Respiratory Syncytial Virus Subgroup B Dominance during One Winter Season between 1987 and 1992 in Vancouver, Canada

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A subgroup analysis of 613 specimens submitted to the British Columbia's Children's Hospital from 1987 to 1992 revealed that subgroups A and B of respiratory syncytial virus (RSV) were both circulating in our community, with some predominance for subgroup A during the period from October 1987 to September 1988 (the 1987–88 season) (64%), 1990–91 (60%), and 1991–92 (62%). During 1989–90 subgroup A represented the majority of isolates (80%). Subgroup B predominated during only one season, 1988–89 (94%). No microheterogeneity within subgroups was apparent as judged by the monoclonal antibody reactivity pattern. More male than female children were affected overall, but no sex-related difference between subgroup infections could be detected (P = 0.28). The majority of patients were less than 1 year of age, and no significant association between age and subgroup was detected after stratifying for year (P = 0.64). This is, to our knowledge, the first comprehensive longitudinal RSV subgroup prevalence study from the Pacific Northwest and from Canada.

Two distinct respiratory syncytial virus (RSV) subgroups, A and B, exist (2, 3, 19), and recent studies support the notion that subgroup A may be more pathogenic than subgroup B (9, 13). This renews the interest in longitudinal epidemiological studies of RSV subgroup prevalence, which have the potential to help identify predictive parameters for yearly subgroup prevalence. To our knowledge, 14 RSV subgroup epidemiological studies from different parts of the world have been published (2, 6, 9, 10, 15, 18, 22, 24–27, 30, 33–35), including one preliminary report from our hospital (35). These studies, with the exception of a few (2, 9, 30, 34), are limited with respect to either the number of seasons or the number of specimens tested, and none cover the Pacific Northwest. The objective of this study was to determine the prevalence of RSV subgroups in our patient population over a 5-year period, and our limited RSV subgroup data from 1987 to 1989 (35) were therefore extended to cover 1987 to 1992.

Patients and specimens. A total of 3,773 respiratory specimens were collected from children (2 weeks to 18 years old) with acute respiratory disease who were admitted to the British Columbia's Children's Hospital from October 1987 to June 1992. The majority of patients were under 1 year of age; most patients had bronchiolitis, and a few had pneumonia. Nasopharyngeal washings constituted 84% of the specimens; the remaining 16% were tracheal aspirates or bronchial washings. The specimens were brought to the laboratory for virus culture no later than 15 min after collection.

Virus isolation and identification. Three commercially available (Connaught Diagnostics, Willowdale, Ontario, Canada) cell types were used: primary rhesus monkey kidney cells, MRC-5 human lung diploid fibroblasts, and HEp-2 epidermoid carcinoma cells. The cells were maintained in a standard fashion as described previously (31, 35). A 1-ml portion of fresh specimen was diluted in 2 ml of Eagle's minimal essential medium containing 100 U of penicillin, 100 μg of streptomycin, and 10 μg of amphotericin B (Fungizone) per ml, incubated for 30 min at room temperature, and centrifuged at 500 × g for 15 min. Two hundred and fifty microliters of the supernatant was added to two tubes each of HEp-2, primary rhesus monkey kidney, and MRC-5 cells and incubated at 35°C for a total of 14 days. Cells were examined every second day for cytopathic effect, which was noted (on average) 5 to 10 days postinoculation. For the period from October 1987 to September 1988 (1987–88) and for the 1990–91 and 1991–92 seasons, two 10-well microscopy slides of infected cells were prepared when cytopathic effect was noted. One slide was used for identification of the isolate as RSV by using a commercially available RSV identification reagent (Ortho Diagnostic Systems, Inc., Raritan, N.J.) and direct immunofluorescence assay. The second slide was frozen at −70°C until subtyping by indirect immunofluorescence assay was performed. For the 1988–89 and 1989–90 seasons, cultured RSV was identified by direct immunofluorescence assay but the second slide for subgroup analysis was prepared upon reculture of the first passage of the original positive culture.

Monoclonal antibodies and indirect immunofluorescence assay. RSV isolates were subgrouped as A or B by using monoclonal antibodies prepared against a member of subgroup B, RS Wv 4843, and characterized as previously described (21). The monoclonal antibodies represented specificities for glycoprotein G, fusion glycoprotein F, and the NP protein; the known epitope specificities are shown in Table 1. The indirect immunofluorescent assay was performed as previously described (35). Briefly, 20 μl of monoclonal antibodies diluted 1:20 in phosphate-buffered saline (PBS) with 1% bovine serum albumin (BSA) (Sigma Chemical Co., St. Louis, Mo.) was added to two wells (Table 1) on
a 10-well slide and incubated for 20 min at 37°C. The slide was rinsed in PBS, and a fluorescein isothiocyanate-conjugated rabbit anti-mouse immunoglobulin G antibody (Zymed Laboratories Inc., South San Francisco, Calif.) was added at a 1:40 dilution in PBS plus 1% BSA. The slides were washed with PBS, air dried, mounted, and assessed by using a Leitz Laborlux 12 microscope with a mercury short-arc lamp. Fluorescence was graded on a scale of 0 to +4; a positive result was fluorescence graded at >1+. The subgroup identity was assigned according to the antibody reactivity outlined in Table 1.

Of 3,773 respiratory specimens received in our laboratory from 1987 to 1992, 980 were RSV positive by culture (Fig. 1). A few specimens which did not grow after being retrieved from storage at −70°C had to be excluded from subgroup analysis; specimens which did not have a prepared 10-well slide (1987–88, 1990–91, and 1991–92) and repeat specimens from the same patient were also excluded. Besides making these exclusions, we selected a limited number of specimens for subgroup analysis, because of the labour-intensive nature of the indirect immunofluorescence assay employed for RSV subgrouping. At least 50% of the isolates from January to March were typed during the first three seasons (Fig. 1 and 2), with the exception of 1988–89, when 54% of the specimens were excluded in January and 61% in February (Fig. 1 and 2). For the 1990–91 and 1991–92 seasons we excluded only 33 and 32 specimens, respectively. These were all either duplicates or specimens with no prepared slide. The monthly distribution of total RSV isolates for the five seasons and the corresponding distribution of the selected subgroup isolates are displayed in Fig. 1 and 2, respectively. Both subgroups showed classical RSV seasonality throughout the study period, with peak isolation months in February (1988–89), March (1987–88, 1990–91, and 1991–92), and February and March (1989–90). The monoclonal antibody reactivity patterns were the same within each season, and no microheterogeneity within subgroups could be detected with the spectrum of monoclonal antibodies used (Table 1). Each of the five epidemics showed a different RSV subgroup pattern (Fig. 2). The percentages of subgroups A and B differed significantly from year to year (P < 0.001), but no trend was discerned.

RSV subgroups for all epidemics were plotted against patient age in Fig. 3. There was no significant association ($\chi^2 = 0.27; P = 0.64$) between age and subgroups after stratifying for year. The median ages for each season are indicated in Fig. 3. The percentage of isolates from children less than 1 year old for each season follows: 1987–88, 82%; 1988–89, 67%; 1989–90, 66%; 1990–91, 75%; and 1991–92, 86%. For the group older than 1 year the age range was 12 months to 18 years, with the majority of children between 2 and 4 years.

<table>
<thead>
<tr>
<th>Season</th>
<th>MAb* (clone no.)</th>
<th>Protein recognized*</th>
<th>Reaction against:</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Subgroup A</td>
</tr>
<tr>
<td></td>
<td>8.25</td>
<td>NP1</td>
<td>Pos.</td>
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<td></td>
<td>8.188</td>
<td>G1</td>
<td>Neg.</td>
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<td></td>
<td>8.296</td>
<td>NP2</td>
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<tr>
<td></td>
<td>9.351</td>
<td>NP3</td>
<td>Pos.</td>
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<td></td>
<td>9.250</td>
<td>F3</td>
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<td></td>
<td>8.305</td>
<td>G1</td>
<td>Neg.</td>
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<td></td>
<td>7.858</td>
<td>F1</td>
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<td>NP2</td>
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<td></td>
<td>7.858</td>
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<td>Neg.</td>
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* MAb, monoclonal antibody.
* The number in each designation represents a particular epitope for the protein. G, glycoprotein; NP, nucleoprotein; F, fusion protein F.
* Neg., negative; pos., positive.
of age. Data for this group are combined in one bar per yearly graph in Fig. 3.

During all seasons more male than female children were affected. In the five consecutive epidemic seasons, 1987–88 to 1991–92, 59% (50 of 85), 67% (55 of 82), 56% (74 of 133), 53% (84 of 159), and 55% (85 of 154), respectively, of the patients were males. There was no significant difference between the sex ratios for infections with each subgroup ($x_1^2 = 1.16; P = 0.28$).

In this study of hospitalized pediatric patients in British Columbia, we have shown that RSV subgroups A and B coexisted, with subgroup B predominant in only one of five winter epidemics. Although geographically varied, such coexistence of the subgroups is common and occurs worldwide as shown by studies available from the United States (9, 10, 15, 18, 22, 27), South America (24–26), Europe (6, 30, 34), and Japan (33). Some authors have observed a cyclical variation pattern with different time intervals (6, 9, 22, 34), whereas others have reported an unpredictable pattern of strain group occurrence (10, 15, 18, 25, 26, 30, 33, 35). RSV epidemics classically occur during the winter period. The peak months of RSV isolation in our study were January to March, with the isolation rate tailing off during April and May to a few sporadic isolates during two summer periods (Fig. 1). Most studies have reported an apparent disappearance of RSV in the summer. Although the spread of RSV is probably interrupted by school vacations and daycare closures during the summer, our occasional isolation of RSV during this time indicates the continuing presence of the virus throughout the year.

Subgroup A is by far the more common isolate overall in subgroup surveys (6, 9, 10, 15, 18, 22, 24–27, 30, 33–35). There are many potential explanations for this, such as intrinsically higher virulence, lower immunogenicity, and
frequent antigenic shifts of subgroup A. Longstanding evidence suggests that subgroup A is more virulent than subgroup B (30, 35), and two recent comprehensive studies appear to confirm this (9, 13); however, a few contradictory reports exist (5, 22, 24). A retrospective chart review of our 5-year material is in progress to address this question as regards our patient population. Another explanation for A dominance would be a predilection for younger age groups, since nonimmune newborn cohorts are available every year to infect. However, this study did not confirm the significant association between early age and subgroup A previously observed in our limited study from 1987 to 1989 (35).

Subgroup B was the predominant isolate during one of five seasons included in this study. Similar observations have been made in other studies (6, 10, 14, 15, 18, 24, 25, 30). In the largest epidemiological subgroup study to date, which spanned 15 years, subgroup B tended to become more frequent every 5th year and to vary from 61 to 85% prevalence (9). At our center, 94% of isolates were subgroup B during the 1988-89 season. At the same time, subgroup B was predominant in Rochester, N.Y. (61%), and Finland (91%) (9, 33). This suggests that RSV epidemiology may have more of a global pattern than was previously thought. On the other hand, in France only 49% of isolates were subgroup B during the same season (6). A sudden B dominance may be a reflection of a slowly waning group immunity to subgroup B concurrent with boosted group immunity to subgroup A after a few consecutive years of subgroup A dominance. Another possibility is that an antigenic shift may occur in subgroup B which would make it transiently more pathogenic. The fact that our limited panel of monoclonal antibodies did not detect any antigenic differences within subgroup B makes this explanation less likely. There may be other explanations for the infrequent dominance of subgroup B throughout the years. Hall et al. have speculated that subgroup B may elicit a more complete immune response and/or a more long-lasting group-specific immunity than subgroup A (9). However, if this were the case, one would expect fewer subgroup B infections with increasing age, which was noted neither in the large Rochester study (9) nor in this study. Subgroup B might also be less prone than subgroup A to shift its antigenic epitopes. Many studies have identified antigenic variants within the two major subgroups (1, 2, 4, 7, 9-11, 15, 16, 20, 21, 26, 28, 29, 32); variations in the G protein appear to be the most common, whereas the other major surface proteins, F and NP, are relatively well conserved (1, 3, 4, 9-11, 15, 16, 20, 26, 28, 29, 32). The detection of antigenic variants is dependent on the panel of monoclonal antibodies used. We could not detect any antigenic difference between the subgroups in the current study, in which we used a panel of five to eight monoclonal antibodies which each recognized either the G1, NP1, NP2, NP3, or F3 epitope (Table 1). Although we cannot currently exclude other microheterogeneities, particularly in other G epitopes, this suggests to us that our locally circulating subtypes are relatively antigenically stable. A study by Freymuth et al. (6), using a limited panel of monoclonal antibodies, also showed a remarkably stable antigenic pattern throughout seasons. The largest number of antigenic variants was recently reported by Anderson et al. (2), in a multicenter study. They used an extended panel of monoclonal antibodies and were able to show geographic clustering of antigenic variants within North America; they pointed out that antigenic patterns are observed at the regional level, at the most, but in many instances are only local (2). A contradictory observation was reported by Cane et al., who used PCR to show that very similar subgroup A viruses are present simultaneously in widely separated countries (5). The infrequent finding of subgroup B compared with subgroup A could also represent a laboratory phenomenon, inasmuch as subgroup B may be less easily detected by culture than subgroup A. This has been suggested by reports which show that subgroup B requires a longer time for growth than subgroup A (15). The choice of permissive cell lines for detection of the virus may therefore have an impact on subgroup prevalence. In our laboratory we have shown that subgroup B on average requires nearly 2 more days than subgroup A (\(X_A = 6.21 \text{ days}; X_B = 8.08 \text{ days}\)) to grow on HEp-2 cells (\(P = 0.001\)) and 1 more day on primary rhesus monkey kidney cells (\(X_A = 6.84 \text{ days}; X_B = 7.85 \text{ days}\) \(P = 0.009\)). The average isolation time on MRC cells is 8.70 days for both subgroups. However, this phenomenon is unlikely to have played a role in our study, since we monitor our cultures for cytopathic effect for 14 days before they are considered negative. Another explanation for the overall lower number of subgroup B infections in our material could be that subgroup B causes less severe infections and is therefore not recognized as often. Our data obtained with material from hospitalized patients do not answer this question. Hall et al. have addressed this issue and could not detect any difference between the A and B proportions in a limited amount of material from outpatients and the proportions in hospital material obtained concurrently (9).

Although 94% of the isolates in our material were subgroup B during the 1988-89 season, subgroup A was still present. This is in concordance with the possibility of an overall increased immunity to subgroup A during that season. The immune response to RSV is classically of short duration, and there is little cross-protection between subtypes, as shown by Mufson et al. in a study of 13 children with two RSV infections in consecutive years (17). They observed that if the first infection was from a subgroup A strain, the second would more likely be a subgroup B strain than would be expected epidemiologically. In this study we have shown that the 1989-90 season, which followed the sole season of group B predominance, contained the highest proportion of subgroup A (80%) during our 5-year period; a successive decline of subgroup A prevalence occurred during the two following seasons. This phenomenon (called "rebound" A predominance) has been reported previously (9, 16). The weak overall immunogenicity (16) of RSV and progressive immunization against one subgroup after several epidemics of this virus could eventually exclude one strain and favor the other to occupy an almost exclusive place, as during our 1988-89 season.

RSV remains the single most important pediatric respiratory viral pathogen worldwide (8). The only currently available antiviral treatment for serious RSV infections is Ribavirin, which is costly and requires administration in an aerosolized form by the use of specialized equipment (12). Subgrouping of RSV has the potential to become an important tool in predicting the next year's predominance: if the more virulent subgroup A were predicted to dominate, appropriate resources in the form of staff, bedspace, and pharmacy funds could be allocated to meet increased seasonal requirements of admissions of infected infants and demand for Ribavirin for serious RSV infections in pediatric hospitals. Epidemiological studies of RSV subgroup prevalence also provide a valuable basis for the design and development of vaccines and improved antiviral agents for RSV.
REFERENCES


