

Human respiratory syncytial virus genomic and antigenic variants isolated in two hospitals during one epidemic, in Santiago, Chile

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Abstract

Background: Human respiratory syncytial virus (HRSV) is a major cause of severe lower respiratory tract infection (LRI) in children. Distinct variants of the viruses have been described.

Objective: The objective was to compare the antigenic and genetic variability of HRSV strains recovered from infants admitted to two hospitals during one epidemic in a big city.

Study design: We analyzed nasopharyngeal aspirates from 201 infants admitted for LRI to two hospitals during 2002 in Santiago, Chile. The analyses were carried out using a panel of monoclonal antibodies against G glycoprotein epitopes (EIA) and RFLP for N and G genes.

Results: No differences in HRSV groups A/B and in N patterns distribution were observed among both hospitals. On the contrary, antigenic and genetic G patterns displayed a wide diversity of strains circulating during one epidemic, in one big city.

Conclusions: RSV variability assessment depended rather on the tool used for analysis than on the geographical location.

Keywords: Human respiratory syncytial virus; RSV molecular epidemiology; RSV diversity; RSV characterization; Chile

1. Introduction

Human respiratory syncytial virus (HRSV) is the principal cause of acute lower respiratory infections (ALRI) in children under 2 years of age (Avendaño et al., 1991; Cane, 2001) causing yearly winter epidemics. The clinical severity of RSV infection has been associated to epidemiological and host factors (Brandenburg et al., 2000). Variability in viral strains might influence in the outcome of the infection. RSV belongs to the family *Paramixoviridae* and displays a non-segmented negative-stranded RNA. The envelope contains glycoproteins

F (gpF) and G (gpG) (Cane, 2001), being G the most variable of the viral proteins. RSV can be divided into two groups, A and B, using monoclonal antibodies (Mab) directed against gpF and gpG (Cane, 2001). RSV strains have also been further assigned to different antigenic subgroups according to reactivity patterns with Mab panels directed against gpG (Galiano et al., 2005a). The analysis of RSV genes by restriction endonuclease digestion (RFLP) and nucleotide sequencing have demonstrated the occurrence of evolutionary intragroup lineages or genotypes, which have worldwide distribution (Cane and Pringle, 1992; Cane, 2001). Distinct variants of groups A and B tend to circulate simultaneously in the world, with their relative proportions varying both geographically and temporally (Cane, 2001; Peret et al., 2000; Sullender, 2000). The clinical and epidemiologic significance of RSV variability still remains unclear (Brandenburg et al., 2000; Cintra et al., 2001; Smith et al., 2002; Walsh et al., 1997). The majority of epidemiologic studies have compared strains recovered in different communities and/or epidemics. Santiago, the capital of Chile, has a population of 6 million and its temperate climate (latitude 33.2°S) yearly constrains RSV's

Abbreviations: HRSV, human respiratory syncytial virus; LRI, lower respiratory tract infection; EIA, enzyme immuno assay; RFLP, restriction fragments length polymorphism; N gene, protein N gene; G gene, attachment glycoprotein gene; NP, patterns of N gene; RNA, ribonucleic acid; Mab, monoclonal antibodies; RR, Roberto del Rio hospital; LCM, Luis Calvo Mackenna hospital; NPA, nasopharyngeal aspirates; IFA, indirect immunofluorescence assay; CPE, cytopathic effect; RT-PCR, reverse transcription-polymerase chain reaction; nM, nanomolar.

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greatest activity to the winter months (Avendaño et al., 2003). The aim of this study was to explore the diversity of RSV strains circulating during one epidemic period in two areas of a big large city.

2. Methods

We studied previously healthy children under 1 year of age, admitted for the first ALRI episode (bronchiolitis and/or pneumonia) in two pediatric hospitals, from May to October 2002, including the hole epidemic RSV season. Roberto del Rio (RR) and Luis Calvo Mackenna (LCM) hospitals attend to middle-low class populations and are located in different areas of the city. Nasopharyngeal aspirates (NPA) were obtained within the first 48 h after admission and processed for viral isolation and indirect immunofluorescence assay (IFA). Samples were inoculated into HEp-2 cells and observed for cytopathic effect (CPE); confirmatory IFA for RSV were performed for cultures with and without CPE, as previously described (Avendaño et al., 2003). The RSV strains obtained from cultures were further submitted to antigenic and genetic characterization.

The antigenic characterization was made by EIA as previously described (Galiano et al., 2005a). Sixteen Mabs (kindly provided by Dr. J.A. Melero, Spain) directed against linear epitopes of gpG and gpF were evaluated: three Mabs against gpG and gpF conserved epitopes in groups A and B (47F, 021/1G and 021/21G); three against group A specific epitopes (2F, 021/2G and 021/19G); two against group B specific epitopes (B1G and B2G); and eight against variable epitopes (021/5G, 021/7G, 021/8G, 021/9G y 25G, 59G, 63G, 68G, 78G). The strains were considered “reactive” if the absorbance was $\geq 25\%$ greater than the absorbance of the reference strains (RSV-A: Mon 3/88 and Long; RSV-B: CH18537). The “antigenic patterns” were defined according to the reactivity to the eight Mabs directed against variable epitopes (Galiano et al., 2005a).

The genetic characterization was done by RT-PCR and RFLP. RNA was extracted from infected HEp-2 cells using a Chomckzynski-phenol solution (Winkler[®]) and chloroform (Chomckzynski and Sacchi, 1987); reverse transcription was performed with primer F844 5'TGTCTAACTATTTGAACA3' (López et al., 1998), 200 μ l reverse transcriptase (M-MLV, Promega[®]), PCR solution 5 \times , 1 mM of dNTPs (Promega[®]), 5 μ l of RNA and water in a final volume of 20 μ l. The mixture was incubated for 60 min at 37 °C, followed by 5 min at 95 °C in a Gene Amp PCR System 2400 thermocycler (PerkinElmer[®]). Fragments of the genes N (nucleotides 858–1135) and G (nucleotides 1–584) were amplified as described previously (Cane and Pringle, 1992). PCR products were purified by columns (Qiagen[®]) and their genetic variability was analyzed by restriction fragments length polymorphism (RFLP) as previously described (Cane and Pringle, 1992). N gene products were digested with Hind III, PstI, BglII, HaeIII and RsaI enzymes, and G

Table 1

Comparison by RFLP of HRSV gene N restriction patterns of strains recovered from two Children's Hospitals, Santiago of Chile, 2002

Gene N patterns	RR hospital (n=51)	LCM hospital (n=47)	p
RSV Group B			
NP1	1	5	0.10
NP3	15	8	0.22
NP11	2	3	0.66
RSV Group A			
NP2	9	12	0.48
NP4	24	19	0.64

gene products, which include the most variable part of the gene, were digested with AluI, TaqI, MboI and MseI enzymes (New England Biolabs[®]), grouping was done in *patterns*. The enzymes used for the gene G analysis were selected with reference to the group A RSV G gene nucleotide sequence.

Chi-square or Fisher tests were carried out when necessary, using the software Epi-Info 6.04 (2001, Center for Diseases Control and Prevention, World Health Organization, Statistical Analysis Software, Geneva, Switzerland). *p*-Values lower than 0.05 were considered as significant.

3. Results

We analyzed 217 NPA obtained from RR hospital and 174 from LCM hospital. HRSV was isolated in 118/217 and 83/174, respectively. We achieved genetic characterization in 51/118 strains and antigenic characterization in 22/118 strains from RR hospital, and in 47/83 and 12/83 strains from LCM hospital, respectively. Only those strains that amplified satisfactory or that grew well enough to provide good protein extracts were included. The HRSV strains reacted with Mabs directed against conserved epitopes of the G and F glycoproteins. The genetic and antigenic classification of RSV isolates into A/B group showed a similar distribution in both hospitals (2/3 A and 1/3 B, *p*=0.93). A total of 11 different antigenic patterns were found, 7 in RR and 5 in LCM hospitals (Fig. 1). Only one pattern was common to both hospitals and accounted for 40.9% (9/22) and 16.6% (2/12) of the RR and LCM strains, respectively. The genetic characterization of N gene made by RFLP showed five patterns: NP2 and NP4 among RSV group A and NP1, NP3 and NP11 corresponding to group B. They were present in both hospitals in similar proportions (Table 1). The variability of the G gene restriction patterns found was very high, suggesting that each strain constitutes an unique pattern; furthermore, some restriction patterns obtained have not been previously described (Table 2).

4. Discussion

Although different HRSV variants may circulate simultaneously during an epidemic, the clinical and epidemiological

Patterns	Mabs				25G	59G	63G	68G	RR Hospital		LCM Hospital	
	021/5G	021/7G	021/8G	021/9G					Nº	(%)	Nº	(%)
Reference strains												
Long												
Mon/3/88												
Studied strains												
IV									1 (4,54%)		5 (41,66%)	
VIII									0 (0%)		1 (8,33%)	
XVIII									6 (27,27%)		0 (0%)	
XXI									0 (0%)		3 (25,0%)	
XXIII									9 (40,90%)		2 (16,66%)	
XXIV									0 (0%)		1 (8,33%)	
XXXII									1 (4,54%)		0 (0%)	
XXXIII									2 (9,10%)		0 (0%)	
XXXIV									1 (4,54%)		0 (0%)	
XXXVI									2 (9,10%)		0 (0%)	
Total									22 (100%)		12 (100%)	

Fig. 1. Antigenic patterns of HRSV isolates obtained from children admitted to two hospitals in Santiago of Chile, 2002. Antigenic patterns were defined by the combination of reactivities with the strain-specific Mabs, according to Galiano et al. (2005a). Reactivity was defined as reactive (■) >25% and no reactive (□) <25% of OD values compared with the prototype strain. Mon/3/88 was the prototype strain for Mabs with prefix 021 and Long was for Mabs 25G, 59G, 63G and 68G.

Table 2

RFLP gene G restriction patterns of HRSV group A strains recovered from infants admitted for ALRI in two Children's Hospitals, Santiago of Chile, 2002

Restriction enzymes	Patterns	RR hospital (n = 34)	LCM hospital (n = 30)	p
Alu I	A	4	6	0.49
	B	0	2	0.21
	C	4	5	0.72
	E	0	1	0.46
	F ^a	26	16	0.092
Taq I	A	10	3	0.10
	B	12	16	0.23
	E ^a	11	8	0.82
	F ^a	0	3	0.09
	G ^a	1	0	1
Mbo I	B	4	0	0.11
	D	1	0	1
	H	22	30	0.001
	J ^a	2	0	0.49
	K ^a	3	0	0.24
	L ^a	2	0	0.49
Mse I	B	3	2	1
	C	0	1	0.46
	D	3	1	0.61
	E	27	16	0.05
	G	1	10	0.003

^a These restriction patterns were not previously described.

significance of this diversity has not been elucidated (Brandenburg et al., 2000; Cintra et al., 2001; Smith et al., 2002; Walsh et al., 1997).

In this study, the analysis of the variability of isolates showed the circulation of many distinct strains in two geographical areas of a big city in one epidemic period.

We did not find differences in the HRSV group A/B distribution among the two hospitals located in different areas of the city, as it has been reported (Hendry et al., 1986). However, we detected a wide diversity among the strains using methods with progressive more discriminatory ability.

Actually, the N gene characterization allowed us to demonstrate the circulation of six genetic patterns previously described (Cane and Pringle, 1992), having similar distribution among both hospitals. The gpG antigenic analyses exhibited a wide variability, detecting only one common pattern for both centers. On the other side, the G gene RFLP study showed so many variants, that there were no common strains among both hospitals. Furthermore, new patterns were obtained, probably on account of the time variability of HRSV, considering that reference patterns were described analyzing isolates from 1988 to 1992 (Cane and Pringle, 1992).

The antigenic characterization by EIA is cumbersome, time consuming and requires the viral isolation. Therefore, it limits the analysis to only those strains that grew well in cell culture, an inherent bias of this technique. Likewise, the great variety of antigenic variants found suggests that this method is not useful for comparing individual strains. Since correlation has been described among genetic and antigenic characterizations (Galiano et al., 2005b), for HRSV strain studies we rather recommend genetic analysis by RFLP or sequencing. The HRSV genetic analysis is commonly made by sequencing the variable portion of G protein (Cane, 2001; Galiano et al., 2005b; Peret et al., 2000). We think it is a tool with intermediate discriminatory ability that could be complemented with the inclusion of other markers for a better comprehension of local epidemiological pattern, as it has been recently shown in influenza viruses analyzing variability of other genes instead of the classical H and N (Nelson et al., 2006).

We conclude that HRSV outbreaks might be conformed by local rather than community patterns. Different strains of HRSV circulate during one season in two areas within a big city. The diversity of HRSV found might be strongly influenced by the discriminatory ability of the techniques used for strain characterization and it must be carefully considered in the study design.

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