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The Parental Strain of the Attenuated Virus (vOka) Used in the Varicella Vaccine Is Circulating in China: Implications for Vaccine Surveillance

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Varicella-zoster virus (VZV) is the etiological agent of both varicella (chickenpox) and herpes zoster (shingles). Primary infection with VZV causes varicella, which usually occurs in childhood. Reactivation of VZV in previously infected individuals results in a herpes zoster that occurs most commonly in the elderly. In 1974, a live-attenuated VZV strain was established and subsequently licensed for vaccination in Japan (1). Comparison of the complete DNA sequences of the Oka vaccine strain (vOka) and the parental Oka strain (pOka) revealed there were only 42 nucleotide substitutions along the whole 125-kbp viral genome, 15 of which were located in the major transactivator ORF62 region (2). The pOka-like wild-type strain constitutes 20–30% of all VZV isolates in Japan (3). In contrast, it seems a rare genotype in other countries such as the U.S. and Australia (4,5). In this study we report a case of herpes zoster infected by the pOka-like wild-type strain in a Chinese woman.

A 27-year-old woman who was 8 months pregnant presented to the First Affiliated Hospital of Anhui Medical University (Hefei, China) with vesicle skin lesions. The diagnosis of zoster was made mainly based on the history and features of the rash. The zoster rashes had developed on the right side of her abdomen, and she had no post-herpetic neuralgia. VZV was isolated in human embryonic lung fibroblasts from vesicle fluid taken from the patient on day 2 after the onset of zoster and was identified by immunocytochemistry with antibodies to VZV glycoprotein E (Biodesign International®, Saco, Maine, USA). This strain is hereafter designated as ZW strain. Serologic assay by fluorescent antibody to the membrane antigen (FAMA) test revealed a specific VZV IgG antibody titer of 1:8 in the serum collected on day 2, and 1:256 in the second serum specimen obtained 16 days after the onset of symptoms.

Viral DNA was extracted from the fluid of the rash vesicles as well as from the viral culture as described (6). For the molecular discrimination between the wild-type and vaccine strains, several well-known single nucleotide polymorphisms (SNPs) in VZV ORF38 (SNP69349, PstI), ORF54 (SNP95241, BglII), and ORF62 (SNP106262, Smal; SNP107136, BssHII; SNP107252, Nael) (14,15) were characterized by restriction fragment length polymorphism (RFLP) analysis (Fig. 1). The following VZV strains were used as controls in the study: vOka (Merek, Whitehouse Station, N.J., USA), pOka, an American wild-type strain (MLS) (provided by Dr. Anne A. Gershon), and the Chinese wild-type strain 18 isolated from a patient with varicella in 2007 (6).

The results showed that the ZW strain is PstI– BglII– in ORF38 and ORF54, respectively (Figs. 1A, 1B), which is the same as pOka and vOka, but different from both the U.S. wild-type MLS and the Chinese wild-type strain 18. Mutations in the vOka genome that create Smal (14), Nael, and BssHII (15) sites in ORF62 were described. These sites have been shown to differentiate the various vaccine preparations including Oka Biken, Oka Merek, and Oka GSK from pOka and the other wild-type VZV strains (14,15). The Smal and Nael sites have been shown to be robust in distinguishing the U.S., U.K., and German wild-type viruses from the vaccine strain (9,15). However, the BssHII+ allele appears to be present in some U.K. genotype C strains (15). The ZW strain in this report shows Smal– Nael– BssHII– in SNP106262, 107252, and 107136, respectively, in ORF62, which demonstrates that the strain is the wild-type virus and not the vOka-like strain.

To further explore the difference between the ZW strain with other well characterized VZV genotypes, vaccine strain associated markers in ORF1 (SNPs 560, 703, 763), ORF22 (SNP 38036), ORF31 (SNPs 58595, 59287), ORF38 (SNP 69349), and ORF62 (SNPs 105310, 105356, 105544, 105705, 105706, 106262, 107136, 107252, 107599, 108111, 108838) were compared to those of reference strains representing different genotypes, including Oka Biken, Oka Merek, and Oka GSK from pOka and the other wild-type VZV strains (14,15). The ZW strain in this report shows Smal– Nael– BssHII– in SNP106262, 107252, and 107136, respectively, in ORF62, which demonstrates that the strain is the wild-type virus and not the vOka-like strain.

Since 1998, the varicella vaccine has been distributed in China, and varicella became a reportable disease in China in 2005. With the introduction of the varicella vaccination, differentiation of infections with the vaccine strain from those infected with the wild-type VZV has become important epidemiologically and can be achieved using molecular genotyping methods. The SNPs in ORFs 38, 54, and 62 have commonly been used in vaccine and epidemiological studies. In the U.S. and Europe the majority of wild-type VZV strains were PstI– in ORF38 (7–10). Therefore, this SNP may be used for differentiating the vaccine strain from wild-type

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strains. However, the dominant VZV strains in Asia, including in China and India, were PstI+ in ORF38 except for those in Japan of which more than 20% of the wild-type VZV strains are PstI– in ORF38. The PstI– wild-type strain described in this article is the first one reported in Asia outside Japan, although such strains were found in the U.S. and Australia with an incidence of <10%. The BglI restriction site in ORF54 could be another marker for differentiating vOka from wild-type VZV because vOka is BglI+, whereas most wild-type isolates in the U.S. and Europe are BglI–. However, this marker does not distinguish vOka from wild-type strains in other parts of the world because the BglI+ wild-type strain is predominant in many countries in Asia, Western Australia, and equatorial Africa.

It was surprising to us that the isolate showed the pOka genotype because this is not a common genotype of VZV isolates in China. The patient history was retrospectively investigated, and neither the patient nor her parents have a travel history outside China. Her family has been living in the suburb of Hefei (capital of Anhui Province, in the middle of eastern China) for at least three generations. It is conceivable that the strain with the pOka genotype might have been transmitted from Japan. This is not only because Japan has the most pOka strains circulating throughout the world, but also because of the long historic relationship between China and Japan, including the invasion of China by Japanese troops for over 8 years during World War II.

Viral genotyping by RFLP and sequencing analysis of ORFs 1, 22, 31, 38, 54, and 62 indicated ZW strain is a PstI– wild-type strain, which is similar to the pOka strain. Thus far we have isolated a total of 50 wild-type strains in China, and 49 of them were genotyped as PstI+ (unpublished data). The only PstI– strain, ZW strain, may represent a rare genotype in China. More specimens or isolates need to be analyzed in order to evaluate the frequency of such strains in China.

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