discrimination of both study groups with a sensitivity of 0.93 and a specificity of 0.83, indicating that the serologic differentiation of children with previous varicella infection and VZV vaccination might be possible.

Conclusion: The VZV line assay enables the detection of anti-VZV IgG with sensitivities comparable to glycoprotein enzyme immunoassays and might be suitable for the serologic discrimination between children with a history of varicella infection and VZV vaccination.

Key Words: varicella, chickenpox, vaccination, serology, specific antigens

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The varicella zoster virus (VZV) is a member of the neurotropic α-herpesvirus subfamily of the Herpesviridae. Upon primary infection, VZV causes varicella (chickenpox) and then persists lifelong in ganglial cells. Later in life after reactivation from latency, VZV may cause herpes zoster (shingles) as secondary disease. Since 1995, a live attenuated vaccine against varicella has been available and officially recommended for all children since 2004 in Germany. Initially, given as a single dose, this recommendation was modified in favor of a 2-dose regimen in Germany by 2009.1–3 In contrast to VZV wild-type infection, which typically confers lifelong immunity, breakthrough infections can occur months to years after VZV immunization and are caused by wild-type virus as a result of vaccine failure.4 Vaccine failure can be divided into 2 types. Whereas primary failure is defined as nonmeasurable immune response on primary vaccination, secondary failure is due to diminishing immune responses over time.5,6 The latter is of particular health interest because it may result in an increased susceptibility later in life when the risk of severe complications can be greater than during childhood.

One major goal of VZV-specific laboratory testing is the measurement of immunologic markers that correlate with protection against varicella. In the prevaccination era, it has been generally accepted that the presence of detectable VZV-specific (IgG-) antibodies in immunocompetent individuals serves as an immune correlate of protection, indicating immunity toward varicella disease. Therefore, laboratory testing for the VZV immunostatus aimed for the detection of VZV-specific (IgG-) antibodies by any means, typically by enzyme immunoassays (EIAs) or enzyme-linked immunosorbent assays (ELISAs). In general, the latter assays can be divided into 2 groups by the nature of the presented VZV antigen: whole cell EIA/ELISA (wCEIA/wCELISA) formats use whole lysates of VZV-infected cells, whereas the apparently more sensitive glycoprotein EIA/ELISA (gpEIA/gpELISA) formats use purified VZV glycoproteins.7,8 Following the introduction of chickenpox vaccination, however, measuring VZV-specific IgG has proven to be difficult because antibody responses following immunization are approximately 10-fold lower than those achieved after natural infections.9–12 As a result, in the postvaccination era, there is an urgent need for the development of sensitive anti-VZV IgG assays and the redefinition of immune correlates of protection against varicella infection.

The goal of our study was to compare the anti-VZV IgG immune responses in children with a history of natural varicella infection and in children with previous VZV vaccination. Whereas the fluorescent antibody to membrane antigen test for the detection of antibodies specific to viral envelope glycoproteins has been considered the serological gold standard for determining immunity to VZV,10 the procedure is labor-intensive and needs considerable expertise in handling VZV. In addition, there is insufficient experience in storages of fluorescent antibody to membrane antigen and the results can only be obtained within several days. Due to logistic reasons, we therefore decided to use the commercially available RIDASCREEN VZV IgG gpEIA (R-Biopharm, Darmstadt, Germany) with a specificity between 0.88 and 0.98 and sensitivity between 0.94 and 1.09 and the just recently developed VZV line assay.11 Both test systems detected anti-VZV IgG antibodies with similar sensitivities. The VZV line assay, however, revealed striking differences in the anti-VZV IgG composition against the VZV open reading frames, 4, 14 and 49, indicating that wild-type varicella infection does not only cause a stronger but also a more diverse antibody response against VZV.
than vaccination. Our results indicate for the first time that the serological differentiation between children with previous varicella infection and chickenpox vaccination is generally possible. Future studies on VZV antigen-specific IgG antibodies might help to identify novel immune correlates of protection against varicella infection in the postvaccination era.

MATERIALS AND METHODS

Ethical approval for this study was obtained as well as fully informed consent from all legal guardians. The study was performed under consideration of the Declaration of Helsinki with amendments from Tokyo 1975, Edinburgh 2000 and Seoul 2008.

Patient Cohort and Sample Collection

Patients for this study were recruited over a period of 9 months (June 2010 to March 2011) at the HELIOS Children’s Hospital Wuppertal. All patients up to an age of 16 years who presented at the emergency department for any reason and had either a positive history for VZV wild-type infection or vaccination were included in this study, except those with primary or secondary immunodeficiency. Epidemiological parameters were obtained using a standardized questionnaire. A blood sample was taken, immediately spun down and the serum was stored at −20°C for later processing. For each patient, the primary care physician was contacted and the date of VZV wild-type infection or alternatively the date of the last VZV vaccination was recorded as documented in patient’s international certificate of vaccination. In the case of uncertainty regarding VZV vaccination of wild-type infection, patients were excluded from the study.

Laboratory Testing of VZV Immune Status

The VZV line assay contains the following 5 VZV gene products: open reading frame (ORF)1, coding for a tail-anchored membrane protein, ORF4 and ORF49 both coding for tegument proteins, ORF14 coding for glycoprotein C (gC) and ORF68 coding for glycoprotein E.

The test was performed following the general instruction manual for recomLine assays (Mikrogen, Neuried, Germany) using the reagents supplied in the kit. In brief, serum samples were applied at 1:100 dilutions and incubated together with the nitrocellulose test strips for 1 hour at room temperature. After washing, a second incubation of 45 minutes with peroxidase-labeled secondary antibody (anti human IgG) was performed. Strips were stained for about 8 minutes using tetramethylbenzidine after 3 additional washing steps of 5 minutes each. The scanner OpticPro S28 (Gwangju, Korea) and recomScan software (Mikrogen) were used according to the manufacturer’s instructions. As control, the RIDASCREEN VZV IgG EIA (R-Biopharm AG)3 and the Calbiotech VZV IgG ELISA (Calbiotech, Spring Valley, CA)4 were performed according to the manufacturer’s instructions. For both tests, borderline results were considered to be positive.

Statistical Analysis

All data are presented as the mean ± standard deviation (SD). Testing for significant differences between groups was performed using unpaired t test for values with Gaussian distribution and the Mann-Whitney test for values without Gaussian distribution. The Kolmogorov-Smirnov test was used to rule out non-Gaussian distribution. Values for \( P < 0.05 \) were considered statistically significant. All analyses were performed using GraphPad version 5.01 (La Jolla, CA).

RESULTS

One hundred twenty-nine patients were included in this study, 72 with previous clinical diagnosis of VZV wild-type infection and 53 with vaccination at least 3 months prior to presentation at the hospital as documented by the primary care physician. Four patients were excluded because they received vaccination even though they were diagnosed with VZV wild-type infection before. Epidemiological characteristics of these patients are provided in the Table (Supplemental Digital Content 1, http://links.lww.com/INF/B306).

Comparison of VZV Line Assay With RIDASCREEN gpEIA and Calbiotech wcELISA

Sixty-four patients (88.9%) with wild-type infection showed positive results on the VZV line assay, defined as being positive for antibodies against ORF68, and 66 patients (91.7%) were positive on the RIDASCREEN EIA (1 borderline positive). All patients positive on the VZV line assay were also positive on the RIDASCREEN EIA. Of the remaining 2 patients positive on the RIDASCREEN, one was negative for any antibodies on the VZV line whereas the other was negative for antibodies against ORF68 but positive for antibodies against ORF49 and ORF4. Because varicella diagnosis was exclusively based on clinical features, patients negative for VZV antibodies in both tests were considered to never have had VZV infection and were excluded from further analysis. Diagnostic certainty of clinical assessment for varicella infection was therefore 91.7%.

Fifty-three patients were vaccinated according to the general practitioner at least 3 months before presentation at the hospital. Data on the number of doses per patient were only available for 22 patients (41.5%). Among these, 14 received one and 8 two doses. All patients received vaccinations based on the Oka strain with at least 1011 plaque forming units. Among these, 42 patients showed a positive and one a borderline positive result on the VZV line assay (79.3% and 1.9%, respectively), which were all positive on the RIDASCREEN EIA (2 borderline positive). From the remaining 10 patients tested negative on the VZV line, 8 (80%) were also negative on the RIDASCREEN EIA whereas 2 (20%) patients were positive. Thus, vaccination failure was calculated to be between 15% and 19%. The exact number of doses per patient tested negative for VZV IgG was only available for 3 patients, 2 received only 1 dose whereas 1 patient received 2 doses of VZV vaccine. Based on the RIDASCREEN EIA data, we calculated the overall sensitivity and specificity of the VZV line to be 0.96 and 1.0, respectively.

A subset of patients with sufficient material was also tested with the wcELISA (Calbiotech), 53 patients post-VZV wild-type infection and 27 postvaccination. Whereas all patients post-VZV wild-type and 26 postvaccination were tested positive both on the RIDASCREEN EIA and VZV line, only 39 patients post-wild-type infection (9 borderline) and 6 postvaccination were positive on the wcELISA (Calbiotech) (Fig. 1A and 1B), yielding a sensitivity of 0.74 and 0.23, respectively.

Epidemiological characteristics did not differ between vaccinated versus patients with wild-type infection except for age and time interval between date of immunization and presentation. Both were significantly longer in patients with wild-type infection (Supplemental Digital Content 1, http://links.lww.com/INF/B306).

Antibody Titers Tend to Be Higher After Wild-type Infection

We then analyzed the antibody responses to the 5 single VZV antigens included in the VZV line assay. In patients with VZV vaccination (n = 53), 42 were positive for antibodies against
the ORF68 (79.3%), 6 patients were positive for ORF49 (11.3%), 2 for ORF14 (3.8%), 1 for ORF4 (1.9%) and none for ORF1. In patients with VZV wild-type infection (n = 66), antibodies against ORF68 were detectable in 64 (96.9%), against ORF49 in 52 (78.8%), against ORF14 in 22 (33.3%), against ORF4 in 18 (27.3%) and against ORF1 in 2 (3.0%) (Fig. 1C). We then assessed antibody responses against different antigens using a semiquantitative approach by comparing signal intensities on the VZV line assay to control sera. As expected, mean antibody responses tended to be higher in patients after wild-type VZV infection when compared with vaccination (Fig. 2) even though the time interval between immunization and vaccination/infection was significantly longer in children with wild-type infection (Supplemental Digital Content 1, http://links.lww.com/INF/B306). Importantly, children immunized with VZV vaccine produced significantly less antibodies against ORF14 coding for gC than children with wild-type infection (Fig. 2C). Subgroup analysis in children with known numbers of VZV vaccination (n = 22) revealed slightly but not significantly lower antibody titers against ORF68 in patients who received 1 (mean, 1.7; 95% confidence interval, 0.5–3.0) when compared with those who received 2 doses (mean, 2.1; 95% confidence interval, 0.3–3.9).

**VZV Line Assay Allows Discrimination Between Wild-type Infection and Vaccination**

Next, we calculated the sensitivity and specificity for different antibody patterns to specifically differentiate between immunization following wild-type infection versus vaccination. All patients tested positive on the RIDASCREEN EIA (n = 66) were included in this analysis. As depicted in Table 1, a positive antibody response against ORF49 in addition to ORF68 had a sensitivity of 0.78 and a specificity of 0.89 to identify wild-type infection. The combination of antibodies against ORF49, ORF14 or both in addition to ORF68 increased the sensitivity to 0.88 with a slightly lower specificity of 0.85. Finally, the inclusion of antibodies against ORF4 yielded a sensitivity of 0.93 with a specificity of 0.83 (Table 1).

**DISCUSSION**

Varicella is generally considered a benign childhood disease. However, severe disease outcomes can occur in the newborn, elderly or immunocompromised hosts.\(^\text{15}\) Since 1995, a life attenuated vaccine against varicella has been available and is currently

![FIGURE 1. Comparison of antibody responses in patients with previous varicella infection versus chickenpox vaccination. Antibody responses against VZV antigens as obtained by (A) gpEIA RIDASCREEN, (B) wcELISA (Calbiotech) and (C) VZV line assay. All patients with varicella infection positive on the RIDASCREEN EIA and all vaccines were included in this analysis.](image)

**TABLE 1. Sensitivity/Specificity of the VZV Line Assay in the Discrimination of a Previous Varicella Infection From a Chickenpox Vaccination by Antigen-specific IgG Combinations**

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<th>Varicella Vaccination Discrimination</th>
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<tr>
<td>ORF68 positive and ORF49 positive</td>
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<tr>
<td>ORF68 positive and ORF49 negative</td>
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<tr>
<td>Sensitivity of ORF49</td>
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<td>Specificity of ORF49</td>
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<td>ORF68 positive and ORF49 or ORF14 positive</td>
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<td>ORF68 positive and ORF49 and ORF14 negative</td>
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<td>Sensitivity of ORF49/ORF14</td>
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<td>Specificity of ORF49/ORF14</td>
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<td>ORF68 positive and ORF4 or ORF14 or ORF4 positive</td>
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<td>ORF68 positive and ORF49 and ORF14 and ORF4 negative</td>
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<td>Sensitivity of ORF49/ORF14/ORF4</td>
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<td>Specificity of ORF49/ORF14/ORF4</td>
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officially recommended in many industrialized countries. However,
in contrast to wild-type infection, the vaccine probably does not
confer lifelong immunity; thus, booster immunization may be nec-
essary in the adult population to prevent varicella outbreaks. Unfor-
tunately, there is currently no reliable serodiagnostic test available
that can differentiate between VZV immunizations due to vaccina-
tion versus wild-type infection and can therefore identify patients
requiring booster vaccinations. In this study, we provide clinical
data on a new recombinant VZV line assay and its reliability in
discriminating between VZV vaccination and wild-type infection.

Diagnostic certainty of clinical diagnosis for acute varicella
infection was 92% in our study population, which is high when
compared with other recent studies from countries with earlier
dates of vaccine introduction than in Germany. Leung et al. for
example reported only confirmation in 53 (57%) of 93 suspected
varicella cases reflecting the decrease in the positive predictive
value of clinical diagnosis with increasing rates of vaccination.
Consequently, diagnosis of acute varicella infection will rely more
and more on laboratory (ie, polymerase chain reaction) testing in
the future.

As expected, individuals after vaccination in general had
lower antibody titers when compared with wild-type infection even
though the time interval between VZV exposure and blood sam-
pling was significantly higher in children with wild-type infection.
This is in-line with previous studies on the currently used vaccine
strain and most likely reflects a stronger immune response against
the wild-type VZV strain. One might argue at this point that this
difference could also be the result of the possibly higher number of
participants having received only one dose of vaccine. However,
comparing antibody titers against ORF68 in patients with known
numbers of vaccine doses did not reveal a significant difference.
Moreover, the mean antibody titers in patients with known 2 doses
of VZV vaccine (mean 2.1) was even lower when compared with all
vaccinated patients (mean 3.0). Thus, other factors seem to be more
important for the magnitude of the immune response.

Importantly, we also observed for the first time a lower
diversity in the antibody response to VZV in vaccines when
compared with patients with wild-type infection. Moreover,
the lower antibody titers after vaccination against ORF14
coding for gC which supposedly plays an important role in viral
pathogenesis might be a partial explanation for the failure of one
VZV vaccination against breakthrough varicella. This hypothesis
is in-line with observations made in herpes simplex virus type 1
infection where anti-gC antibodies have been found to neutralize the
virus. Further analysis also showed that the observed differences
in antibody patterns might be used to differentiate between
patients immunized with the vaccine VZV versus wild-type strain.
Sensitivity and specificity for this differentiation were calculated to
be 0.76-0.91 and 0.83-0.89, respectively. The best performance,
providing a good balance between sensitivity and specificity, was
obtained when using a combination of antibody responses against
the 3 antigens ORF49, 14 and 4. To our knowledge, no currently
used serodiagnostic test allows a similar differentiation of the
origin of the VZV immune status in any kind of juvenile patients.
In addition, the VZV line assay is relatively fast (2.5 hours) and
easy to perform and to interpret. It seems to be a promising tool for
differentiation between VZV immunity due to wild-type infection
versus vaccination at least in children and thus might help to
identify children requiring a booster vaccination to keep up their
immunization status. Moreover, it might facilitate clinical and
epidemiological studies trying to elucidate the relative importance
of individual protein-specific antibodies in the prevention of
reinfection. Further studies are needed to assess whether these
results are also applicable in adult patients.

FIGURE 2. Relative antibody titers in patients with previous varicella infection versus chickenpox vaccination. Antibody titers
against VZV antigens as obtained by (A) gpEIA (RIDASCREEN), (B) wcELISA (Calbiotech) and (C) VZV line assay. All patients
with varicella infection positive on the RIDASCREEN gpEIA and all vaccines were included in this analysis. Red arrows indicate
the threshold when the test is considered positive according to the manufacturer’s recommendations. Data are expressed as
the mean ± 2 standard deviations (SD). Significance testing was performed using unpaired Student’s t test and values for
P < 0.05 were considered to be statistically significant.
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REFERENCES