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Genotypic and Phylogenetic Analysis of the G Gene of Respiratory Syncytial Virus Isolates in Okinawa, Japan, 2008

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Respiratory syncytial virus (RSV) is an important causative agent of acute respiratory infections (ARIs) in infants and young children (1). The prevalent season of RSV infection is from May to September in Okinawa Prefecture (Ryukyu Islands), while it is from November to February in other areas of Japan (2). This suggests that the subgroup of RSV prevalent in Okinawa may be different from those prevalent in the rest of Japan (1). The present study was performed to understand the molecular epidemiology of RSV in Okinawa.

Eight RSV isolates were obtained from children in Okinawa with ARIs in June and July 2008. The isolates were propagated in HEp-2, LLC-MK2, or Vero9013 cells. Virus RNA was extracted from the isolates using a QIAamp Viral RNA Mini kit (Qiagen, Germantown, Md., USA) and suspended in DNase/RNase-free water. After RNA extraction, reverse transcriptase-polymerase chain reaction (PCR) was performed as described previously (3). Amplicons were purified using a QIAquick PCR Purification kit (Qiagen) and the nucleotide sequences were determined by direct sequencing (3). Partial nucleotide sequences (270 nt) of the G gene of RSV were analyzed phylogenetically using Molecular Evolutionary Genetics Analysis (MEGA) software version 4 (4). Evolutionary distances were estimated using Kimura’s two-parameter method, and phylogenetic trees were constructed using the neighbor-joining (NJ) method (5). The reliability of the tree was estimated based on 1,000 bootstrap replications.

A phylogenetic tree was constructed including the Okinawa isolates (Fig. 1). Of the 8 new isolates, 7 were classified into subgroup A, and one into B. In subgroup A, the 7 isolates were classified into genotype GA2, and further into 2 subclusters: 5 with a Brazilian (JU1780/2007) isolate and 2 with Brazilian (SP1044/2006) and Belgian (BE/9600/05) isolates. In subgroup B, the Okinawa isolate was classified into genotype BA, and further in the subcluster with Indian (DEL/ AFF/05), Belgian (BE/9382/05), and Brazilian (JU1324/2006) isolates. Two other isolates in Japan, S02-71 isolated in Sapporo in 2002 and NG-004-03 in Niigata in 2003, were isolates. Two other isolates in Japan, S02-71 isolated in Sapporo in 2002 and NG-004-03 in Niigata in 2003, were isolates.

This unique duplication have recently spread in the world (6,7). Further, there was a novel stop codon which leads to a nucleotide substitution in the present isolate, corresponding to nucleotide position 881 in the reference strain (strain 18537, Genbank accession no. M17213); TTA to TAA; Leu to stop codon. The results suggest that RSV strains belonging to subgroup A were dominant in Okinawa in 2008, with those belonging to subgroup B as minor. These results were consistent with those of previous reports (7,8).

The prevalent season of RSV infection in Okinawa is summer, unlike that in other areas of Japan. In tropical regions of Asia, such as Hong Kong, the Philippines, and Thailand, epidemics of RSV infection occur in the rainy season (9). In northern tropical regions of South America, as observed in Cali, Colombia, RSV infection is epidemic year round (1). These reports suggest that the prevalent season of RSV infection differs depending on global region and climate (1,9) rather than to strain per se. Additional studies are needed in order to further understand the molecular epidemiology of RSV in Okinawa.

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REFERENCES


