Short Communication

Molecular Characterization of Clinical Varicella-Zoster Strains from India and Differentiation from the Oka Vaccine Strain

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SUMMARY: With the introduction of varicella vaccination in India, surveillance of circulating varicella-zoster strains has gained significance. The aim of the present study was to achieve molecular characterization of circulating varicella-zoster virus (VZV) strains and differentiation from the Oka vaccine strain. In this study, the genotype of 100 clinical VZV strains was analyzed. Vesicle fluid was collected from patients with VZV infections (92 cases of varicella and 8 cases of herpes zoster). The PCR-RFLP analysis of two polymorphic loci—a PstI restriction site in ORF 38 and a BglI restriction site in ORF 54 was used to characterize and differentiate them from the vaccine strain. All the wild-type strains were positive for the PstI restriction site in ORF 38. This differentiated them from the Oka vaccine strain, which is PstI negative. The wild-type strains as well as the Oka vaccine strain were positive for the BglI restriction site in ORF 54. Thus, the genotype of all the VZV strains examined had the wild-type pattern represented as PstI+BglI+. None of the strains had the PstI- BglI+ genotype characteristic of the Oka strain or the PstI+BglI- wild-type pattern. To conclude, PstI and BglI serve as good reference markers in the genotyping of circulating varicella strains in India and serve to differentiate them from the vaccine strain as well as other wild-type strains.

Varicella-zoster virus (VZV) causes chicken pox (varicella), a common disease in children that is characterized by a disseminated vesicular rash with fever (1). Following the primary infection, a lifelong latent infection is established, and the virus often reactivates in adulthood to cause shingles (zoster). Usually self-limiting, the illness may be complicated by secondary bacterial skin infections, pneumonitis and encephalitis. The incidence of serious complications increases with age, and adult infections are often associated with higher mortality (2). A live attenuated vaccine against varicella using the Japanese Oka strain was first developed in 1974 (3). This vaccine was licensed for use in the United States in 1995 for the prevention of chicken pox (4). Since then it has been shown to confer protective immunity in more than 90% of healthy adult recipients (5). However, a small number of healthy recipients may develop a vaccine-strain-related chicken pox or subsequent breakthrough chicken pox caused by the circulating wild-type virus (6). In addition, reactivation of the vaccine strain to cause zoster and reactivation of the wild-type VZV in a previously vaccinated individual has also been observed (7). A range of post-vaccination complications from non-specific skin eruptions to life-threatening erythema multiforme have been documented (8). With the introduction of varicella vaccination in India, surveillance of the circulating VZV strains and differentiating them from the vaccine strain is important epidemiologically and can be achieved only by molecular genotyping methods. The VZV genome consists of 125 kb of linear, double-stranded DNA and contains 71 open reading frames (ORFs), which encode proteins with various functions (9). Genotyping methods based on polymerase chain reaction (PCR) amplification and restriction fragment length polymorphism (RFLP) analysis of several ORFs have been described previously (10-13). Two well characterized genetic differences in ORFs 38 and 54 leading to single nucleotide polymorphisms (SNPs) have been analyzed to distinguish the vaccine strain from wild-type strains (11,12,14). The PstI site results from a G-to-A substitution at position 69360 of ORF 38, and the BglI site arises from a C-to-T change at position 95241 of ORF 54 (12,15). These differences lead to the loss of a PstI site in ORF 38 and the gain of a BglI site in ORF 54 of the Oka vaccine strain designated PstI- BglI+. Studies from the United States and the United Kingdom have revealed the presence of PstI restriction sites in all the wild-type strains investigated, which distinguishes them from the Oka vaccine strain (15). Furthermore, using the amplification of a DNA fragment located in ORF 54, wild-type VZV strains could be differentiated into BglI+ and BglI- strains. Nearly 80% of these wild-type isolates lacked the BglI site in ORF 54. However, this site is present in the vaccine Oka strain. Thus, based on a combination of these differences, nearly all the wild-type strains could be differentiated from the Oka vaccine strain. However, about 20% of the wild-type strains were found to be positive for the BglI restriction site (PstI- BglI+). In the United Kingdom, these BglI+ wild-type strains were found with increasing frequency among Asian immigrants from countries with low adult immunity to varicella (16). This PstI- BglI+ genotype has been found to predominate in strains circulating in countries with a history of European colonization and is, thus, the predominant genotype in equatorial Africa, Bangladesh and Western Australia (17). The aim of the present study was to analyze the genotype of circulating VZV strains in India and to differentiate them from the Oka vaccine strain. Vesicle fluid specimens from 100 consecutive patients with VZV infections who presented to the Department of Dermatology of a tertiary care hospital during the period from 2005 to 2006 were analyzed. They comprised 92 cases of varicella

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and 8 cases of herpes zoster. Twelve of the 100 patients with varicella gave a history of vaccination with the Oka strain. All of them were cases of varicella. The amount of time since vaccination in these patients ranged from 2 to 5 years. To differentiate it from wild-type viruses and as a control, the VZV Oka strain from the vaccine preparation Varivax (GenBioTech, Mumbai, India; batch no. 20050832; date of expiry, 03/07/06) was processed in parallel with the clinical strains. Viral DNA was extracted from the clinical specimens and the vaccine using the QIAamp DNA mini kit according to the body fluid spin protocol (Qiagen, Hilden, Germany). PCR and RFLP analysis of DNA fragments for ORF 38 and 54 were performed as previously described (11,12). The amplified product was confirmed by 2% agarose gel electrophoresis. The amplified product was further divided equally and subjected to restriction enzyme digestion with restriction enzymes *Pst*I (ORF 38) and *Bgl*II (ORF 54). The digests were separated by electrophoresis on a 4% agarose gel. DNA sequencing was performed for 50 selected strains and the Oka vaccine strain by the dideoxy-chain termination technique with a T7 Sequencing kit according to the manufacturer's instructions. Sequenced products were run on a 3730 DNA Automated Sequencer (Applied Biosystems, Birmingham, UK). The BLAST program was used for the database searches. The sequences of the clinical strains were compared with that of the vaccine strain to identify the SNPs.

Amplification of the VZV ORF 38 and ORF 54 regions of the viral genome was achieved in 82 of the 100 samples processed. All the specimens collected from vaccinees were successfully amplified. VZV strains that possessed the specific *Pst*I restriction site for the 647-bp PCR product of ORF 38 were cut by the *Pst*I endonuclease into two fragments that were 357- and 290-bp in size. In contrast, the 647-bp amplified product of the Oka vaccine strain was not digested by *Pst*I endonuclease. *Bgl*II endonuclease digestion of the 222-bp PCR of the ORF 54 product of both the wild-type and Oka vaccine strains resulted in two fragments that were 137- and 85-bp in size (Fig. 1). Thus, all the clinical varicella-zoster strains examined were positive for the *Pst*I restriction site in ORF 38. The Oka vaccine strain was negative for the *Pst*I restriction site. All the clinical strains as well as the Oka strain were positive for the *Bgl*II restriction site in ORF 54. The genotype of all the clinical VZV strains in the study had the wild-type pattern *Pst*I+ *Bgl*II+. The Oka vaccine strain showed the *Pst*I+ *Bgl*II+ genotype. Circulating strains of the genotype *Pst*I+ *Bgl*II+ or of the Oka strain pattern (*Pst*I+ *Bgl*II-) were not detected. Upon analysis of the gene sequences, all the wild-type VZV strains analyzed showed a G-to-A substitution at position 69360 and were thus positive for the *Pst*I restriction site in ORF 38. This SNP was not detected in the Oka vaccine strain, and it was confirmed to be *Pst*I-. The SNP that causes the C-to-T substitution at position 95241 leading to the elimination of the *Bgl*II restriction site in ORF 54 could not be detected in any of the wild-type VZV strains or the Oka vaccine strain. They were thus confirmed to be positive for the *Bgl*II restriction site. SNP analysis confirmed the genotype of the Oka vaccine strain to be *Pst*I+ *Bgl*II+ and that of all the wild VZV strains to be *Pst*I+ *Bgl*II+. Nucleotide sequences of the 50 clinical VZV strains were submitted to GenBank (DQ656706-656755). For ORF 38, the sequences of 23 of the 25 samples were different from each other, and the differences ranged from 1 to 4 nucleotide alterations in the sequenced 647-bp region. For ORF 54, the sequences of 18 of the 25 samples were different from each other, and the differences ranged from 1 to 8 nucleotide alterations in the sequenced 222-bp region.

With the introduction of varicella vaccination in India, molecular surveillance of circulating VZV strains gained significance. In this study, the VZV genotype could be identified in 82 out of the 100 specimens processed. Viral DNA in the vesicle fluid of 18 patients could not be detected. These patients presented to the hospital rather late after the appearance of the rash (>5 days), and most of the lesions were in the crusting stage. The results of this study demonstrate that all the wild-type strains, unlike the Oka vaccine strain, were positive for the *Pst*I restriction site in ORF 38. Wild-type VZV strains can thus be reliably distinguished from the Oka vaccine strain on the basis of this marker. All the wild-type VZV strains as well as the Oka strain were found to be positive for the *Bgl*II restriction site in ORF 54. These data suggest that viruses that are indistinguishable from the Oka strain at certain loci are circulating in the community. Earlier studies that investigated Indian isolates found the *Pst*I+ *Bgl*II+ genotype to be predominant (17,18). Our study using a larger number of clinical isolates is the first full-length investigation to characterize circulating VZV strains in India. Thus, it may be concluded that the *Pst*I+ *Bgl*II+ genotype is the common genotype among Indian VZV strains (100%) and that other strains are rarely detected. This genotype was also detected in the 12 vaccinees with breakthrough varicella. The seroconversion rate after varicella vaccination in India is estimated to range from 95-99% (19). In this study, vaccinated individuals experienced varicella caused by circulating wild-type VZV strains. However, we do not have the data to determine whether these breakthrough cases resulted from primary or secondary vaccine failure. While the genetic markers used in this study effectively distinguished circulating Indian strains (*Pst*I+) from the Oka vaccine strain (*Pst*I−), they failed to distinguish some Japanese wild-type isolates (10). These isolates carried a *Pst*I site less mutation and were thus indistinguishable from the Oka strain at this site. More extensive genotyping methods were required to distinguish these isolates from the Oka strain (20). However, this genetic variation has not been detected in any VZV strains outside Japan and hence is unlikely to be detected in any circulating strains in India as well. In addition to the *Pst*I and *Bgl*II sites used in this study, several other genetic markers have been found to characterize VZV strains and differentiate them from the vaccine strain. The more appropriate markers that have been analyzed include the Smal and *Nae*I sites in ORF 62 (13,21). These markers appear to be more robust for distin-

**Fig. 1.** Agarose gel electrophoresis of restriction digestion products from wild-type strains showing (A) *Pst*I digestion of ORF 38 amplified products (lane 1 through 7) and (B) *Bgl*II digestion of ORF 54 amplified products (lane 8 through 14). Lane M: 100-bp molecular weight ladder.
guishing the vaccine strain from wild-type stains and can be used confidently to characterize VZV strains. Several direct methods for the genotyping of varicella-zoster strains and differentiating them from the vaccine strain have been developed (22,23). Given the constraints on molecular biology techniques in most medical laboratories in India, the PCR-RFLP technique using genetic markers ORF 38 and 54 used in this study will continue to be useful in distinguishing wild-type Indian varicella-zoster strains from the Oka vaccine strain. Genotyping strategies using these two markers will also be useful in detecting BglII strains in India when a minor-type virus infection occurs. Investigation of the molecular epidemiology of Indian VZV strains may have important implications in the use of the Oka vaccine in India and in the monitoring of vaccine-related adverse events. The availability of technically accessible, reliable methods for the genotyping of varicella-zoster strains will serve the critical function of effectively monitoring vaccine impact in countries with broad varicella vaccination policies.

REFERENCES