Molecular Characterization of an Outbreak of Respiratory Syncytial Virus (Subgroup A) in Havana, Cuba, by Monoclonal Antibodies and Restriction Mapping (N Gene)

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Received 15 July 1996/Returned for modification 20 February 1997/Accepted 3 June 1997

Twenty-one respiratory syncytial virus (RSV) strains isolated from one outbreak in Havana, Cuba (1994 to 1995), were analyzed to determine their relatedness. All isolated strains were classified as subgroup A by monoclonal antibodies. Of 21 RSV strains examined, 20 were classified as having restriction pattern NP4 and only 1 was classified as having restriction pattern NP5.

Respiratory syncytial virus (RSV) is the major cause of lower respiratory tract infection in infants. Annual epidemics in temperate climates usually peak during the winter months, placing considerable pressure on the provision of pediatric hospital beds (11, 14, 17). Two subgroups, A and B, of RSV exist; these were originally defined serologically (1, 8, 15) and later shown to be distinct at the nucleotide sequence level (2, 9, 10).

The isolates of subgroups A and B of RSV can be further subdivided into distinct groupings or lineages by three methods: restriction mapping of part of the N gene, nucleotide sequencing of part of the small hydrophobic (SH) gene, and nucleotide sequencing of the G protein gene (2). In this study we applied the use of monoclonal antibodies and restriction analysis of the N gene for the rapid classification of RSV of subgroup A into these various lineages in one outbreak in Havana, Cuba.

Twenty-one virus isolates were examined in this study; they were from 2- to 9-month-old children admitted to the respiratory ward of “William Soler” Pediatric National Hospital in Havana with the clinical diagnosis of bronchiolitis. The samples used in this study were from the 15 municipalities forming Havana. Isolated viruses were cultured in HEP-2 cells in plastic flasks (Costar, Cambridge, Mass.) with a surface area of 25 cm² and containing 5 ml of minimum essential medium (with glutamine, penicillin, streptomycin, and 5% fetal bovine serum). When extensive cytotoxic effect was present, the cells were detached into the tissue culture medium by shaking with sterile glass beads, and 0.5 ml of each suspension was dispensed and stored at −70°C until further use for PCR analysis. The rest was centrifuged at 2,000 rpm. The cell pellet was employed for indirect immunofluorescence with monoclonal antibodies (specific for subgroups A and B). Identification with monoclonal antibodies was conducted according to previously reported procedures (16). The monoclonal antibodies used in this study were 021/1G, 021/19G, 021/68G, and 021/78G. These monoclonal antibodies have identified three types of epitopes in the G molecule (6, 7): (i) variable epitopes, (ii) subgroup-specific epitopes, and (iii) conserved epitopes, shared by subgroups A and B.

Nucleic acid extraction, reverse transcriptase reaction, and PCR were performed as previously described (2–4).

Distilled water, mixed buffer solutions, a full-time open vial with a final buffer mixture, a nasopharyngeal aspirate from an asymptomatic individual, and RNA from HEP-2 cells were included as negative controls. RNA from an RSV reference strain was prepared as a positive control (12, 18). The cDNA reaction and the PCR were carried out by using a strict protocol, with precautions to prevent contamination (13). After the last cycle of amplification, 10 μl of each amplified product was analyzed by electrophoresis using 2% agarose gels with Tris-borate buffer. The rest of the products were digested with 100 μl of H₂O, extracted with 150 μl of phenol-chloroform, ethanol precipitated, and then digested with HindIII and BglII. The correct interpretation and classification of the restriction patterns obtained were performed according to previous reports (2–5, 10). The primers used for the N gene were as previously described (2–5).

All 21 of the clinical specimens were positive by cell culture and were identified as subgroup A. Primers N1 and N2 yielded a PCR product of 278 bp from all strains of RSV examined. PCR products were digested only with HindIII and BglII since all samples belonged to subgroup A. Of the 21 RSV isolates obtained during the period from 1994 to 1995 in Havana, 20 (95%) of the strains gave an N gene restriction pattern of NP4 and only 1 strain (5%) showed a gene restriction pattern of NP5. Restriction patterns for 19 of the strains are shown in Fig. 1. The PCR products digested from strains 105 and 128 were tested in another gel and showed the NP4 pattern (data not shown).

In the present work we combined the use of monoclonal antibodies and restriction analysis of PCR products of a selected region of the N gene to obtain a complete characterization of an outbreak of RSV in Havana. All the isolated strains were identified as RSV subgroup A by monoclonal antibodies. Restriction analysis was then done for the 21 isolates, which all gave N gene fragment restriction patterns of NP4 or NP5. Moreover, 95% of them gave an NP4 restriction pattern; only one (5%) strain gave a pattern of NP5. These results confirm that two lineages of RSV were present in Havana at the same time (cocirculated locally). The phenomenon of local cocirculation of different lineages of this virus in a single epidemic has frequently been reported by other authors (2–4). For example, the restriction patterns of 46 strains isolated in Birmingham, United Kingdom, were determined by Cane and Pringle in...
1991 (2). Twenty-nine of them were of subgroup A, and 17 were of subgroup B. Of the subgroup A strains, 20 showed the NP2 pattern, 5 showed the NP4 pattern, and 2 showed the NP5 pattern. The patterns of two of them were not determined. It will be interesting to examine strains from this city and other regions in the same country in subsequent years to determine when and where the new NP groups appear or reappear. Analysis of sequential epidemics in Cuba are continuing to explore this question further. Similar to previous data, the results reported in this study were derived from the use of monoclonal antibodies and the restriction analysis of a fragment of the N gene obtained by PCR; the latter method could be used in conjunction with G gene analysis to obtain a more definitive identification of RSV subgroups. Nevertheless, the classification proposed by Cane and Pringle in 1991 for the amplified fragment in this study is very easy to interpret. Thus, it could offer important information regarding molecular epidemiology that may be obtained in laboratories in developing countries. Hence, the method applied in this work is rapid, economical, and convenient for this purpose; it involves reagents that are commonly available commercially; and it is technically much simpler and quicker than nucleotide sequencing.

We thank Jean Handy and Orquidea Biart for critically reviewing the manuscript, J. A. Melero for kindly providing the monoclonal antibodies, and P. Perez-Brena for kindly providing the primers.

REFERENCES