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Subgrouping of respiratory syncytial virus strains from Australia and Papua New Guinea by biological and antigenic characteristics

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Summary. Strains of respiratory syncytial virus from 3 major areas of Australia and Papua New Guinea (PNG) were analyzed for variations in their antigenic and biological properties and in the molecular weights of their major structural proteins. Seventy-eight strains from infants and young children with LRI were collected from 1981–1984. The RSV season in the Australian cities lasted from April through September, with major peaks in July of each year, while the RSV season in tropical PNG was year-round, with small peaks in March and October of each year coinciding with excessive rainfall. Fifty-six strains were analyzed in detail; 40 were typed by time-resolved fluoroimmunoassay with monoclonal antibodies as group A strains and 16 were group B; both groups were concurrent. Three children of one family had sequential RSV infections 13 months apart, and the etiologic group A strain was identical both years in terms of growth and antigenic properties with strain-specific ferret antisera; the second infection was milder in all three children. On average, the group A strains replicated considerably better than group B strains in HEp2 cells, producing 53% more syncytia and 99% higher infectious virus titers in 31% less time in culture. Ten group A and B reference strains exhibited the same growth patterns as the A and B regional strains, respectively. Differences in antigenicity as measured with hyperimmune antisera to prototype Long strain were even greater. Group A strains exhibited a mean 68% greater IFA staining than B strains, a 71% greater EIA reaction, and were neutralized to 69% higher serum titers than B strains. Again, the reference A and B strains included as controls gave patterns identical to those of the regional strains. Finally, the P phosphoprotein had consistently higher molecular weight in A strains (mean

35 900) than B strains (mean 33 100). Small variations in the sizes of the F and G glycoproteins were not sufficient to suggest grouping on this basis.

Introduction

Respiratory syncytial virus (RSV) is well documented as the major cause of acute lower respiratory tract illness (LRI) in infants and young children in many parts of the world [5, 8, 23, 35, 48, 56]. Two major antigenic groups of RSV were noted by cross-neutralization tests soon after the virus was discovered [12, 14, 16, 51, 59], and these groups, now called A and B, were later further characterized by monoclonal antibodies to the F, G, M, P, and N proteins of RSV [4–7, 19, 21, 38–40, 44, 54, 57] and by sequence information from the F, G, SH, and N genes [8, 33, 49]. These data have been recently reviewed [3, 10, 35, 56].

Only limited information is available on the prevalence and strain pattern of RSV in countries of the Southern Hemisphere, however. RSV is thought to be a major cause of LRI in these countries, but the seasonal pattern and other epidemiologic features may be different from Northern Hemisphere countries [15, 17, 24]. Little strain data of any sort is available. In this study, we analyzed a large number of RSV strains from selected Southern Hemisphere areas to determine a) the antigenic grouping of strains involved in RSV disease; b) the extent of antigenic, biologic, and polypeptide pattern variability among them; c) a comparison of Australian strains with reference RSV strains from around the world; and d) the epidemiology and growth characteristics of these strains.

Materials and methods

Viruses and cell culture

The prototype strains of RSV group A (Long) and group B (CH-18537) were obtained from our reference virus collection at the $KB_6L_2HEp2_1$ and $xHEp2_8$ passage levels from the mid-1960s, respectively. Other reference RSV strains also were obtained from this collection (see Table 2). Isolates of RSV from 1981–1984 were made in various cells depending on originating laboratory: HEp2, MRC5, BSC1, and CV1 (Newcastle, N.S.W., Australia); HeLa, HEL, and MK (Melbourne, Victoria, Australia); and HEp2, HF human embryonic lung fibroblast, LLC-MK2, and MDCK (Goroka, Eastern Highlands Province, Papua New Guinea). All isolates were identified in the initial laboratory by IFA and EIA tests. All passages used in this study were made in HEp2 cells under Medium 199 maintenance medium with 2% heat-inactivated fetal calf serum (FCS) and incubated on a roller or rocker platform at 36 °C for 5–14 days as previously described [30, 52]. Tubes and flasks were harvested at 4+ cytopathology (CPE) by scraping and then stored at −80 °C in aliquots as crude working stocks for the preparation of test antigens and for antibody production. Due to the lability of RSV, an aliquot was used only once, and tests were run in a manner to minimize the exposure of virus to ambient temperatures.

Antibodies

Horse anti-RSV hyperimmune serum and bovine anti-RSV serum, both against Long strain, were obtained from Flow Laboratories, McLean, VA, U.S.A. and Wellcome Research

Laboratories, Beckenham, England, respectively. RSV monoclonal antibodies (MAbs) were previously described [5–7] and were used as purified IgG from mouse ascitic fluids; some antibodies were labeled with europium as described [27, 28]. Ferret hyperimmune antisera were prepared in non-castrated, unvaccinated juvenile ferrets (Jack Forbes, Dungog, NSW, Australia) that were given three biweekly intranasal administrations of live RSV, 0.8 ml/nostril, after mild sedation with 0.3–0.5 ml ketamine, and exsanguinated 9 days after the third inoculation. The viruses used for antiserum production had minimum infectivity titers of $10^{8.5}$ $TCID_{50}$/ml in HEp2 tube cultures in 12 days and gave maximum readings in IFA and EIA tests with polyclonal antisera. Goat anti-ferret-IgG serum was prepared at CDC by two subcutaneous injections, 6 weeks apart, of 4 mg purified ferret IgG mixed with Freund's incomplete adjuvant, followed by a volume bleed 2 weeks later; the resulting goat IgG was labeled with peroxidase for use in western blot assays.

Indirect fluorescent antibody test (IFA)

IFA was carried out as previously described [22, 26] using spots of infected washed cells that were fixed in cold acetone and reacted with bovine anti-RSV serum at a 1:8 dilution (30 µl/spot, incubated 30 min at 37 °C in a humidified chamber), followed by extensive rinsing in PBS, air-drying, and reaction with fluorescein-coupled goat anti-bovine IgG (Kirkegaard and Perry, Gaithersburg, MD, U.S.A.) at a 1:40 dilution (30 µl/spot, incubated 30 min as above). The slides were washed 3 × as above, air dried, and read at 250 × without oil under incident fluorescence.

Enzyme immunoassays (EIA)

The EIA procedures were modifications of those previously described [7, 29]. Capture antibody was purified horse anti-RSV IgG at 10^{-3} dilution in 0.01 M carbonate buffer, pH 9.6 (100 µl/well in flat-bottom plates), with incubation overnight at 4 °C in a moist box. Wells were next saturated with 0.5% gelatin for 30 min at 37 °C. Virus was added for 1.5 h at 37 °C as a 1:3 dilution in PBS-GT (0.01 M PBS, pH 7.2 with 0.5% gelatin and 0.15% Tween-20), which was also the wash solution used between each step. The detector antibody was bovine anti-RSV IgG at 10^{-3} dilution in PBS-GT with 2% normal horse serum (PBS-GTH), also incubated 1.5 h at 37 °C. The conjugate was goat anti-bovine IgG H & L-peroxidase (Nordic Immunology, Tilburg, The Netherlands) at 10^{-4} dilution in the PBS/GTH diluent, incubated 1 h 37 °C. Finally, the *o*-phenylenediamine/H_2O_2 substrate was added (125 µl/well, incubated 45 min at room temperature in the dark), the reaction stopped with 25 µl of 3.5 M HCl, and the test read at 492 nm.

Time-resolved fluoroimmunoassay (TR-FIA)

The purification and europium labeling of monoclonal antibodies from mouse ascitic fluids was done as described [27, 28]. All antibodies were titrated in various capture/detector combinations to determine the most sensitive format and the optimal dilution of each antibody. The one-incubation TR-FIA procedure also was done as described [28]. Briefly, purified monoclonal IgG as capture antibody was diluted to optimal concentration in pH 9.6 carbonate buffer, added to wells of 12-well polystyrene strips in 250 µl volumes, and adsorbed overnight at ambient temperature in a moist chamber. The wells were washed 3 × with aqueous 0.9% NaCl/0.05% Tween-20, and then saturated with 250 µl of 0.1% gelatin in 0.05 M Tris/0.9% NaCl/0.05% NaN_3 buffer, pH 7.75. For the test, the wells were washed 3 ×, and 100 µl each of antigen and Eu^{3+}-labeled detector antibody were added to appropriate wells. The antigen (as cell culture suspension) was diluted 1:3 in specimen

diluent, consisting of 50 mM Tris, pH 7.75, 0.9% NaCl, 0.01% NaN_3, 0.5% gelatin, 0.01% Tween-40, 20 µM DTPA, and 2% BSA; the detector antibody was diluted to the appropriate concentration in the same diluent. The strips were then incubated for 1 h at 37 °C in a moist chamber, washed 6 ×, and 200 µl per well of enhancement solution was added. The plates were gently agitated on a shaker for 10 min at ambient temperature and then placed in a fluorometer for counting [27, 28].

For group A strains, the optimal format was MAb 130–8F at 0.5 µg/well for capture antibody and MAb 131–2A at 25 ng/well for detector, which was the most sensitive format previously reported for RSV [27, 29]. For group B strains, the optimal format was MAb 133–1H at 0.5 µg/well for capture and MAb 102–10B at 50 ng/well for detector. MAbs 130–8F, 131–2A, and 133–1H were derived from strain A2 (group A) immunogen, and MAb 102–10B was derived from strain 18537 (group B) virus; all antibodies are directed to the F protein (5–7).

Serum neutralization (SN) test

SN tests were carried out in HEp2 cells by standard procedures, using stock passages that were first titrated for infectivity. The serum titer was defined as the reciprocal of the highest dilution of serum inhibiting 30–70 $TCID_{50}$ of virus per 0.1 ml for 12 days.

PAGE and western blots

Slab gel electrophoresis of RSV polypeptides from purified virus was done as previously described [30]. For western blots, the viruses were grown in HEp2 cells under EMEM with 2% FCS for 3–7 days (before any CPE was evident), then the monolayers were washed 1 × with PBS, overlayed with PBS, and incubated 12 additional h. The cultures were harvested by 2 × freeze-thaws and light centrifugation. The supernatant fluids were then solubilized, electrophoresed under both reducing and nonreducing conditions, and the proteins identified by the blotting technique of Tsang et al. [53] as previously described [28].

Results

Virus strains

Seventy-eight isolates of RSV were initially studied for epidemiologic purposes; all of these strains were identified by IFA and EIA in the originating laboratory and then confirmed as RSV by the polyclonal EIA test in this study. Twenty-two strains picked at random were then excluded from the detailed study to render the number more manageable. RSV isolates from Newcastle (1981–1984), Melbourne (1983–1984), Fiji (1984), and the eastern highlands of Papua New Guinea (Goroka plus 15 villages around Goroka, 1982–1984) were recovered from nasopharyngeal aspirate (NPA), nasal swab, and throat swab specimens from infants and children with various lower respiratory tract infections (Table 1). Most of the children studied were admitted to hospital for pneumonia. The 56 strains studied in detail were compared with recognized strains used as controls in our laboratory, 5 group A and 5 group B strains (Table 2); these strains were isolated between 1956 and 1979.

Table 1. Source of 78 RSV strains from the Australia region, 1981–1984

Strains	Locale	Number of strains			Date	Age (yrs.)
		total/studied		illness	month/year	mean (range)
V1890/A–C	Newcastle	3	3	bronchitis	Jun–Jul, 1981	1.5 (0.5–2.5)
V1891/A–T	Newcastle	21	11	LRI	Jun–Aug, 1982	1.6 (0.5–3.4)
V1883/D–M	Newcastle	11	8	bronchitis, LRI, bronchiolitis, pneumonia	Jun–Jul, 1983	1.7 (0.6–3.2)
V1884/I–P	Newcastle	8	8	LRI	Jul–Aug, 1984	1.8 (0.4–3.5)
V1885/A–E	eastern PNG	5	3	pneumonia	Aug–Dec, 1982	0.7 (0.3–1.2)
V1886/A–M	eastern PNG	13	9	,,	Jan–Oct, 1983	0.8 (0.2–2.3)
V1888/A–F	eastern PNG	6	3	,,	Jan–Apr, 1984	0.5 (0.2–1.0)
V1889/B–H	Melbourne	7	7	bronchiolitis, URI[a], pneumonia, whooping cough	Jun–Aug, 1983	1.9 (0.3–3.5)
V1889/I–K	Melbourne	3	3	pneumonia	Jun–Jul, 1984	1.6 (0.5–3.0)
V1889/L	Fiji	1	1	pneumonia	July, 1984	0.5

[a] Includes 1 18-year old female and 1 57-year old female with URI who were not counted in the age column

Table 2. Reference strains used for comparative analyses

Group	Strain	Origin	Citation
A	Long	Baltimore, MD, 1956	11
	159/59	Gothenburg, Sweden, 1959	16
	11657	Washington, DC, 1960	12
	A1	Melbourne, Victoria, 1961	34
	V617/B	San Paulo, Brazil, 1979	7
B	18537	Washington, DC, 1962	14
	8/60	Gothenburg, Sweden, 1960	16
	RSN-2	Newcastle-upon-Tyne, England, 1972	9
	RSN-1599	Newcastle-upon-Tyne, England, 1975	9
	RU-9320	Boston, MA, 1977	31, 42

Antigenic grouping of strains by TR-FIA

Using MAbs to F protein and carefully optimized test formats, the 56 Australia-region strains and 10 control strains were tested by group A- and group B-specific TR-FIA for division into appropriate group (Table 3). Group A strains gave fluorometric cps values ranging from 2 627 to 142 719 with the group A format, and values of 1 to 297 with the group B format, for a mean 500-fold difference in counts/second (cps) between the A and B formats. Group B strains gave lower values, ranging from 1 608 to 32 528 cps with the group B format and 10 to 1 387 cps with the group A format, for a mean 26-fold

Table 3. Grouping of 56 strains by TR-FIA

Locale	Number	130–8F/131–2A cps[a]		133–1H/102–10B cps[a]	
		mean	(range)	mean	(range)
Group A strains					
Newcastle	25	56,534	(21,188–95,091)	102	(34–252)
Eastern PNG	7	43,110	(2,627–142,719)	82	(70–126)
Melbourne/Fiji	8	41,704	(13,821–56,435)	93	(55–134)
Controls	5	60,927	(29,689–99,385)	128	(1–297)
Group B strains					
Newcastle	5	637	(37–1,387)	13,245	(1,622–25,617)
Eastern PNG	8	363	(40–793)	11,982	(1,608–23,927)
Melbourne/Fiji	3	460	(43–889)	12,618	(1,778–22,113)
Controls	5	765	(10–1,336)	18,893	(1,810–32,528)

[a] Fluorometric counts per second (cps) with group A-specific format and group B-specific format, respectively; all values included testing with 2–3 passages of each strain

Table 4. Prevalence of RSV A and B strains by locale and year

Locale	Year	Group A	Group B
Newcastle	1981	1	2
	1982	11	0
	1983	7	1
	1984	6	2
Eastern PNG	1982	1	2
	1983	4	5
	1984	2	1
Melbourne/Fiji	1983	5	2
	1984	3	1
Total		40	16

difference between A and B. Within each A and B group, the control strains gave similar cps values as the Australia-region strains. Although substantial variation in counts was observed within each locale and group, all strains clearly typed out as belonging to group A or B. The low cps values were felt to be due to poorly grown or inadequately stored cultures because fresh culture supernatants invariably gave higher counts.

Both A and B strains were found throughout the Australia region in each of the 4 years surveyed (Table 4). Group A predominated from 1982 through 1984; the first year sampled (1981) was not sufficiently represented to determine prevalence. Infections with group A RSV thus outnumbered group B infections by more than 2:1.

Table 5. Biological and gross antigenic properties of A and B strains[a]

Group	Number	Syncytial formation[b]	Culture time[c]	Infectivity titer[d]	Versus Long antiserum		
					IFA[e]	EIA[f]	SN[g]
A	40	2.6 (1.8–4.0)	6.1 (3.1–7.8)	6.8 (5.0–8.5)	3.6 (2.8–4.0)	2.5 (1.4–2.8)	80 (20–320)
A controls	5	2.7 (1.8–4.0)	6.1 (4.0–7.6)	6.9 (5.3–8.3)	3.8 (3.0–4.0)	2.6 (1.4–2.8)	160 (40–320)
B	16	1.2 (0.2–2.8)	8.8 (5.5–14.0)	4.5 (3.3–6.3)	1.1 (0.1–2.0)	0.6 (0.2–1.9)	5 (<5–10)
B controls	5	1.3 (0.4–2.6)	8.7 (5.6–13.1)	4.8 (3.4–6.5)	1.2 (0.3–2.4)	0.8 (0.4–2.4)	5 (<5–10)

[a] For each property, the value given is the mean (and range) of 3–7 determinations for each strain in the category

[b] Degree of syncytial formation at the end of the culture period, on a scale of 0 (no syncytia observed) to 4 (syncytia over entire monolayer)

[c] Days for complete (4+) CPE to develop in HEp2 cells after inoculation with a standard dose of virus (MOI = 0.1) and incubation at 36 °C in roller culture

[d] Standard infectivity titer in HEp2 cells after 12 days of incubation at 36 °C in roller culture, expressed as $\log_{10}TCID_{50}$/ml

[e] Indirect fluorescent antibody test with virus culture vs. bovine anti-Long serum followed by FITC-labeled goat anti-bovine-IgG; readings were scored on a scale from 0 (no fluorescence) to 4 (the degree of fluorescence with Long virus in the test)

[f] Enzyme immunoassay with virus cultures vs. horse anti-Long capture antibody followed by bovine detector antibody and goat anti-bovine IgG-peroxidase; readings were spectrophotometric absorbance values at 492 nm, from 0 to 3000 maximum

[g] Standard serum neutralization test with serial dilutions of horse anti-Long serum; values are reciprocals of highest dilution of serum inhibiting 30–70 $TCID_{50}$'s of virus per 0.1 ml after 12 days of incubation at 36 °C in roller culture

Variation in biological properties

Throughout the study, some strains were found to replicate to higher infectivity titers and in less time than other strains. Further, differences in degree of syncytial formation were seen and were repeatable in subsequent cultures. This had been observed in our laboratory previously and was one of the stated goals of this study. The growth characteristics of the strains are summarized after division into group A and B strains (Table 5). According to the definitions given in Table 5, the mean scoring of syncytia developing during culture ranged from 1.8 to 4.0 for group A regional and control strains, and from 0.2 to 2.8 for group B strains; the duration of culture to 4+ CPE after inoculation with 0.1 M.O.I. of virus ranged from 3.1 to 7.8 days for group A and from 5.5 to 14.0 days for group B strains. Infectious virus titers also were higher in group A cultures, ranging from $10^{5.0}$ to $10^{8.5}$ $TCID_{50}$/ml for group A regional and control strains, and from $10^{3.3}$ to $10^{6.5}$ $TCID_{50}$/ml for group B strains. Thus, group A strains (40 Australia-region isolates and 5 control strains) produced 53% more syncytia than group B strains (16 regional and 5 control strains)

and 99% higher infectious virus titers while replicating to complete (4+) CPE 31% faster.

Variation in antigenic properties

Reactions of the strains against prototype Long antisera were even more distinct (Table 5). IFA tests scored against prototype Long virus as the maximum fluorescence gave 68% higher values with group A strains compared to group B on the average, and polyclonal EIA tests using horse capture and bovine detector antibodies to Long strain gave a mean 71% higher absorbance with group A strains compared to group B. Crude neutralization titers were also much higher with group A strains, ranging from 1:20 to 1:320 for regional group A strains and 1:40 to 1:320 with reference group A strains, but only <1:5 to 1:10 for all group B strains, a 69% difference. These data are important in the clinical laboratory, where these are the tests commonly used to identify RSV isolates.

Re-infection in three children

Of particular interest was one family of three children, ages 1–3 years, who developed RSV infection in June 1983 and again in July 1984. The youngest two children were hospitalized with severe pneumonia the first year; none were hospitalized the second time. Fever and duration of illness were also reduced in the second infection. Both infections were caused by group A strains, and the viruses were identical as judged by a) identical growth and antigenic properties, as in Table 5; b) identical subgrouping (subgroup A4) when tested by EIA with a panel of MAbs (5); and c) identical cross-neutralization titers in ferret antisera made against these specific strains. Thus, the 1984 RSV strain which infected these siblings just 13 months later appeared to be identical to the 1983 virus in all three children.

Variation in protein sizes

The final criterion used to compare RSV strains from the Australia region was the size of the structural proteins in purified virions as analyzed by PAGE under reducing conditions and western blots with polyclonal and monoclonal antibodies. Western blots to compare the L, F, G, M, P, and N proteins utilized horse anti-Long serum and ferret antisera to all 10 reference strains listed in Table 2, followed by the appropriate anti-species IgG-peroxidase. Blots to further compare the F and G glycoproteins and the N nucleoprotein utilized MAbs 63–10F and 92–11C (anti-Long G and F, respectively); 100–7G and 102–10B (anti-18537 N and F, respectively); and 130–2G (G), 130–8F (F), 131–2A (F), 131–4G (N), and 133–1H (F) (all anti-A2 strain) [7], followed by anti-mouse peroxidase. As expected, the F and G proteins of group A strains reacted more intensely with polyclonal group A antibodies than with group B antibodies, and F and G of group B strains likewise stained more strongly with polyclonal group B antibodies. With MAbs to F and G, the staining intensity

could only distinguish groups A and B if the particular epitopes on the antibodies were group-specific; thus, MAbs 92–11C, 130–8F, and 131–2A clearly indicated group A, and 102–10B was specific for group B strains, as previously described in other test systems [4–7]. No distinction between A and B groups could be seen in migration distance of the G, F1, or F2 reduced polypeptides, although variability was evident.

Staining intensity in the other protein bands was equivalent in both RSV groups. Also, variations in the molecular weights of the L, N, and M polypeptides from group A viruses were within the same range as the MW variations in group B virus proteins. Only the size of the P phosphoprotein was a distinguishing property. The mean MW of P from group A strains was 35 900 (range 34 800–37 100), while the mean MW of P from group B strains was 33 100 (range 30 700–34 200).

Discussion

RSV is the major cause of childhood pneumonia and other LRI in both hemispheres [17, 35, 48]. Survey data we obtained in the Goroka region of the eastern highlands of PNG for calendar year 1983 revealed that 38% of all etiologically-diagnosed LRI was caused by RSV, followed by CMV, influenza A, measles, parainfluenzaviruses, adenoviruses, and picornaviruses, in decreasing order. RSV was isolated every month, with small peaks during the rainiest months of March and October, similar to the rainfall-associated incidence of RSV in Hawaii [43]. During a 5-year period (1980–1984) in Sydney and Newcastle, the two largest metropolitan areas of New South Wales, RSV averaged 36% of all NPA isolates (from patients of all ages), followed by herpes simplex (19%), adenoviruses (10%), entero-, influenza-, and parainfluenzaviruses (each 8%), rhinoviruses (6%), CMV and measles (each 2%), and mumps and reoviruses (each $<1\%$). The "RSV season" in Melbourne, Sydney, and Newcastle extended from April through September each year, with the major peak always in July (early winter) in all three cities. This exactly matches the winter peak of RSV in temperate zones of the Northern Hemisphere.

Antigenic differences between strains of RSV were recognized almost as soon as the virus was first described. The first indications of antigenic variation were by cross-neutralization tests with hyperimmune animal antisera [12–14, 16, 51, 59], and these studies were later expanded by EIA and IFA tests with monoclonal antibodies directed to specific RSV proteins [5, 7, 21, 40, 44]. More recently, strain differences have been analyzed by F and G protein cross-immunity studies [33], group-specific cDNA probes [49], nucleotide sequencing of the SH and N genes [8], and other methods not cited here.

Multiple studies of RSV strains from the Northern Hemisphere have shown that two major antigenic groups exist, group A and B (formerly 1 and 2). Many of these studies also revealed antigenic or nucleotide diversity within groups, which has resulted in several subgrouping schemes [2, 4–8, 18, 23, 38, 47, 49, 50]. Patterns of A and B outbreaks or infection by diverse strains have been

described from Canada [5], Finland [8, 57, 58], France [20], Germany [8], Japan [54], Malaysia [8], Russia [60], Sweden [1], the UK [8, 21, 37, 48], and the U.S.A. [5, 23, 25, 36, 38–40, 43]. Both antigenic groups exhibit similar epidemiologic and clinical disease patterns [3, 10, 25, 32, 35, 48, 56], although some studies have reported more severe disease with group A than with group B viruses [23, 38]. In all of these studies, group A infections usually exceeded group B both in numbers of epidemics and in numbers of cases.

Little information is available on strain differences among RSV isolates from the Southern Hemisphere. RSV without strain differentiation has been reported from American Samoa [24], Sydney, Australia [15], and throughout the South Pacific [17]. Groups A and B have been identified in Argentina [46], Uruguay [8, 45], and southern Brazil [47] during the particular years those studies were done, with group A predominating most years. We also found both antigenic groups in co-circulation throughout the 1981–1984 time period of this study in Australia and Papua New Guinea, with group A strains predominating by a ratio of 2.5:1. All 56 regional and 10 reference strains in this study were grouped by the monoclonal TR-FIA test, but any subgrouping of strains beyond A and B was not suggested by our data and was not done.

No differences in clinical severity could be attributed to either group on the basis of the limited records available; most cases were in infants <2 years of age who had been hospitalized with pneumonia. The family sequentially infected with a group A strain 13 months apart did experience milder disease during the second infection, similar to a finding by Mufson et al. [39].

Differences in growth characteristics among RSV strains have been noted before but not systematically studied [12, 60]. Coates et al. found that plaques developed in HEp2 cells faster and to increasing size with Long strain compared to 18537 virus [12]. Yurlova et al. described extensive differences in the ability of RSV strains to interfere with the growth of a challenge virus (VSV) in a HeLa cell line at 37 °C; strains were divided into low-, moderate-, and high-interfering capability, which was somewhat correlated with growth rate in HEL cells [60]. Also, syncytial development and infectious virus titer are generally observed to be greater with Long and A2 viruses than with 18537-like viruses [56].

By quantifying selected growth characteristics on the basis of empirical scales, we have found in this study that differences between strains are clearly group-associated and are reproducible. Using 10 well-studied reference strains as controls throughout the study, we showed that group A strains on the average produce higher infectious virus titers, with greater development of syncytia, and in less culture time than group B strains.

We also quantified the one-way antigenic relationship between group A and B strains. Using reference antisera to Long strain, the group A strains reacted significantly more strongly than group B strains by IFA, EIA, and SN tests. Greater reactions of A strains with the anti-Long sera would be expected because these tests with polyclonal antibodies reflect multiple antigenic sites

on the virus and viral proteins *plus* multiple antibodies (to all epitopes of all the RSV structural proteins) likely to be in the hyperimmune antisera. Any reaction with other group A strains would thus be enhanced. The net differences between A and B strains would then be magnified when B strains are reacted against polyclonal A antisera, and the degree of difference noted would be dependent on the sensitivity of the test system used. These differences may also account for the lower number of B strains usually isolated; because group B strains replicate more slowly and to lower titers and appear to be more labile in the laboratory than group A strains, their presence in a culture may be missed entirely. Thus the number of B strains studied here may be artificially low.

Differentiation of RSV groups has also been shown by IFA and EIA tests with MAbs, which are not only protein-specific but epitope-specific as well [6, 7, 18, 21, 40, 44]. These studies fully support the existence of two major groups of RSV which share only limited antigenic identity. Two-directional SN tests with hyperimmune antisera also showed this differentiation between representative strains of groups A and B, which ranged from 2% to 43% relatedness [12, 16, 31, 59]; SN tests with MAbs similarly showed major distinctions between groups A and B [4, 44].

The protein MW patterns of the strains in our study were not as distinctive as we had projected from obvious antigenic and structural differences in the F and G glycoproteins between groups. The G, F1, and F2 bands showed only minor fluctuations in MW among all strains, regardless of group; thus, these proteins did not allow grouping on the basis of size. Our data also did not confirm the small difference in MW of the F1 polypeptide previously reported in A and B strains [41]. The L, N, and M bands were constant throughout. Only the P phosphoprotein exhibited significant variations in size, ranging from 34800 to 37100 for group A and 30700 to 34200 for group B stains. This finding confirms previous reports of P ranging from 34000 to 36000 in group A strains and 31000 to 34000 in group B strains [2, 9, 21, 37, 41, 55], which has proven useful as a stand-alone grouping method [55].

Thus, the antigenic and growth characteristics and protein patterns of the Australia-region RSV strains isolated during 1981–1984 allowed classification of the strains into groups A and B, with major differences observed between the groups. This study extends the predominance of group A over B to the Southern Hemisphere, where both viruses were concurrent in the regions surveyed.

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