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Antigenic Characterization of Respiratory Syncytial Virus Associated with Acute Respiratory Infections in Uruguayan Children from 1985 to 1987

J. C. RUSSI,[1] A. DELFRARO,[1] J. R. ARBIZA,[1] H. CHIPARELLI,[1] C. ÖRVELL,[2] M. GRANDIEN,[2]* AND M. HORTAL[1]

Virus Laboratory, Microbiology Unit, Dept. de Laboratorios de S. Publica Ministerio de Salud Publica, 8 de Octubre 2720, Montevideo, Uruguay,[1] and Virus Departments, National Bacteriological Laboratory and Karolinska Institute, Fack, S-105 21 Stockholm, Sweden[2]

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The occurrence of subgroup A and B strains of respiratory syncytial virus (RSV) was studied during three epidemic years, 1985 to 1987, in Uruguay. A set of monoclonal antibodies was selected according to their reactivity with local RSV isolates and used for the typing of RSV directly in nasopharyngeal cells by indirect immunofluorescence. Of 77 specimens, 69 could be typed as belonging to subgroup A or B, 5 could not be typed with the restricted set of monoclonal antibodies employed, and 3 reacted with both subgroup-specific antibodies. In 1985 and 1986 subgroup A predominated, accounting for 65.7% of all typed specimens, but in 1987 subgroup B surpassed subgroup A, accounting for 82.4% of the samples.

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infections in infants and young children around the world (12).

In Uruguay RSV infections occur annually in the late fall, winter, and early spring and spread widely among infants and children in the community (7). RSV accounts for 40 to 60% of serious lower respiratory tract disease in children less than 1 year old who require hospitalization (8).

Recently, different groups of investigators (2, 4, 10), using monoclonal antibodies (MAbs) against virus structural proteins, have recognized two major antigenic groups: A and B or 1 and 2.

Since the identification of the two RSV subgroups, only limited studies of their epidemiology have been reported. All of them originated in the developed countries from the northern hemisphere (1, 6, 9) and showed that subgroup A strains occurred more often than subgroup B strains, but both circulated together during the epidemic season.

Because of the potential importance for both vaccine development and an understanding of the epidemiology of RSV subgroups in a temperate area of the southern hemisphere, antigenic characterization of RSV strains isolated in our country was performed by immunofluorescence (IF) with MAbs. Typing with MAbs directly in cell smears from nasopharyngeal aspirates (NPA) was also done, and the results were compared with those obtained by typing tissue culture-isolated strains.

MATERIALS AND METHODS

Specimen processing and virus isolation. Virus isolation was performed in HEp-2 cells inoculated with supernatants of NPA obtained from infants and children below 5 years of age with acute lower respiratory tract infections.

Specimens were sampled as described by Gardner and McQuillan with minor modifications (3, 5). Briefly, NPA were obtained from both nostrils 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 supplemented with 0.5% gelatin. The secretions were transported under refrigeration (melting ice) to be processed in the laboratory within 4 h of collection.

NPA were centrifuged at 350 × *g* for 10 min at 4°C, and the pelleted cells were carefully washed twice with phosphate-buffered saline, 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 processed.

Antibiotics were added to the NPA supernatant, and the specimens were inoculated in HEp-2 cells. RSV isolates were recognized by their characteristic cytopathic effect, and the specificity was confirmed by indirect IF, using bovine anti-RSV serum (Wellcome Ltd., Beckenham, England) and an antibovine fluorescein isothiocyanate conjugate (Wellcome Ltd.). All RSV isolates were stored at −70°C and passaged on HEp-2 cells once before the preparation of cell deposit slides for IF typing.

MAbs. Seventeen MAbs were selected from a collection prepared against RSV subgroups A and B (10, 11). MAbs against subgroup A included four anti-G antibodies specific for two epitopes (G1 antibody C793 and G2 antibodies B14, B18, and B23) and two anti-NP MAbs specific for two epitopes (NP1 antibody B90 and NP2 antibody B60). Antibodies B14, B18, B23, and B90 reacted with subgroup A but not with subgroup B, whereas antibodies C793 and B60 reacted with both subgroups in the IF technique (10).

Antibodies prepared against subgroup B included two anti-G antibodies specific for two epitopes (G1 antibody 8188 and G2 antibody 8943), four anti-F antibodies specific for three epitopes (F1 antibody 7858, F2 antibody 7945, and F3 antibodies 8913 and 9250), three anti-NP antibodies specific for three epitopes (NP1 antibody 8251, NP2 antibody 8296, and NP3 antibody 8275), and two anti-M antibodies specific for two epitopes (M antibodies 9532 and 9115). Antibodies 8188, 8943, and 7858 were specific for subgroup B, whereas the remaining antibodies reacted with both subgroups (11). None of the 17 antibodies used in the study could neutralize the infectivity of RSV in in vitro tests (10, 11).

IF. RSV subgroups were identified by the indirect IF

* Corresponding author.

TABLE 1. Antigenic characterization of 11 RSV strains by IF

RSV strain	IF[a] characterization with following MAb:												Subgroup
	C793 (AB)[b]	7945 (AB)	8913 (AB)	9250 (AB)	8296 (AB)	8275 (AB)	B14 (A)	B18 (A)	B23 (A)	B90 (A)	8188 (B)	8943 (B)	
H45	−	+	+	++	−	+	−	−	−	−	+	−	B
H30	+	++	+	+	+	−	−	−	−	−	++	−	B
60/1	−	++	+++	+++	++	++	−	−	−	−	+	−	B
H49	+	+	+	+	+	+	−	−	−	−	+	+	B
H66	+++	++	+	+	+	+	−	−	−	−	+	−	B
H101	++	+	+	+++	−	+	−	−	−	−	+	+	B
H12	+++	++	++	+++	+	++	−	−	−	−	++	+++	B
421	+	+	+	+	±	+	+	+	+	+	−	−	A
CE275	+	+++	+	+	+	++	++	++	+	+	−	−	A
CE267	+	±	+	+	±	+	−	+	−	−	−	−	A
72/1	+	±	−	−	+	±	±	+	+	−	−	−	A

[a] IF was graded ± to +++ according to intensity.
[b] Subgroup(s) for which MAb is specific.

technique. HEp-2 cells were grown in tubes, and when confluent, the monolayer was infected with RSV and incubated at 37°C. When approximately 50% of the cells showed cytopathic effect, they were washed three times with phosphate-buffered saline and detached with a cell scraper in a few drops of phosphate-buffered saline. Cell suspensions were dispersed on microscope glass slides, dried at room temperature, fixed with cold acetone at 4°C for 5 min, and kept at −70°C until processed.

MAbs diluted 1:15 (except B18 and 8188, which were diluted 1:30) were applied to the fixed cells and allowed to react at 37°C for 30 min. The slides were subsequently washed; fluorescein isothiocyanate-conjugated $F(ab')_2$ fragments of rabbit immunoglobulin against mouse immunoglobulin were added to the spots for 30 min at 37°C. Finally, the slides were washed, mounted with glycerol, and read in an IF microscope. Controls consisted of uninfected HEp-2 cells. All RSV strains were passaged in HEp-2 cells at least two times before being typed.

The identification of RSV subgroups directly in NPA cells was performed with a restricted set of MAbs (B90, B18, 8188, and 8943), using the same general procedure as described for IF.

RESULTS

The pattern of reactivity of local strains was investigated to select a limited number of antibodies for characterization of RSV antigenic variants directly in NPA cells.

Table 1 shows the reactivity of 11 strains with 12 of the MAbs. The reactivity with different MAbs varied, but two virus subgroups could be characterized as previously described (subgroups A and B). Three strains, not included in Table 1, reacted with specific antibodies against both subgroups.

The subgrouping of RSV determined directly in NPA cells, as well as after isolation and passage in tissue culture, was done with seven specimens. There was a total agreement of subgroup classification results by the two procedures. Thus, the investigation was extended to 77 specimens obtained from 1985 to 1987. Of the samples, 69 were associated with subgroup A or B (Table 2), 5 were not possible to type, and 3 reacted with both subgroup-specific antibodies.

In 1985 and 1986 subgroup A predominated over subgroup B (65.7 and 34.3%, respectively), but in 1987 subgroup B was associated with 83.4% of the samples and subgroup A was associated with only 17.6%. The monthly distribution of RSV subgroups is shown in Fig. 1.

DISCUSSION

The advantage of subgrouping RSV directly in NPA cells by IF is that it overcomes differences in recovery efficiency of the RSV strains and constitutes a useful tool for laboratories with limited resources in handling cell cultures and virus isolation.

Our results, as well as a previously published report (6), have shown that subgroup designation by IF in NPA cells is possible. In contrast to the results observed with the isolated strains which were all characterized by MAbs as belonging to subgroup A or B or both, five RSV-positive NPA specimens could not be characterized. It is not known why it was not possible to type the specimens. These specimens contained only a few cells, and when only one MAb was used at the same time, the numbers of epitopes present in the specimen may have been too sparse to allow detection.

Three samples reacted with antibodies specific for both subgroups. The reason for this is not known. The existence of a new subgroup or the existence of a dual infection with two subgroups in these patients cannot be excluded at present. Intermediate strains have been isolated by other investigators (6); they may represent a new antigenic subgroup or a mixture of strains from dual infections.

In each of the three annual epidemics that were studied, subgroup A and B strains circulated together throughout the epidemic, as has been previously described in the northern hemisphere (1, 6, 9). Subgroup A strains predominated in the epidemics of 1985 and 1986, but were surpassed by subgroup B strains in 1987.

Previous reports had shown that although RSV subgroup A strains are on average, three times as common as sub-

TABLE 2. Antigenic characterization of RSV in NPA cells by indirect IF with MAbs[a]

Subgroup	No. of specimens (%)		
	1985	1986	1987
A	12 (70.6)	11 (61.1)	6 (17.6)
B	5 (29.4)	7 (38.9)	28 (82.4)

[a] MAbs B90, B18, 8188, and 8943. Nontypable and intermediate reactivities were excluded from the table.

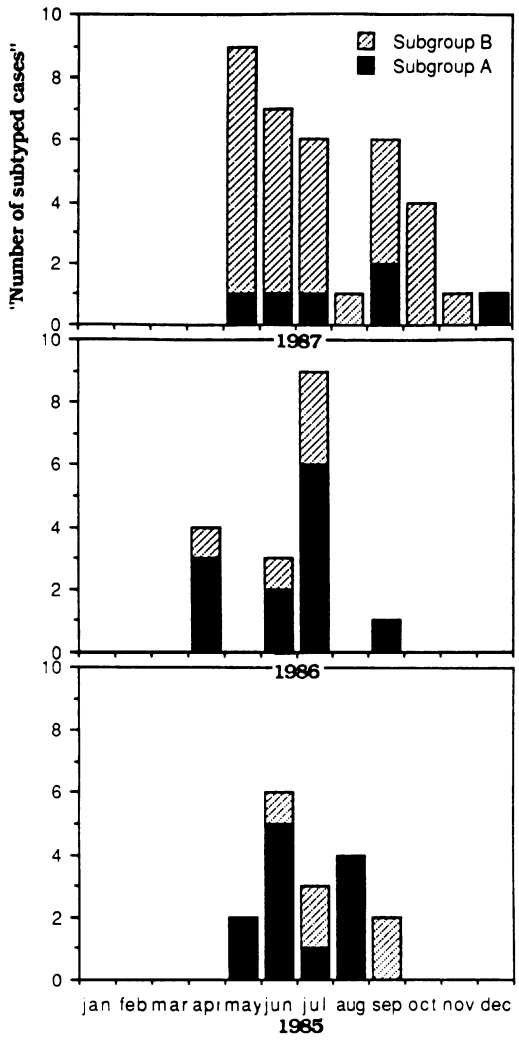

FIG. 1. Monthly distribution of RSV subgroups.

group B strains (6, 9), in some epidemic outbreaks subgroup B strains were more frequent than subgroup A strains (9). Reasons for such differences in the epidemiology of RSV have not been elucidated.

The variable reactivity of RSV strains with the different MAbs is an important fact that must be considered in order to select the most appropriate antibodies for the rapid diagnosis and typing of RSV. A larger number of strains from different regions of the world must be investigated to select the best set.

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