Polymerase Chain Reaction and Restriction Fragment Length Polymorphism Analysis of Varicella-Zoster Virus Isolates from the United States and Other Parts of the World

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A polymerase chain reaction (PCR) assay that identifies and differentiates wild-type (wt) and vaccine strains of varicella-zoster virus (VZV) was used to determine if VZV strains with restriction fragment length polymorphisms resembling those of the Japanese Oka vaccine strain were present in the wt pool outside of Japan. Virus samples (n = 114) from patients with chickenpox and zoster from various parts of the United States and Australia were analyzed. The assay correctly identified 113 samples as wt strain. The 1 sample identified as Oka vaccine strain came from a child with leukemia who developed a vaccine-associated rash after receiving the live attenuated varicella vaccine. At this point, there is no evidence that wt strains resembling the vaccine are circulating outside of Japan. This indicates that this PCR assay can be utilized to distinguish rashes due to vaccine and wt VZV.

Live attenuated varicella vaccine was approved for use in the United States (US) on 17 March 1995 and became available in the US in May of that year. Studies are underway to more clearly define the risk of infrequently reported vaccine-associated complications. During this preliminary phase of vaccine introduction, there is still widespread circulation of the wild-type (wt) varicella virus in the general population. To clearly determine the etiology of adverse events attributed to vaccine, it is important to be able to differentiate wt virus from vaccine virus. We have previously described [1] a polymerase chain reaction (PCR) assay that identifies and differentiates varicella-zoster virus (VZV) wt and vaccine (Oka) strains by utilizing restriction fragment length polymorphisms (RFLPs) present in American and Japanese strains. It is important to note that these differences do not represent markers of attenuation of vaccine virus but rather the geographic clustering of these RFLPs.

In brief, the parental wt Oka strain, the attenuated Oka vaccine strain, and other Japanese wt isolates contain a BglI restriction site not present in most American strains. In addition, all wt strains tested also contain a PstI site, which is not present in Oka. We have classified American isolates as either wt (BglI−, PstI−), BglI wt (BglI+, PstI−), or Oka (BglI+, PstI+) on the basis of the presence (+) or absence (−) of these sites in amplified products. The American wt pool consists of both the wt and BglI wt patterns. The vaccine strain and some Japanese wt strains are represented by the Oka pattern.

Concern has been expressed that due to the ease of international travel, Japanese wt strains resembling Oka may be present in the North American VZV wt pool and may incorrectly be identified as vaccine strain by this assay. In order to address these concerns, we collected and amplified VZV isolates obtained from the US (northern and southern California, Rochester and New York City, NY), eastern and western Australia, and Japan prior to widespread use of the vaccine in the US. The goal of the current study was to determine whether the Oka RFLP pattern is present at this time in the American wt pool and in additional locations easily accessible to travelers from Japan (i.e., Australia).

Materials and Methods

Samples consisted of lesion swabs, infected cells, or extracted DNA from patients with varicella or zoster. The samples were collected from persons in the United States (northern and southern California, Rochester and New York, New York), eastern and western Australia, and Japan prior the introduction of varicella vaccine in the US (May 1995). Researchers in the PCR laboratory were blinded to the pertinent clinical histories associated with these samples.

PCR was done as previously described [1]. In brief, two sets of primer pairs were used to amplify a 222-bp fragment in gene 54 (primer pair Nla/Fok) and a 350-bp fragment in gene 38 (Pst I/A/B) of the VZV genome; the fragments asymmetrically straddle the BglI and PstI restriction sites described above. Thirty-five cycles of amplification were done at 94°C for 1 min, 55°C for 1 min, and 72°C for 2 min, followed by one cycle at 72°C for 5 min, using a 9600 series thermocycler (Perkin-Elmer, Foster City, CA).
Table 1. Algorithm used to interpret results of the VZV polymerase chain reaction assay.

<table>
<thead>
<tr>
<th>VZV DNA amplified</th>
<th>β-globin DNA amplified</th>
<th>222-bp product cuts with BglI</th>
<th>350-bp product cuts with PstI</th>
<th>VZV strain assignment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>ND</td>
<td>No</td>
<td>Yes</td>
<td>wt</td>
</tr>
<tr>
<td>Yes</td>
<td>ND</td>
<td>Yes</td>
<td>Yes</td>
<td>wt (BglI wt)</td>
</tr>
<tr>
<td>Yes</td>
<td>ND</td>
<td>No</td>
<td>Insufficient*</td>
<td>wt</td>
</tr>
<tr>
<td>Yes</td>
<td>ND</td>
<td>Yes</td>
<td>No</td>
<td>Oka</td>
</tr>
<tr>
<td>Yes</td>
<td>ND</td>
<td>Yes</td>
<td>Insufficient*</td>
<td>Indeterminate</td>
</tr>
<tr>
<td>No</td>
<td>Yes</td>
<td>NA</td>
<td>NA</td>
<td>Negative</td>
</tr>
<tr>
<td>No</td>
<td>No</td>
<td>NA</td>
<td>NA</td>
<td>Inadequate</td>
</tr>
</tbody>
</table>

NOTE. ND = not done, wt = wild-type strain, Oka = vaccine strain, NA = not applicable.

* Occasionally, there is inadequate amplification with Pst A/B primer pair, resulting in insufficient DNA to perform restriction analysis with PstI.

Amplified product was separated by gel electrophoresis in a 1.8% agarose gel and detected by UV light illumination after ethidium bromide staining. If amplified product was detected, the remaining amplicon was divided into two aliquots, which were subjected to restriction endonuclease digestion with BglI or PstI, respectively. The products of the two digestion reactions were precipitated (0.03 M NaC2H3O2, 100% ethanol), suspended in 8 μL of water, and separated by electrophoresis in a 4% agarose gel.

The pattern of the bands produced by the two digestions was compared with those of wt and vaccine strains, and strain was assigned on the basis of the algorithm shown in table 1. To verify the presence of amplifiable DNA in samples that failed to give positive results for VZV DNA, a second amplification reaction was done with a primer pair (Beta-globin Primers; Perkin-Elmer) that amplifies a 268-bp fragment of the human β-globin gene. This assay has a sensitivity of 97% and a specificity of 100% for samples from skin lesions from patients with varicella or zoster, compared with standard viral culture [1, 2].

Results

Of 114 samples obtained from patients outside of Japan, 113 exhibited one of the wt patterns (i.e., either wt or BglI wt) (table 2). The only non-Japanese sample showing the Oka configuration was obtained from a child with leukemia who developed a vaccine-associated rash 18 days after receiving live attenuated varicella vaccine as part of a study [3]. This isolate previously had been determined to be the Oka vaccine type, using the technique of multiple restriction endonucleases of VZV DNA extracted from virus isolated in tissue culture [4].

Discussion

This VZV PCR assay correctly identified all non-Japanese clinical varicella isolates from the US and Australia as wt strain (either wt or BglI wt). One isolate from northern California from a leukemic child with a varicella vaccine-associated rash was correctly identified as vaccine type (Oka). One of the Japanese varicella isolates exhibited the Oka restriction pattern.

This is to be expected given the fact that the RFLP pattern exhibited by Oka is not associated with attenuation and that the parental Oka strain attenuated by M. Takahashi, the developer of the chickenpox vaccine, was a circulating wt strain isolated from a child with chickenpox. It would be useful to analyze larger numbers of Japanese varicella isolates to better determine the frequency of this pattern in the Japanese wt pool. This would allow us to speculate on the probability of spread of this pattern to areas outside Japan.

About 22% (25/113) of the wt isolates tested exhibited the BglI wt pattern. The percentage of BglI wt isolated varied from none in eastern Australia to 64% in western Australia. We had previously reported a frequency of ~20% in isolates collected in the New York City area [1].

Although it is encouraging that there is no evidence of the Oka RFLP pattern in Australia or the West Coast of the US, it would be useful to study isolates from Hawaii and Southeast Asia to monitor the spread of the Oka pattern to areas outside of Japan. At present, given the lack of evidence of the Oka pattern outside of Japan, the PCR assay used in this study will

Table 2. Results of VZV polymerase chain reaction analysis of 123 samples from patients with varicella or zoster.

<table>
<thead>
<tr>
<th>Source (country)</th>
<th>n</th>
<th>wt</th>
<th>BglI wt</th>
<th>Oka</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northern California</td>
<td>17</td>
<td>15</td>
<td>1 (6)</td>
<td>1*</td>
</tr>
<tr>
<td>Southern California</td>
<td>25</td>
<td>22</td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td>Rochester, NY</td>
<td>25</td>
<td>22</td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td>New York City</td>
<td>26</td>
<td>15</td>
<td>11 (42)</td>
<td></td>
</tr>
<tr>
<td>Australia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eastern Australia</td>
<td>10</td>
<td>10</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Western Australia</td>
<td>11</td>
<td>4</td>
<td>7 (64)</td>
<td>—</td>
</tr>
<tr>
<td>Japan</td>
<td>9</td>
<td>8</td>
<td>8 (100)</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>123</td>
<td>88</td>
<td>33</td>
<td>2</td>
</tr>
</tbody>
</table>

NOTE. wt = wild-type strain, Oka = vaccine strain. Data are no. (% of wt isolates that were BglI wt).

* From child with leukemia who had vaccine-associated rash.
continue to be useful in distinguishing US and Australian wt strains from Oka. Use of this assay in the study of vaccine-related adverse events in other parts of the world should first be preceded by an analysis of the wt pool in those areas.

References