Simultaneous Cocirculation of Both European Varicella-Zoster Virus Genotypes (E1 and E2) in Mexico City[▽]

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Full-length genome analysis of varicella-zoster virus (VZV) has shown that viral strains can be classified into seven different genotypes: European (E), Mosaic (M), and Japanese (J), and the E and M genotypes can be further subclassified into E1, E2, and M1 through 4, respectively. The distribution of the main VZV genotypes in Mexico was described earlier, demonstrating the predominance of E genotype, although other genotypes (M1 and M4) were also identified. However, no information regarding the circulation of either E genotype in the country is available. In the present study, we confirm the presence of both E1 and E2 genotypes in the country and explore the possibility of coinfection as the triggering factor for increased virulence among severe cases. A total of 61 different European VZV isolates collected in the Mexico City metropolitan area from 2005 to 2006 were typed by using a PCR method based on genotype-specific primer amplification. Fifty isolates belonged to the E1 genotype, and the eleven remaining samples were classified as E2 genotypes. No coinfection with both E genotypes was identified among these specimens. We provide here new information on the distribution of VZV genotypes circulating in Mexico City.

Varicella-zoster virus (VZV), or human herpesvirus 3, is a member of the subfamily *Alphaherpesvirinae* within the family of *Herpesviridae* and is the causal agent of varicella (chickenpox), a highly contagious illness characterized by generalized exanthematous rash (24). Varicella is usually benign in children; however, it may also result in complications that might include pneumonia, encephalitis, multiple sclerosis, and death (18, 21, 24). Primary infection is commonly followed by the establishment of lifelong latent infection of the sensory ganglia, which can be reactivated later in life, resulting in herpes zoster (shingles).

VZV is a highly conserved virus exhibiting little nucleotide sequence variation, its genome (double-stranded DNA) of ~125 kb in length consists of ~70 open reading frames (ORFs). The modest genomic variation along the genome is predominantly due to punctual mutation scattered uniformly across all ORFs. Nucleotide sequence variability analysis based on whole-genome sequences has demonstrated the presence of generic and specific markers for each genotype, a characteristic that has helped implement and unify VZV genotyping strategy (9).

Viral genotyping based on single-nucleotide polymorphism (SNP) along ORF22 has successfully established the existence of seven different viral genotypes: European (E), Mosaic (M), and Japanese (J); the E and M genotypes are further classified into E1, E2 and M1 through M4, respectively (6–8, 19, 20). Molecular epidemiology of VZV varies geographically, dis-

playing a distinctive distribution of the major genotypes in relationship with climate (6, 14, 23). Thus, the predominance of certain genotypes in different geographical regions might be due to multiple factors, including environmental, host, and viral components. In addition, introduction of new genotypes to naive populations through immigration or international travelers might also play role in VZV global genotype distribution.

The E genotype is the most predominant worldwide and is largely spread in Western Europe, the United States, Mexico, and South America. On the other hand, the J genotype has been detected only in Japan, China, and Korea or countries with immigrants from those nations. M genotype has been found in Africa, Indochina, Australia, Mexico, and several European countries, including Iceland, Germany, France, Italy, and Spain (5, 6, 7, 9, 17, 20).

Immigration and vaccination are two important factors that can potentially contribute to the introduction of new genotypes or emergence of recombinant viruses into otherwise naive populations. The distribution of VZV strains in Mexico City has been reported previously, demonstrating the presence of E, M1, and M4 genotypes (17). Immigration and international travelers from regions where circulating VZV genotypes differ to those present in the country, such as European or Asian nations, are not unusual in Mexico. Moreover, the varicella vaccine is readily accessible in the country, and immunization against VZV in private medical practices is common. In spite of the availability of the vaccine, impact studies addressing the effect of introducing the Oka strain in the population and its repercussion on the molecular epidemiology of VZV in Mexico are nonexistent. Thus, molecular surveillance is necessary to understand the implications of vaccination on the dynamics of VZV, as well as to better monitor the emergence of recom-

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binant strains in the population. Furthermore, no information regarding the circulation of E genotypes in Mexico has been documented thus far, and their association with severity is not clear (9, 22).

In the present study, we further address and analyze the presence of E VZV genotypes in Mexico City. A total of 69 different VZV isolates belonging to the E genotype collected in Mexico City metropolitan area from 2005 to 2006 were typed by using a PCR approach based on genotype-specific primer amplification. We provide here new information on the distribution of VZV isolates circulating in Mexico and elucidate the VZV global molecular epidemiology. Continuous molecular epidemiologic surveillance of VZV in Mexico should be conducted to better assess the current status of VZV in the country and to monitor the emergence of recombinant strains.

MATERIALS AND METHODS

Patients. Patients and samples used during the course of the present study were collected as part of a cross-sectional, descriptive study conducted in Mexico City between February 2005 and June 2006. VZV infection was defined as vesicular eruption with generalized onset. Clinical samples from patients with VZV were identified in different hospitals and primary health clinics in Mexico City. All subjects enrolled in the study were residents of the Mexico City metropolitan area, with no previous vaccination against VZV or history of any recent travel within the country or abroad. There were no epidemiological links between patients or secondary infections among the household contacts. None of the VZV cases were members of the same family group, knew each other, attended the same school, or worked together. All infections were reported as sporadic cases occurring in different areas of the city. Ethical review and informed consent approval was obtained from the Ethical Committee of the Mexican Institute for Epidemiological Diagnosis and Reference (InDRE). Informed consent was obtained from all subjects who agreed to participate in the study.

Genotyping. All clinical samples were subjected to DNA extraction by standard methods. Total DNA was used to amplify VZV-specific sequences located on ORF22. Genotyping was performed as described previously (7). This classification is based on four distinctive SNPs occurring at positions 37902, 38055, 38081, and 38177.

Design of genotype-specific primers. The Kalign algorithm (<http://www.ebi.ac.uk/Tools/kalign/>) was used to analyze 19 full-length genome VZV genomic sequences currently available from GenBank (<http://www.ncbi.nlm.nih.gov/GenBank/index.html>). The alignment was inspected and refined manually. Genomic regions belonging to ORF21 and ORF22 were analyzed by using Lasergene DNA & Protein analysis package v8.0.2 (DNASTAR, Inc., Madison, WI) in order to design two different primer sets (E generic [Eg] and E2) capable of differentiating between the two E genotypes, based on their respective nucleotide patterns in SNPs 33725, 33728, and 38081.

PCR amplification. Extracted DNA (5 μ l) was subjected to PCR using the genotype-specific primer described above. PCR amplification was carried out in PCR tubes in a final reaction volume of 50 μ l (PCR mix; 50 mM KCl, 10 mM Tris [pH 8.5], 1.5 mM MgCl₂, 0.01% gelatin, 200 mM deoxynucleoside triphosphate, 5 mM dithiothreitol, 1 μ M concentrations of all four primers, and 5 U of *Taq* polymerase). The DNA was then amplified during 40 cycles at 95°C for 10 s, 60°C for 10 s, and 72°C for 30 s. PCR amplicons were resolved by using a Bioanalyzer 2100 (Agilent Technologies, Inc., Santa Clara, CA).

Endpoint limiting dilution PCR (EPLD). Analysis of the viral population was performed as described elsewhere (16). Briefly, serially diluted DNA (log dilutions) was prepared in quadruplicates for each sample where coinfection was suspected. All DNA dilutions (1 μ l) were subjected to real-time PCR amplification (final reaction volume, 10 μ l) using specific primers for the ORF22 (p22R1f, GGGTTTGTATGAGCGTTGG; p22R1r, CCCCCGAGGTTTCGTAATATC) and a LightCycler 480 SYBR green I master kit (Roche Applied Sciences, Indianapolis, IN) in a Stratagene MX3005P instrument (Agilent Technologies). The endpoint was defined as the last dilution where two of four reactions were successfully amplified. Thus, the target DNA at that dilution was expected to be amplified in only 50% of all reactions. Under these conditions, the amplicons are most likely to be amplified from a single molecule. A total of 96 aliquots of the corresponding DNA at the endpoint dilution were prepared on a PCR plate, and 1 μ l from each dilution was then PCR amplified using the

ORF22-specific primers as described above in order to obtain ~48 PCR-positive clones for each sample. All DNA aliquots corresponding to PCR-positive clones were subjected to three independent real-time PCR amplifications using the following three primer sets: (i) Eg, (ii) E2, and (iii) Var-E2-FWD and the E1 genotype-specific reverse primer (Var-E1-RVS, CGTCTCTGACCGTTGTCGT TATTTTAACATTA). Thus, coinfection was defined as a PCR-positive reaction with all three primer sets in any given clone.

RESULTS

Genotyping. A total of 61 different clinical samples were genotyped by using the ORF22 scheme described elsewhere (7). SNP analysis along the ORF22 showed that all isolates belonged to the E genotype.

Primer design. In order to further classify the E genotypes strains into their corresponding subtype, an alignment including 19 different VZV whole-genome sequences available from GenBank was used to design genotype-specific primers on the VZV ORF21 and ORF22 genomic regions. The strategy used for the design was based on the SNPs located on both regions (33725 to 33728 and 38081 for ORF21 and ORF22, respectively). Thus, two primer sets were designed in such way that the amplicon patterns obtained after simultaneous amplification of both genomic regions (multiplex PCR) would be adequate to successfully differentiate between both E genotypes (E1 and E2). In this case, both forward primers are generic and can anneal to more than one genotype; on the other hand, the last nucleotide in the 3' end of both reverse primers aligns with the SNPs responsible for distinguishing the viral genotypes (Var-E2-FWD [CAACAGGAGAAAATATACCCGGTCTTGC], Var-E2-RVS [CGTCTCTGACCGTTGTCGTTATTTT AACGTTG], Var-Eg-FWD [CCGATTGTGAACGTGCACA TAAACAAC], and Var-Eg-RVS [ACCTCTTAACAAAGC CAAAGGCGGT] for ORF21 and ORF22, respectively). Hence, the amplification can only proceed if the right nucleotide is present in the target sequence. The primer set targeting the ORF21 specifically binds sequences containing "C" at nucleotide positions 33725 and 33728 (E2, M1, M2, M4, and J). Likewise, the second primer set binding ORF22 would exclusively extend sequences bearing "A" in nucleotide position 38081 (E1 and E2). An additional mismatch (C instead of T), three nucleotide positions upstream to the 3' end, was incorporated into the reverse Eg primer to help differentiate between E genotypes from other genotypes. In consequence, a single amplicon (~300 bp) is indicative of E1 genotype, whereas two PCR fragments (~300 and ~350 bp) demonstrate the presence of E2 genotype or possible coinfection with both E genotypes.

Subgenotyping. After validation and optimization of the PCR, all 61 strains belonging to E genotype were subjected to genotype-specific PCR amplification. E1 genotype was confirmed in 50 cases, while E2 was found among 11 isolates.

Coinfection analysis. Identification of coinfection by EPLD analysis was conducted in order to address whether coinfection with both E genotypes could be used as a marker for increased virulence among severe varicella isolates. For this purpose, all 50 samples exclusively infected with E1 genotype (Eg+, E2-primer amplification) were excluded from the analysis. EPLD analysis was conducted in the remaining 11 specimens (Eg+, E2+). Multiple PCR clones from each and every sample were tested for all Eg, E1, and E2 primer sets. Considering that

EPLD analysis is likely to amplify fragments from single molecules, the criteria to establish the infecting genotype among the PCR clones was as follows: (i) Eg+, E1+, E2- = E1; (ii) Eg+, E1-, E2+ = E2; and (iii) Eg-, E1+, E2- = M3. Multiple PCR clones (30 to 50 per sample) were generated by EPLD analysis. All clones analyzed were PCR positive for both Eg and E2 primer sets, indicating the exclusive presence of the E2 genotype in all 11 specimens. None of the clones was PCR positive when the E1-specific primers were used for amplification, ruling out coinfection with E1 or M3 genotypes. Thus, in the present study no coinfection between E2 and any other genotype could be established among patients infected with the E2 genotype.

DISCUSSION

In this study, the circulation of both E1 and E2 genotypes in Mexico City's metropolitan area was confirmed. In conjunction with previously reported information (17), we provide here a comprehensive analysis of the distribution of VZV strains in Mexico City. Consequently, we can propose that VZV isolates in Mexico City are of European origin.

It has been suggested that the E2 genotype is the result of recombination events between the parental E1 and J or M strains (8). This relatively new viral genotype has successfully managed to outcompete its original European ancestor, at least in some geographical regions, allowing its establishment as the dominant VZV genotype (9). The growing evidence regarding the occurrence of recombinant events among wild-type strains (7, 10, 12, 13), the relative closeness between E2 and M4 genotypes (9), and the identification of the first M4 isolate in Mexico (17) might together favor the appearance of recombinant strains in the country.

The presence of E2 strains can be at least partially explained by the high number of travelers and immigrants from European countries in which this genotype has been identified. Moreover, this particular genotype has also been reported to circulate in other North American countries with which Mexico has an active traffic of persons including, especially, the United States due to the extensive border shared by the two countries, making it one of the most frequently crossed international boundaries in the world.

Recently, a fatal case due to E2 VZV infection in an immunocompromised adult was reported (22). This particular isolate exhibited distinctive SNPs in ORF21, ORF51, and ORF54; however, not enough information is currently available regarding these nucleotide substitutions to associate them with an increased virulence. Others have also reported associations between nucleotide changes and augmented pathogenicity (11). In Europe, 38% of European isolates are associated with the development of shingles, although age also seems to play an important role (9). Here, no association between the infecting genotype and the severity of disease was observed. Thus, whether E2 strains are intrinsically more virulent than E1 isolates requires further studies.

Subclades within the two E genotypes have been described elsewhere (19). This phylogenetic analysis based on a subgenomic region ~7,482 nucleotides in length, including ORF5, ORF37, and ORF62, seems to contain sufficient information to identify different variants (E1a, E2a, and E2b). This novel

classification of E strains may help us to understand the molecular evolution of VZV and might facilitate the identification of markers of virulence among E VZV isolates.

VZV coinfection with the two different E genotypes has been described by others (15). Coinfections might occur in regions where cocirculation of two or more different genotypes has been documented. In Mexico, the transmission of multiple VZV genotypes has been demonstrated, thus increasing the opportunity to observe binomial infections (17). In the present study, coinfection between E2 and other genotypes could not be demonstrated, and therefore further inference of the role of coinfection in disease severity could not be suggested. Nevertheless, the "ability" of the EPLD method used here to detect minor variants is limited, and therefore the identification of viral strains represented in low proportions (>1%) within the viral population would be extremely challenging. Thus, although multiple PCR clones per patient were analyzed in order to establish the simultaneous presence of both genotypes, the possibility of coinfection cannot be completely ruled out. Unfortunately, EPLD is a time-consuming, cumbersome, and expensive methodology that significantly limits the extent of viral population and complexity analysis. The use of new platforms able to identify minor variant with higher sensitivity might allow us to identify and understand better the dynamics of viral variants composition and their implications in pathogenesis (3).

Interestingly, no J genotypes have been reported to be circulating in the country thus far. The absence of this genotype might be, at least partially, explained by the relatively small sample size and/or the lack of strains representative of the entire country. Importantly, in the present study none of the subjects enrolled had a recent travel history within the country or abroad, reducing the opportunities to identify imported strains and biasing the sampling toward autochthonous isolates. Alternatively, considering that temperature and climate seem to play an important role in the geographical distribution of VZV (1, 23), we propose that host and environmental factors are important in dictating the spreading and displacement of VZV variants in any given population. It is also noteworthy that Mexico, a former Spanish colony, demonstrates a VZV distribution similar to that seen in Spain (9). Thus, long-established viral populations in the country might have a fitness advantage over new VZV viral genotypes, impeding their displacement by other genotypes.

Currently, VZV vaccination is not considered as part of the National Program of Immunization in Mexico. However, the vaccine is licensed and commonly administered in private medical practices. The lack of a national program that precisely records and controls the number of vaccine doses applied significantly affects the opportunities to study the impact of vaccination on the dynamics of VZV transmission. Despite the fact that varicella vaccine seems to be highly effective, a number of viral meningitis attributed to varicella vaccine have been documented (2). Nucleotide changes in the vaccine strain characteristic of wild-type strains have been reported by others (4), leading to the development of herpes zoster with meningitis. Therefore, special emphasis should be made to implement an effective surveillance that can accurately identify such strains. The need to establish surveillance systems that can monitor the emergence of recombinant VZV strains is particularly impor-

tant, considering that not only are multiple genotypes present in the region but vaccination with the live attenuated Oka varicella vaccine is also routinely administered.

In summary, the circulation of E1 and E2 genotypes in Mexico City's metropolitan area was established. Molecular surveillance should be implemented in Mexico in order to identify probable recombinant viral strains and to assess the impact of varicella vaccination in the country.

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REFERENCES

- Barrett-Muir, W., F. T. Scott, P. Aaby, J. John, P. Matondo, Q. L. Chaudhry, M. Siqueira, A. Poulsen, K. Yaminishi, and J. Breuer. 2003. Genetic variation of varicella-zoster virus: evidence for geographical separation of strains. *J. Med. Virol.* **70**(Suppl. 1):S42–S47.
- Chaves, S. S., P. Haber, K. Walton, R. P. Wise, H. S. Izurieta, D. S. Schmid, and J. F. Seward. 2008. Safety of varicella vaccine after licensure in the United States: experience from reports to the vaccine adverse event reporting system, 1995–2005. *J. Infect. Dis.* **197**(Suppl. 2):S170–S177.
- Le, T., J. Chiarella, B. B. Simen, B. Hanczaruk, M. Egholm, M. L. Landry, K. Dieckhaus, M. I. Rosen, and M. J. Kozal. 2009. Low-abundance HIV drug-resistant viral variants in treatment-experienced persons correlate with historical antiretroviral use. *PLoS One* **4**:e6079.
- Levin, M. J., R. L. DeBiasi, V. Bostik, and D. S. Schmid. 2008. Herpes zoster with skin lesions and meningitis caused by 2 different genotypes of the Oka varicella-zoster virus vaccine. *J. Infect. Dis.* **198**:1444–1447.
- Liu, J., M. Wang, L. Gan, S. Yang, and J. Chen. 2009. Genotyping of clinical varicella-zoster virus isolates collected in China. *J. Clin. Microbiol.* **47**:1418–1423.
- Loparev, V., E. Martro, E. Rubtcova, C. Rodrigo, J. C. Piette, E. Caumes, J. P. Vernant, D. S. Schmid, and A. M. Fillet. 2007. Toward universal varicella-zoster virus (VZV) genotyping: diversity of VZV strains from France and Spain. *J. Clin. Microbiol.* **45**:559–563.
- Loparev, V. N., A. Gonzalez, M. Deleon-Carnes, G. Tipples, H. Fickenscher, E. G. Torfason, and D. S. Schmid. 2004. Global identification of three major genotypes of varicella-zoster virus: longitudinal clustering and strategies for genotyping. *J. Virol.* **78**:8349–8358.
- Loparev, V. N., E. N. Rubtcova, V. Bostik, D. Govil, C. J. Birch, J. D. Druce, D. S. Schmid, and M. C. Croxson. 2007. Identification of five major and two minor genotypes of varicella-zoster virus strains: a practical two-amplicon approach used to genotype clinical isolates in Australia and New Zealand. *J. Virol.* **81**:12758–12765.
- Loparev, V. N., E. N. Rubtcova, V. Bostik, V. Tzaneva, A. Sauerbrei, A. Robo, E. Sattler-Dornbacher, I. Hanovcova, V. Stepanova, M. Splino, V. Eremin, M. Koskiniemi, O. E. Vankova, and D. S. Schmid. 2009. Distribution of varicella-zoster virus (VZV) wild-type genotypes in northern and southern Europe: evidence for high conservation of circulating genotypes. *Virology* **383**:216–225.
- Lopez, A. S., A. Burnett-Hartman, R. Nambiar, L. Ritz, P. Owens, V. N. Loparev, D. Guris, and D. S. Schmid. 2008. Transmission of a newly characterized strain of varicella-zoster virus from a patient with herpes zoster in a long-term-care facility, West Virginia, 2004. *J. Infect. Dis.* **197**:646–653.
- Natoli, S., M. Ciotti, P. Paba, G. P. Testore, G. Palmieri, A. Orlandi, A. F. Sabato, and F. Leonardi. 2006. A novel mutation of varicella-zoster virus associated to fatal hepatitis. *J. Clin. Virol.* **37**:72–74.
- Norberg, P., J. A. Liljeqvist, T. Bergstrom, S. Sammons, D. S. Schmid, and V. N. Loparev. 2006. Complete-genome phylogenetic approach to varicella-zoster virus evolution: genetic divergence and evidence for recombination. *J. Virol.* **80**:9569–9576.
- Peters, G. A., S. D. Tyler, C. Grose, A. Severini, M. J. Gray, C. Upton, and G. A. Tipples. 2006. A full-genome phylogenetic analysis of varicella-zoster virus reveals a novel origin of replication-based genotyping scheme and evidence of recombination between major circulating clades. *J. Virol.* **80**:9850–9860.
- Quinlivan, M., K. Hawrami, W. Barrett-Muir, P. Aaby, A. Arvin, V. T. Chow, T. J. John, P. Matondo, M. Peiris, A. Poulsen, M. Siqueira, M. Takahashi, Y. Talukder, K. Yamanishi, M. Leedham-Green, F. T. Scott, S. L. Thomas, and J. Breuer. 2002. The molecular epidemiology of varicella-zoster virus: evidence for geographic segregation. *J. Infect. Dis.* **186**:888–894.
- Quinlivan, M., N. Sengupta, and J. Breuer. 2009. A case of varicella caused by coinfection with two different genotypes of varicella-zoster virus. *J. Clin. Virol.* **44**:66–69.
- Ramachandran, S., G. L. Xia, L. M. Ganova-Raeva, O. V. Nainan, and Y. Khudyakov. 2008. End-point limiting-dilution real-time PCR assay for evaluation of hepatitis C virus quasispecies in serum: performance under optimal and suboptimal conditions. *J. Virol. Methods* **151**:217–224.
- Rodriguez-Castillo, A., G. Vaughan, J. E. Ramirez-Gonzalez, E. Gonzalez-Duran, J. C. Gudino-Rosales, and A. Escobar-Gutierrez. 2009. Genetic variation of varicella-zoster virus strains circulating in Mexico City. *J. Clin. Virol.* **46**:349–353.
- Rodriguez-Violante, M., G. Ordonez, J. R. Bermudez, J. Sotelo, and T. Corona. 2009. Association of a history of varicella virus infection with multiple sclerosis. *Clin. Neurol. Neurosurg.* **111**:54–56.
- Schmidt-Chanasit, J., S. Olschlager, A. Bialonski, P. Heinemann, K. Bleyemehl, G. Gross, S. Gunther, R. G. Ulrich, and H. W. Doerr. 2009. Novel approach to differentiate subclades of varicella-zoster virus genotypes E1 and E2 in Germany. *Virus Res.* **145**:347–349.
- Sergeev, N., E. Rubtcova, V. Chizikov, D. S. Schmid, and V. N. Loparev. 2006. New mosaic subgenotype of varicella-zoster virus in the U.S.A.: VZV detection and genotyping by oligonucleotide-microarray. *J. Virol. Methods* **136**:8–16.
- Sotelo, J., A. Martinez-Palomo, G. Ordonez, and B. Pineda. 2008. Varicella-zoster virus in cerebrospinal fluid at relapses of multiple sclerosis. *Ann. Neurol.* **63**:303–311.
- Springfield, C., A. Sauerbrei, A. Filusch, M. Konstandin, W. Hartschuh, P. Sauer, J. Encke, W. Stremmel, and P. Schnitzler. 2009. Fatal varicella in an immunocompromised adult associated with a European genotype E2 variant of varicella zoster virus. *J. Clin. Virol.* **44**:70–73.
- Wagenaar, T. R., V. T. Chow, C. Buranathai, P. Thawatsupha, and C. Grose. 2003. The out of Africa model of varicella-zoster virus evolution: single nucleotide polymorphisms and private alleles distinguish Asian clades from European/North American clades. *Vaccine* **21**:1072–1081.
- Whitley, R. 2009. Varicella-zoster virus, p. 1963–1969. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 7th ed., vol. 2. Churchill Livingstone, New York, NY.