
ABSTRACT

We examined the distribution of group A and B strains from respiratory syncytial virus (RSV) during fourteen epidemic years from 1988 to 2001 in Montevideo, Uruguay. IFI or ELISA test with conserved and group specific monoclonal antibodies were used to determine the group of RSV isolates obtained from infants hospitalized due to acute respiratory infection. The predominance of groups varied both between and within yearly outbreaks. Thus, two patterns were determined: (1) strong predominance of group A strains (11 years with 64.7%-100% A strains), (2) strong predominance of B group strains (3 years with 73.3%-100% B strains). The pattern of highly dominant A strains occurred in cycles of up to 4 consecutive years followed by a single intervening year in which B strains were dominant.
INTRODUCTION

Respiratory syncytial virus (RSV) is a major cause of lower tract infections in infants and young children around the world (Collins et al. 2001). Virus isolates were first classified into two antigenic groups, A and B by their reactivities with panels of monoclonal antibodies (Anderson et al. 1985, Mufson et al. 1985). For each group, the attachment protein (G) showed the highest degree of antigenic and genetic diversity (Melero et al. 1997). The antigenic and genetic variability of RSV G glycoproteins from isolates of both antigenic groups have been studied in great detail around the world (Garcia et al. 1994, Martinez et al. 1999, Peret et al. 1998, Peret et al. 2000, Roca et al. 2001, Venter et al. 2001). Several evolutionary lineages were identified among isolates of both groups. Viruses from different lineages were isolated during the same epidemic and in the same place. Moreover, very similar viruses have been isolated in distant places and in different years (Garcia et al. 1994, Cane & Pringle 1995).

In Uruguay, we reported the antigenic and genetic variability of RSV group A isolated during 1987-1992 (Garcia et al. 1994), 1993-2000 (Frabasile et al. 2003) and genetic variability of group B isolated between 1987-1996 (Martinez et al. 1999). Only the occurrence of RSV group A and B during three epidemic years, 1985 to 1987, were examined previously (Russi et al. 1989). The aim of the present study was to analyze the occurrence of antigenic groups A and B during fourteen consecutive years in Uruguay (1988-2001).

MATERIAL AND METHODS

Specimen processing and virus isolation.

Nasopharyngeal aspirates (NPA) were obtained from infants and children under 5 years of age with acute respiratory tract infections, and specimens were sampled as described by Russi et al. (1989). Briefly, NPAs were obtained with a polyethylene suction tube attached to a mucous trap. The mucus remaining in the tube was washed out with 1.5 ml of phosphate-buffered saline (PBS) supplemented with 0.5% gelatin. The samples were transported under refrigeration (melting ice) to be processed in the laboratory within 4h of collection.

NPAs were centrifuged at 350x g for 10 min at 4°C, and the pelleted cells were carefully washed twice with PBS, suspended in a few drops of buffer, spread on six-spot Teflon-coated slides, dried at room temperature, fixed with cold acetone for 5 min, and kept at -70°C until processing.

Antibiotics were added to the NPA supernatant, and the specimens were inoculated into HEp-2 monolayers. RSV isolates were recognized by their characteristic cytopathic effect, and the specificity was confirmed by indirect immunofluorescence (IFI). When approximately 50% of the cells showed cytopathic effect, they were detached with a scraper, washed three times with PBS and the pellet was resuspended in a few drops of PBS. Cell suspensions were spread on six-spot Teflon-coated slides, dried at room temperature, fixed with cold acetone for 5 min, and kept at -70°C until processing.

Immunofluorescence (IF).

In some cases the identification of RSV groups was performed directly in NPA cells or HEp-2 cells infected with each strain (in the period 1988-1990), as was explained above, using a set of monoclonal antibodies (Mabs) using an indirect IF as was described previously by Russi et al., 1989. The Mabs used in the IFI (B90, B18, 8188 and 8943) were generously provided by Dr. Grandien from National Bacteriological Laboratory and Karolinska Institute, Stockholm, Sweden. (Mufson et al. 1985,

**Enzyme-linked immunosorbent assay (ELISA).**

Extracts of infected cells were used as antigens in an ELISA test. Thus, HEp-2 cells were infected with each strain (in the period 1991-2001). When an extensive cytopathic effect was evident by the formation of syncytia, the cells were scraped off with a scraper, pelleted by low-speed centrifugation (3,000x g, 5min), and washed with PBS. The cell pellets were then resuspended in 300 l of lysis buffer (10mM Tris [pH 7.6], 5mM EDTA, 140mM NaCl, 1% octyl-glucopyranosid). The extracts were clarified by centrifugation at 10,000x g for 5min and supernatants were used as antigen in the ELISA assay. The extracts were appropriately diluted in PBS and coated over night at 4 C in a microtiter ELISA plate. Plates were blocked with PBS-bovine seroalbumin 1%.

Mabs were diluted in 0,1% PBS-bovine seroalbumin and incubated with the viral extracts for 1h at 37°C. The Mabs used in this assay raised against the Long F protein (47F and 2F) were kindly provided by Dr. Melero from Instituto de Salud Carlos III, Majadahonda, Spain. One of them, 47F recognized both groups, while the Mab 2F detected only group A strains (Garcia Barreno et al. 1989). The reaction was revealed using biotinylated antimouse immunoglobulin, streptavidin-peroxidase and ortho-phenylenediamine (OPD) - H2O2 citrate phosphate buffer.

**RESULTS AND DISCUSSION**

RSV isolates from Montevideo, Uruguay, spanning a 14-year collection period (1988-2001) were classified into principal antigenic groups A and B (Table 1), and the distribution of groups was examined (Figure 1).

<table>
<thead>
<tr>
<th>Year</th>
<th>Nr. of specimens (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Group A</td>
</tr>
<tr>
<td>1988</td>
<td>36 (94,7%)</td>
</tr>
<tr>
<td>1989</td>
<td>14 (77,7%)</td>
</tr>
<tr>
<td>1990</td>
<td>11 (84,6%)</td>
</tr>
<tr>
<td>1991</td>
<td>4 (26,6%)</td>
</tr>
<tr>
<td>1992</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>1993</td>
<td>4 (100%)</td>
</tr>
<tr>
<td>1994</td>
<td>5 (21,7%)</td>
</tr>
<tr>
<td>1995</td>
<td>5 (71,4%)</td>
</tr>
<tr>
<td>1996</td>
<td>17 (77,2%)</td>
</tr>
<tr>
<td>1997</td>
<td>11 (84,6%)</td>
</tr>
<tr>
<td>1998</td>
<td>10 (100%)</td>
</tr>
<tr>
<td>1999</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>7 (100%)</td>
</tr>
<tr>
<td>2001</td>
<td>11 (64,7%)</td>
</tr>
</tbody>
</table>
In most of the years studied, both groups A and B circulated together during the epidemic season. This result is consistent with previous findings of our country (Russi et al. 1989) and other countries in the northern (Akerlind & Norby 1986; Waris 1991) and southern hemisphere (Siqueira et al. 1991, Carballal et al. 2000, Salomon et al. 1988) where the co-circulation of both groups has been detected during the same epidemic. According to McIntosh et al. (1993), when a sufficient number of strains are examined, both groups can be detected, thus the small number of samples available for 1993, 1998, 1999 and 2000 in our study may explain why one group was not detected. However, in 1995 although only seven strains were isolated and typified, both groups were detected (Table 1, Figure 1).

A wide predominance of RSV group A strains (eleven of fourteen studied) was observed. The occurrence of group A more often than group B is in agreement with several previous reports. In some countries, although both groups were found, group A was always predominant (Storch & Park 1987, Morgan et al. 1987, Carballal et al. 2000).

Two patterns of group distributions were seen, (a) group A predominance (11 years), and group B predominance (3 years). The patterns observed in our study are the same described previously for our country by Russi et al. (1989), in a study of the occurrence of RSV groups A and B during three epidemic years 1985-87. In this study group A predominated during the epidemics of 1985 and 1986, and group B in 1987.

The results of the distribution of the RSV groups obtained in our study during 1988-2001 and previously reported by Russi et al. (1989) during 1985-87, showed a pattern of alternating predominance between A and B groups in Uruguay. Thus, overall, the pattern of 2 to 4 consecutive years of group A predominance was followed by a single intervening year in which group B predominated.

Alternating predominance between the A and B groups, has been reported in several places, as in Japan (Tsutsumi et al. 1988) or Boston, USA (Hendry et al. 1989). But very few reported a clear alternating predominance in
cycles as was observed in our country. For example, in Rochester, USA (Hall et al. 1990) in a study over 15 years the pattern was that 1 or 2 consecutive years of group A predominance were followed by a year with an important increase of group B isolates. In Finland, a regular alteration of group prevalence was observed with a 2-year cycle (Waris 1991).

This pattern of variable dominance in the occurrence of different RSV group strains may suggest a possible role of the group-specific immune response in RSV epidemics.

On the other hand, the results obtained previously from the antigenic and genetic variability within the major antigenic groups of the strains analyzed in this work (García et al. 1994, Martínez et al. 1999, Frabasile et al. 2003) and by others authors (Cane et al. 1994, Peret et al. 1998, Peret et al. 2000) indicate that although a single antigenic group may be dominant for more than one year, a genotypically different virus of that group could be dominant each year.

Future analysis of the occurrence of the major antigenic groups and their antigenic and genetic diversity around the world should facilitate the rational design of vaccines against RSV.

ACKNOWLEDGMENTS

This study was supported in part by grants: CL1*CT94-0012 and IC18CT980374 from the European Union. C. Negro was supported by a grant from Laboratório Santa Elena. We acknowledge Roque Cámara and Ana Navarro for technical assistance.

REFERENCES


Orvell C, Norrby E, Mufson MA 1987. Preparation and characterization of monoclonal antibodies directed against five structural components of human respiratory syncytial virus sub-


