Genetic Analysis of Measles Virus in São Paulo, Brazil.

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Despite the availability of an effective vaccine, sporadic and epidemic measles virus (MV) infections continue to occur. The World Health Organization estimates that worldwide, 45 million cases of measles continue to occur annually of which >1 million are fatal. In developing countries, 3%-15% of children who contract measles die.1,2,3 In the pre-immunization era, the measles were among the major public health problems in Brazil, representing in some regions the main cause of death in the 1 to 4 years old group. Despite the use of several strategies, routine vaccination did not prevent two extensive epidemics, one for them in 1984 and other in 1986, with respective rates of incidence of 62.9/100,000 and 97.2/100,000 habitants. As a result of measles-control efforts a 93.1% reduction in the incidence of the disease was observed from 1981 to 1996, in 1997, the resurgence of measles in São Paulo resulted in more than 21,465 cases.4

In order to explore the reasons for the resurgence, a genetic characterization of the isolated wild-type virus was done.

Most of the studies to describe the genetic characteristics of wild-type measles viruses have been conducted by sequencing the gene coding for the hemagglutinin (H) protein and the nucleoprotein (N). Of the six genes on the viral genome, the H and N genes are the most variable. Over their protein coding regions, the H and N genes contain up to a 7% variability at the nucleotide level. The single most variable part of the measles genome is the 450 nucleotides that code for the COOH-terminus of the N protein where nucleotide variability can approach 12% between various wild-type viruses. Many laboratories have conducted sequence analyses of wild-type measles viruses and assigned the viruses to various genetic groups.5-8,10,11,

This work describes isolated viruses and the molecular surveillance of the measles virus associated with the outbreak in São Paulo in 1997. Samples were collected during the measles outbreak. From 36 patients we obtained information of dates of the onset of the rashes and fevers, and some clinical symptoms such as coughs, coriza, conjunctivitis and encephalitis. The mean number of days from the onset of the rash to the collection of the acute phase specimen was 3 days. The serum was tested by ELISA and virus isolation was achieved by peripherial blood lymphocytes which were separated from heparinized blood with Ficoll-Hypaque gradients and suspended in DMEM supplemented with 5% fetal bovine. The propagation of the measles virus was in Vero cells, an established cell line of African Green Monkey Kidney cells10 and B95A cells, a cell line of Epstein-Barr virus-transformed marmoset B-lymphoblastoid cell line.11

The RNA extraction methods have been described 12, using guanidinium thiocyanate.13 The RT-PCR was done with primers that amplify either the coding region of the H gene or the carboxyl-terminus of the N gene as previously described.14,15 PCR products were sequenced directly by cycle sequencing (373 DNA Sequencer; Perkin-
Elmer, Applied Biosystems Division, Foster City, CA - Phylogenetic analysis. Different sequence data were analyzed as previously described as well as PAUP (phylogenetic analysis using parsimony, version 3.1.1)\(^{16}\). All phenograms were drawn as unrooted trees.

The 36 viruses isolated from outbreaks during 1997 in São Paulo, Brazil, were genetically heterogeneous and represented many of the measles genetic groups that have been described previously\(^{7,15}\).

Among these was

- The group A viruses, which have limited sequence variation relative to the wild type measles viruses isolated in the 1950s and 1960s, as well as to all of the vaccine strains.
- group D5 which was associated with viruses imported from Thailand and Southeast Asia and Japan.
- group D6, which was associated with imported viruses from western Europe.

Viral sequences from measles cases that were known be imported viruses, or from cases that no known sources were compared with, nor with the previously described sequence data, were used to identify or confirm the course of the virus sequence analysis of measles viruses in an integral part of the surveillance of measles cases. This information became the data on transmission patterns which gave indications of the status of worldwide measles-control and elimination programs.

The molecular epidemiologic studies have made significant contributions to measles control efforts by enabling investigators to identify the source and transmission pathways of the virus.

References