ELSEVIER

Archives of Medical Research 37 (2006) 543–547

Archives of Medical Research

PRELIMINARY REPORT

Respiratory Syncytial Virus Group A and B Genotypes and Disease Severity among Cuban Children

Clara Savón,[a] Angel Goyenechea,[a] Odalys Valdés,[a] José Aguilar,[b] Grehete González,[a] Lídice Palerm,[a] Guelsys Gonzalez,[a] and Pilar Pérez Breña[b]

[a]*Instituto de Medicina Tropical ''Pedro Kourí'', Havana, Cuba*
[b]*Instituto de Salud Carlos III, Majadahonda, Madrid, Spain*

Received for publication October 20, 2004; accepted August 24, 2005 (ARCMED-D-05-00123).

Background. Respiratory syncytial virus (RSV) is the leading cause of serious lower tract infections in infants. Comorbid conditions such as chronic diseases and prematurity have been associated with greater severity illness, but virus genotypes and disease severity is still unknown.

Methods. Forty selected strains of RSV group A and B from Cuban infants with acute respiratory disease (ARD) over five seasons were studied. Viral RNA was extracted and polymerase chain reaction (PCR) was carried out using direct primers directed to parts of the nucleoprotein (N) and fusion (F) genes, respectively. Amplicons were digested using restriction fragment length polymorphism (RFLP) to define the association between virus and disease severity. Disease severity was assessed as very mild, mild, moderate, and severe.

Results. Three of six known N genotypes were detected. NP4 and NP3 were found more frequently; moreover, it was difficult to establish a relationship between N genotypes and disease severity. Five genotypes in F gene were found: F1, F2, F5, F9 and F11; F9 and F11 were associated with very mild disease, but F1 genotype appears to be associated with moderate to severe disease.

Conclusions. At least five combinations of N and F genotypes circulated in the studied infants in Cuba. Patients with F1NP4 genotype showed moderate to severe disease. Relationship between genotypes and disease severity was established.

Key Words: Respiratory syncytial virus, Genotypes, PCR, Illness severity.

Introduction

Respiratory syncytial virus (RSV) is the leading cause of serious lower respiratory tract infection in infants and young children (1). Infection is manifested across a spectrum from mild upper respiratory tract disease to bronchiolitis and pneumonia. This virus is responsible for 50% of all bronchiolitis cases during the first month of life (2).

During primary RSV infection, host factors such as young age (6 months), prematurity and chronic diseases have been associated with severe disease. In addition, lower socioeconomic status, exposure to cigarette smoke, crowded households and lack of breastfeeding have also been associated with greater disease severity. Finally, viral factors associated with virulence, virus genotypes and disease severity are not sufficiently understood (3).

RSV isolates can be divided into two groups: group A and group B by antigenic and genetic characteristics (4). These two groups circulate independently in the human population, with group A being the most prevalent (5,6). Studies that compared severity of infants infected with group A and group B have not revealed significant clinical differences. However, some authors have concluded that infection with group A generally can be associated with more severe illness (6).

Address reprint requests to: Dr. Clara Savón, Instituto de Medicina Tropical Pedro Kourí, Aptdo. Postal 601, Marianao 13, Havana, Cuba; E-mail: clara@ipk.sld.cu

0188-4409/06 $–see front matter.
doi: 10.1016/j.arcmed.2005.08.007

This virus is an enveloped virus with nonsegmented negative-strand RNA genome coding for ten genes. Surface glycoproteins F and G are considered the main antigens responsible for cell attachment and induction of neutralizing immune response, respectively (7).

Fletcher et al., in 1997, reported for the first time the association between virus genotype and disease severity using restriction fragment length polymorphism (RFLP) (8). This analysis was done over PCR products of N and G genes. The gene N is relatively well conserved among virus isolates, but the homology between prototype A and B of the F gene is about 96% (9,10).

Although most studies described the association between G and N genes, in this report we studied the possible association between N and F genotypes and disease severity, taking into account parameters such as age, days of hospitalization, prematurity, and birthweight.

Materials and Methods

Patients and Strains

A selection of 40 isolated strains of RSV group A and B from Cuban children with acute respiratory disease (ARD) over five epidemic seasons were studied.

Selection Criteria

To be enrolled in the study, patients must be <1 year old. In order to rule out a nosocomial infection, the clinical sample for viral isolation, nasopharyngeal swabs should be taken within the first 24 h of admission and 4 days after symptom onset. Detailed clinical histories were obtained from each patient upon admission to the respiratory ward of pediatric hospitals throughout the country. In all cases, an informed consent was signed by parents or guardians.

Immunofluorescence Assay (IFA) and Virus Classification in Antigenic Groups

Initial classification in antigenic group was performed by indirect immunofluorescence assay with monoclonal antibodies for respiratory viruses (Chemicon International, Temecula, CA) (11).

Primers

Primers encoding fusion protein between nucleotides (310–730) and N protein between (760–1033) were used to perform nested and semi-nested PCR according to the protocols proposed by Coiras et al. (12). F1 and F2 were used in the RT-PCR and FA3, FA4, FB3 and FB4 in the nested PCR, which resulted in an amplified fragment of 356 pb for RSV group A and 294 for RSV group B. The primers used in the N gene during the first amplification step were N17 and N18 and in the second step were N18 and N23. The amplified fragment was 278 pb (Table 1).

Reference Strains

The reference strains used in this study were the Long/56 strain for RSV group A and CH18536/62 for RSV group B. Both strains were kindly supplied by Dr. José A. Melero (Centro de Biología Fundamental Instituto de Salud Carlos III, Madrid, Spain).

RNA Extraction, RT and First Amplification Step

RNA was extracted from 1 mL of infected tissue-cultured Hep-2 cells, using the guanidinium thiocyanate method previously described by Casas et al. (13). After processing, the dried pellet was resuspended in 30 μL of RNAse-free sterile water. A single-step RT amplification reaction was carried out with the Promega Access RT system Kit (Promega, Madison, WI), which consisted of a PCR mixture containing 2 mM $MgSO_4$, 300 μM each of dATP, dGTP, dCTP and dTTP; 0.5 μM of specific primers, 10 μL of 5X reaction buffer; 5 U of *AMV* reverse transcriptase (RT); and 5 U of *TFL* DNA polymerase. An aliquot of 5 μL of extracted RNA was added to a final volume of 50 μL. Amplifications were carried out into thin wall tubes (Sorenson Bioscience, Salt Lake City, UT) in a PTC-200 (Peltier Thermal Cycler, MJ Research, Watertown, MA). Samples were subjected to an initial cycle of 48°C for 45 min and 94°C for 3 min. Cycling conditions of the PCR were as follows: 45 cycles of 94°C for 30 sec, 50°C for 2 min; 68°C for 1 min, and a final incubation of 68°C for 10 min.

Semi-nested and Nested Amplification

The PCR nested amplification mixture contained 2 mM $MgCl_2$ (Perkin Elmer, Branchburg, NJ), 200 μM each of

Table 1. Primers of the RT–PCR and nested PCR formerly used to subtype RSV into A and B

F gene primers	Position	Sequence 5′ → 3′
F1 (+)	310−329	ACAATCGRGCCAGAAGAGAA
F2 (−)	730−708	GTTACACCTGCATTAACACTRAA
FA3 (+)	351−372	ACACTCAACAATACCAAAAAWAC
FA4 (−)	707−687	TTCCCTGGTAATCTCTAGTAG
FB3 (+)	320−340	CAGAAGAGAAGCACCACAGTA
FB4 (−)	614−593	GGGTAATAATCGGTTATTTATG
N gene		
N17	760−778	CCTATGGTGTCAGGGCAAGT
N18	846−865	GCAGAAATGGAACAAGTTGT
N23	1033−1015	TTCTTCTGCTGTC/TAAGTCTA

RT-PCR, reverse transcription–polymerase chain reaction; RSV, respiratory syncytial virus.
1 forward; 2 reverse in the first round RT-PCR.
A3 and B3 forward in nested PCR. A4 and B4 reverse in the nested PCR.
N17 reverse; N18 reverse in the first round.
N18 forward and N23 reverse in the semi-nested PCR.