Primary Vaccine Failure after 1 Dose of Varicella Vaccine in Healthy Children

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Universal immunization of young children with 1 dose of varicella vaccine was recommended in the United States in 1995, and it has significantly decreased the incidence of chickenpox. Outbreaks of varicella, however, are reported among vaccinated children. Although vaccine effectiveness has usually been ~85%, rates as low as 44% have been observed. Whether this is from primary or secondary vaccine failure—or both—is unclear. We tested serum samples from 148 healthy children immunized against varicella in New York, Tennessee, and California to determine their seroconversion rates, before and after 1 dose of Merck/Oka varicella vaccine. The median age at vaccination was 12.5 months; postvaccination serum samples were obtained on average 4 months later. Serum was tested for antibodies against varicella-zoster virus (VZV) by use of the previously validated sensitive and specific fluorescent antibody to membrane antigen (FAMA) assay. Of 148 healthy child vaccinees, 113 (76%) seroconverted, and 24% had no detectable VZV FAMA antibodies. Our data contrast with reported seroconversion rates of 86%–96% by other VZV antibody tests and suggest that many cases of varicella in immunized children are due to primary vaccine failure. A second dose of varicella vaccine is expected to increase seroconversion rates and vaccine effectiveness.

Varicella-zoster virus (VZV) is an alphaherpesvirus that causes chickenpox during primary infection and zoster with recurrence. Immunity to VZV consists of both VZV-specific antibodies and CD4 and CD8 T cells [1]. Both natural VZV infection and vaccination with live attenuated Oka virus induce humoral and cell-mediated responses that appear to be long lasting, although the persistence of these responses after vaccination has been evaluated only in the setting of continued circulation of wild-type VZV [2–4].

The primary modality by which VZV immunity, whether elicited by natural infection or vaccination, is determined is by detection of VZV antibodies. Methods of evaluating cell-mediated immunity are available but are expensive and impractical for use in children. Measuring VZV IgG antibodies is challenging because many available serologic techniques lack sensitivity and specificity [5, 6]. Prelicensure studies of the varicella vaccine used the glycoprotein ELISA (gpELISA), for which the threshold for seroconversion was initially 0.625 gpELISA units/mL [7, 8]. In later studies, a response of ≥5 gpELISA units/mL was defined as an approximate correlate of protection against varicella. Using this value, reported seroconversion rates have ranged from 86% to 96% [4, 9, 10].

The fluorescent antibody to membrane antigen (FAMA) assay, developed in 1974 and used consistently for several decades to assess immunity to VZV, is a highly sensitive and specific assay for VZV antibodies [6, 11, 12]. This assay is validated by demonstrating the ab-
sence of detectable antibodies in individuals before they develop varicella, which then appear after contracting the disease. In addition, antibodies to VZV measured by the FAMA assay correlate with protection from varicella after household exposure. The FAMA assay was used to evaluate the immunogenicity of varicella immunization in children with acute lymphoblastic leukemia in remission and as an immune correlate of efficacy, studies which subsequently led to widespread testing of the varicella vaccine in healthy US children [13].

In an effort to determine whether recent reports of breakthrough varicella [14–24] might represent higher-than-expected rates of primary vaccine failure, we studied VZV antibody titers using the FAMA assay in 148 immunized children before and after receiving 1 dose of vaccine. Primary vaccine failure is defined as failure to mount a protective immune response after a dose of vaccine, and secondary vaccine failure is defined as a gradual loss of immunity after an initial immune response over a period of years after vaccination (waning immunity).

METHODS

Patients. We studied serum from 148 healthy children receiving primary varicella immunization between the years 1998 and 2003. The pre- and postvaccination titers of antibody to VZV were determined using the FAMA assay. Serum samples were collected from pediatric practices at the Vanderbilt University Medical Center (Nashville, TN), the Palo Alto Medical Foundation in conjunction with Stanford University Medical Center (Palo Alto, CA), and the Columbia University Medical Center (New York, NY) (table 1). All children were immunized with 0.5 mL of the Merck/Oka strain of varicella vaccine, which contains a minimum of 1350 pfu/dose. The dates of birth, serum collection, and vaccine administration for all children were documented. Patient identifiers were unknown to the authors. Institutional Review Boards at Columbia University, Vanderbilt University, Stanford University, and the Palo Alto Medical Foundation approved this study.

FAMA technique. Serum samples were diluted serially in 2-fold aliquots (from 1:2 to 1:128 of each sample) in 96-well polystyrene plates, to which unfixed VZV-infected human embryonic lung fibroblasts were then added [11, 25]. VZV antibodies in patient serum bind to glycoprotein antigens on the surface of unfixed infected live cells. It is hypothesized that using cells in this manner does not alter the conformation of surface VZV antigens, which is responsible for the accuracy of the assay. Subsequent addition of fluorescein-conjugated goat anti–human IgG specifically interacts with antigen-antibody complexes present on the membrane of infected cells. Fluorescence around the perimeter of cells demonstrates the presence of VZV antibodies, indicating a positive result. Lack of membrane fluorescence indicates a negative result. The end-point titer is the highest dilution that yields a positive response. In most fields, at least 6–10 cells are visible, with most showing a positive reaction in serum from protected individuals. Fluorescence at a 1:4 dilution of test serum and higher is considered positive. There are no positive cells when serum from a susceptible person is tested. PBS (pH 7.4) without serum is used as a negative control. All specimens were analyzed by the microscopist in a blinded fashion.

Statistics. The seroconversion rates between the 3 groups were compared using the χ2 test with continuity correction. The z test for proportions was used for comparison of FAMA data from this study with gpELISA data from previous studies. A Kruskal-Wallis rank-sum test was used to analyze the geometric mean titer (GMT) of each of the groups with and without results for seronegative subjects. This test ranks the values and does not make distribution assumptions. Analysis of variance was not done because there is a normality assumption and values were skewed even with log transformation. For pairwise comparisons between the GMT of each group, a Wilcoxon rank-sum test with Bonferroni adjustment was applied.

RESULTS

The analysis of serum from the 148 children by FAMA assay showed that all were seronegative before vaccination. The median age of children was 12.5 months (range, 11 months–7 years). Among the 3 groups, only 3 patients were <12 months old and all were seronegative before vaccination. Serum was obtained just before and on average 4 months after immunization (range, 4 weeks–11 months). The comparison of pre- and postvaccination serum specimens demonstrated an overall seroconversion rate by FAMA assay of 76% (113/148) (95% confidence interval [CI], 68.6%–82.9%), indicating a primary vaccine failure rate of 24% (figure 1). Of the 35 children from New York, 30 (86%) were positive by FAMA assay after a median interval of 3 months since vaccination. In the group from Tennessee, 76% (61/80) of children were FAMA positive after a median interval of 3.1 months, and, in the group from California, 67% (22/33) of children were FAMA positive after a median interval of 6 months. Despite differences in the average interval between vac-

Table 1. Comparison of patients from each of the 3 sites.

<table>
<thead>
<tr>
<th>Location</th>
<th>New York (n = 35)</th>
<th>Tennessee (n = 80)</th>
<th>California (n = 33)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean (range)</td>
<td>16.7 (12–84)</td>
<td>16.2 (11.2–51.2)</td>
<td>12 (12–13)</td>
</tr>
<tr>
<td>Median</td>
<td>12.5</td>
<td>13.4</td>
<td>12</td>
</tr>
<tr>
<td>Months since vaccination</td>
<td>2.7 (1.5–6)</td>
<td>4.1 (1.5–11)</td>
<td>5.7 (1–7)</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>3.1</td>
<td>6</td>
</tr>
</tbody>
</table>

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Seroconversion rates among the 3 groups were not significantly different ($P = .29$, $\chi^2$ test).

Table 2. Aggregate distribution of fluorescent antibody to membrane antigen (FAMA) titer among the 3 study groups, with corresponding geometric mean titers (GMTs).

<table>
<thead>
<tr>
<th>Group (mean time after vaccine)</th>
<th>Location</th>
<th>No. (%) with FAMA titer of</th>
<th>GMT (CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;4</td>
<td>4</td>
</tr>
<tr>
<td>3 months ($n = 35$) New York</td>
<td></td>
<td>5 (14)</td>
<td>3 (9)</td>
</tr>
<tr>
<td>4 months ($n = 80$) Tennessee</td>
<td></td>
<td>19 (24)</td>
<td>8 (10)</td>
</tr>
<tr>
<td>6 months ($n = 33$) California</td>
<td></td>
<td>11 (33)</td>
<td>3 (9)</td>
</tr>
</tbody>
</table>

NOTE. CI, confidence interval; SN, seronegative.

DISCUSSION

Primary vaccine failure—24% in the present study—is evidently common in healthy young children after a single dose of monovalent varicella vaccine. The 76% VZV seroconversion rate, determined with the FAMA assay, is significantly lower than rates obtained with the gpELISA assay, which is commonly used because it is easy to conduct and can be automated [7]. Most reported gpELISA seroconversion rates have been >95% when measured 6 weeks after 1 dose of monovalent varicella vaccine and using a value of ≥5 gpELISA units/mL to indicate a positive response [9, 10]. Only one study found a lower rate, of 86% [4]. These high gpELISA-determined seroconversion rates do not correspond well with the demonstrated effectiveness of varicella vaccine, which is 80%–85% [25–27]. The current observations made using the FAMA assay to determine immunity are consistent with epidemiologic experience and indicate that most breakthrough cases of varicella after a single dose of vaccine can be attributed to primary vaccine failure.

The FAMA assay was initially validated as an indicator of immunity to varicella in 1974 [11] and was the first serologic assay that made it possible to determine susceptibility to varicella. As such, the FAMA assay is considered the gold standard to which the sensitivity and specificity of other assays of varicella immunity are compared [25]. A seroconversion rate of 100% has been found by the FAMA assay to occur in >1000 children and adults after developing clinical varicella [11, 12, 28–32]. Because varicella is highly contagious [33], protection after household exposure was used to evaluate immunity by means of the FAMA assay. A positive FAMA titer (≥1:4) predicts protection from infection after household exposure to VZV with rare exceptions [34, 35]. The attack rate of chickenpox in FAMA-positive individuals after household exposure in an aggregate of multiple studies is 2% (3/126), whereas that in FAMA-negative individuals is 59% (37/63) [11, 12, 34, 36–38]. Among the FAMA-negative population, the attack rate in unvaccinated individuals with no history of varicella is 77% (20/26) [11, 36, 37]. The attack rate in FAMA-negative individuals with no history of varicella is 77% (20/26) [11, 36, 37].
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We have been unable to compare directly gpELISA and FAMA titers in vaccinated children because we do not have access to the reagents to perform the gpELISA. A relevant comparison, however, performed some years ago, was done in adults. In a series of serum samples from 17 adults who developed varicella, all were FAMA-negative before becoming ill [12]. When the same serum samples were tested by gpELISA in another laboratory, 12% were found to exhibit titers compatible with immunity to varicella (≥5 gpELISA units/mL) (A.A.G. and S.P.S., unpublished data). In a published series of serum samples from vaccinees tested by other investigators, only 60% of 41 postvaccine titers judged to be moderately positive by gpELISA were also found to be positive by FAMA assay. Of serum samples from 16 patients with high postimmunization gpELISA titers, only 94% were FAMA positive [7]. These results are consistent with the idea that, when judged by the FAMA comparator, the gpELISA overestimates immunity to varicella. Consideration of the value of the gpELISA assay has been made more difficult because the threshold used for considering the test to be read as positive has varied from 0.625 to ≥5 gpELISA units/mL in different publications [4, 7–10, 42]. A published correlation between neutralization of VZV and gpELISA used 0.3 units/mL as a negative value and 0.625 units/mL as a positive value [8]. The positive value of 0.625 units/mL, however, has now been abandoned and does not predict immunity. The earlier validation of the gpELISA using a VZV neutralization assay thus cannot be relied on; neutralization tests for VZV are difficult to perform [25].

Numerous outbreaks of varicella have occurred in vaccinated populations in the United States since the vaccine was licensed, indicating that protection from varicella is not as effective as was originally expected. Since 1997, 18 outbreaks have been reported [14–25]. Many other outbreaks of varicella have undoubtedly not been reported. Vaccine effectiveness in reported outbreaks ranged from 44% to 100%, with an average of 79% [25]. In only 1 of 18 outbreaks was effectiveness >90%, despite seroconversion rates being as high as 96% by gpELISA. Protection in the setting of an exposure is thus significantly lower than the gpELISA seroconversion data would lead one to expect.

The most plausible explanations for the observed degree of vaccine failure after 1 dose of vaccine are that immunized children either do not develop humoral immunity to VZV at all or that there is an initial immune "burst" of immunity that is enough to generate a positive gpELISA result but is inadequate to generate a sustained memory T cell response. This concept is consistent with data from a case-control study in which vaccine effectiveness was 97% though the first year after vaccination but fell to 86% in the second year. Although there was some small decline of effectiveness in subsequent years, these declines were not statistically significant [26].

The present study suggests that VZV antibody titers may decrease during the first year after vaccination. There may be a rapid initial fall in VZV GMTs in a subset of patients. Although there is some overlap in ages of children in different locales, children tested 3 months after vaccination had higher median FAMA GMTs (1:12.4) than did those tested 6 months after vaccination (1:4.9). Further studies should be undertaken to see whether these findings can be confirmed. Whether or not a decline continues beyond 6 months, moreover, remains to be demonstrated. Secondary vaccine failure, defined as a demonstrable immune response to vaccination that gradually wanes over a long period of time, cannot explain the high percentage of children who lack FAMA-detectable VZV antibodies in the early weeks and months after immunization. It is possible that secondary vaccine failure occurs, but it has not been consistently demonstrated [26, 40]. Our present data, however, do not directly address the issue of whether or not secondary vaccine failure occurs. However, because primary vaccine failure occurs in 24% of children after a single dose of vaccine, if secondary vaccine failure does occur, it is likely to be less significant than primary vaccine failure given the recognized vaccine effectiveness of 85%. It should be noted that other published data showing a lower rate of primary immune failure by use of the FAMA assay did not use our exact procedure for the FAMA test. In the assay
used by Johnson et al. [43], VZV-infected cells used as the anti-
gen were dried on slides. Our assay uses live, unfixed cells, pre-
sumably preserving the protein conformation of VZV glycopro-
teins on the cell surface [11].

It is conceivable that improper storage of the vaccine explains
the relatively high rate of primary vaccine failure found in the
present study. Low FAMA seroconversion rates, however, were
detected in 3 different practice settings. It seems unlikely that all
3 would have allowed the vaccine to deteriorate. Still, the overall
number of patients investigated is not large, and the number of
sites, although multiple, may not be totally reflective of the uni-
verse of sites at which vaccination is done. A larger study of
vaccines in widely distributed sites should thus be conducted to
evaluate the rate of primary vaccine failure in the entire United
States.

Primary vaccine failure in just 10% of vaccinees after a single
dose could result in progressive accumulation of susceptible in-
dividuals over time and lead to an increased incidence of var-
cella in young adults. Such an increment is potentially danger-
ous. Approximately 4 million infants are vaccinated annually in
the United States. A primary vaccine failure rate of 10% would
thus lead to 400,000 vaccinated but susceptible infants every
year. Within 5 years, there would be 2 million vaccinated but
susceptible individuals. The present findings therefore strongly
support the use of a second dose of vaccine for all children with-
out a history of disease. We thus support the recent recommen-
dation made by the Advisory Committee on Immunization
Practices of the Centers for Disease Control and Prevention that
a second dose of varicella vaccine be given to all children rou-
tinely. The unacceptably high rate of primary vaccine failure
suggests that the interval between the first and second doses of
vaccine should be a matter of months rather than years. Correc-
tion of the problem of primary vaccine failure is thus urgent; if
done it will probably prevent not only the current phenomenon
of isolated outbreaks of breakthrough disease but a subsequent
epidemic of serious varicella in vaccinated but unprotected
adults.

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