Circulation Patterns of Group A and B Human Respiratory Syncytial Virus Genotypes in 5 Communities in North America

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Human respiratory syncytial virus (HRSV) is a major cause of serious lower respiratory tract illness in infants, young children, and the elderly. To characterize the circulation patterns of HRSV strains, nucleotide sequencing of the C-terminal region of the G protein gene was performed on 34–53 isolates obtained from 5 communities during 1 epidemic year, representing distinct geographical locations in North America. Phylogenetic analysis revealed that 5–7 HRSV genotypes, including 1 or 2 predominant strains, circulated in each community. The patterns of genotypes were distinct between communities, and less diversity was seen between strains of the same genotype within than between communities. These findings are consistent with HRSV outbreaks' being community based in nature, although transmission of viruses between communities may occur. Several strains are probably introduced or circulate endemically in communities each year, and local factors—possibly immunity induced by previous years' strains—determine which strains predominate during an HRSV season.

As a main cause of serious lower respiratory tract disease in infants and young children worldwide, human respiratory syncytial virus (HRSV) is a high priority for vaccine development [1]. Because RSV infection induces only partially protective immune responses, repeated infections and disease occur throughout life, especially in immunocompromised patients [2, 3] and the elderly [4–6]. The impact of the HRSV disease among the elderly has been considered sufficiently extensive to merit vaccine development [7].

Variability between HRSV strains is one of the features of HRSV infections that might contribute to the ability of the virus to infect people repeatedly and cause yearly outbreaks. Initially, investigators used monoclonal antibodies to characterize differences between HRSV strains and identified 2 major groups of strains, A and B, as well as different strains within each group [8–14]. More recently, genetic characterization has been used to differentiate HRSV strains [14–22].

Both antigenic and genetic studies have demonstrated that several distinct strains from groups A and B often circulate during the same season [9, 10, 12, 21–23]. Genetic studies, primarily using the G protein gene, have demonstrated that distinct strains within each group cluster in groups of phylogenetically related strains or genotypes [14, 15, 18, 19, 21, 22].

The importance of strain differences to clinical and epidemiological features of HRSV and to vaccine development has not yet been elucidated. Some of the characteristics related to strain heterogeneity, however, suggest that diversity is likely to be important to HRSV infection and disease. The G protein, the most variable of the HRSV proteins and a major constituent in the protective immune response to the virus, shows a high percentage of nucleotide substitutions that lead to amino acid changes, suggesting that amino acid changes provide an advantage to the virus, possibly by altering epitopes and helping it to escape preexisting immunity. Studies in animals and limited data in humans imply that antigenic differences between the 2 groups are sufficiently extensive to affect susceptibility to infection or disease [24–26]. In addition, community-based studies have revealed a displacement in the predominant circulating lineages or genotypes in successive HRSV seasons [18, 21, 22]. These consistent shifts in the predominating strains suggest that change may confer a selective advantage by helping the virus to evade preexisting immunity.

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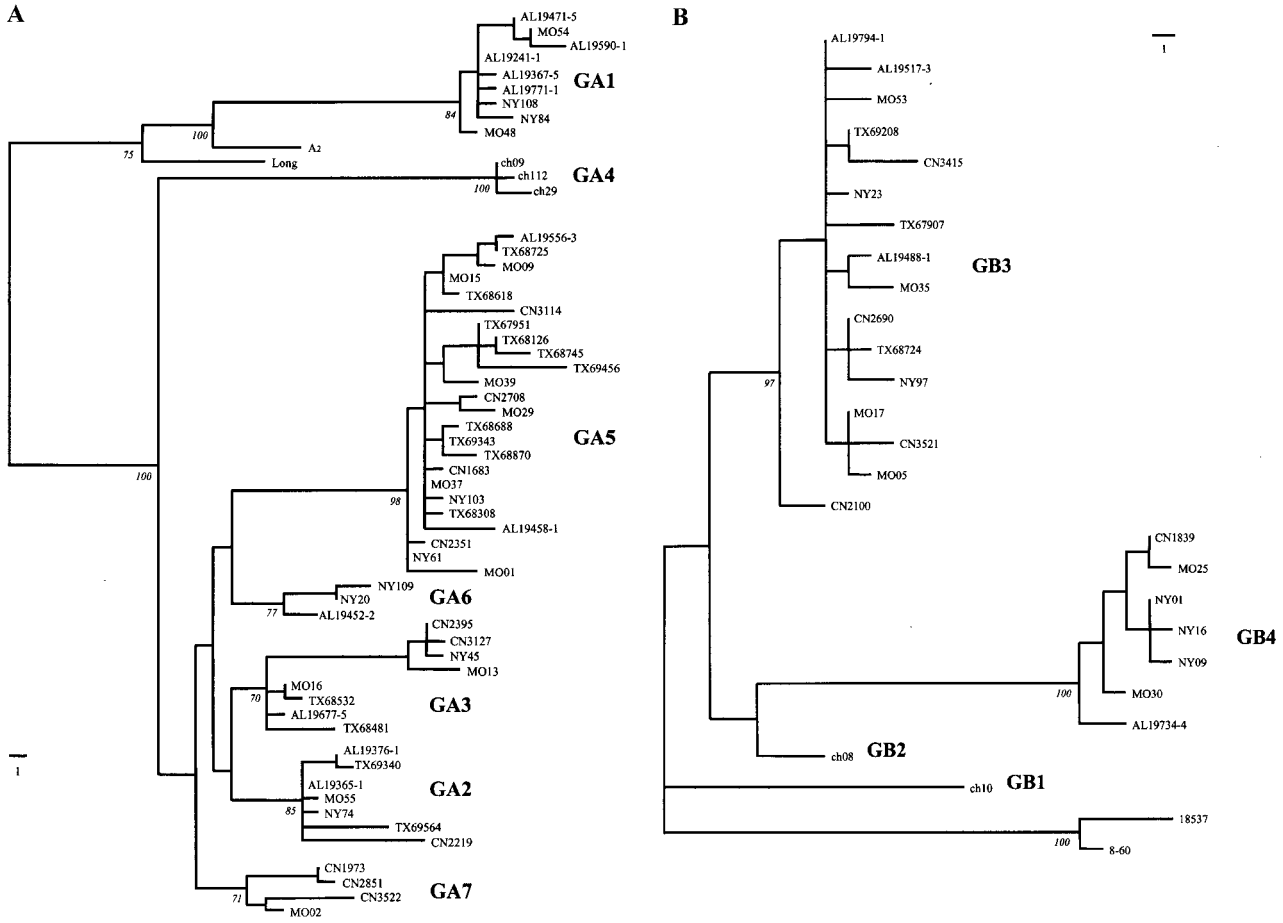

Figure 1. Estimated maximum parsimony (MP) trees, drawn to scale, showing the genetic clusters obtained in the analysis of the human respiratory syncytial virus group A *(A)* and group B *(B)* nucleotide sequences. The phylograms were the best trees under the Kishino-Hasegawa test. The bootstrap proportions, obtained from a 50% majority rule consensus tree, were plotted at the main internal branches of the phylogram to show the support values. For the bootstrap analysis under the MP assumption, sequences were added randomly, and 1 tree was held at each step (100 replicates and 100 bootstrap replicates), applying the tree bisection-reconnection branch-swapping algorithm. Prototype strains (A2 and Long for group A, 8–60 and 18537 for group B) were included in the analysis. The trees were midpoint-rooted, using minimum *F*-value optimization. Bar, 1 nucleotide change. Genotypes are shown in boldface. AL, Alabama; MO, Missouri; NY, New York; TX, Texas; CN, Canada.

The multiplicity of HRSV strains and the constant shift in circulating strains in a community have made it difficult to define circulation patterns of strains and to understand the relationships between strains and HRSV disease [15, 18, 21, 22, 27–30].

In the current work, we looked at the circulation patterns of HRSV strains in 5 distinct communities during 1 epidemic season in North America (1994–1995) by analysis of the G protein gene of group A and B HRSV strains. This study was designed to help us understand the circulation of HRSV strains within and between communities. Our results demonstrate that the patterns of circulating strains in communities are often distinct, suggesting that HRSV outbreaks are a community phenomenon, not a national one. We also identified instances in which strains probably spread between communities.

Materials and Methods

Viruses. HRSV isolates were obtained during 1 epidemic season (1994–1995) from 5 communities, representing distinct geographical locations in North America. A total of 220 isolates were studied: 43 isolates from the Children's Hospital of Alabama, University of Alabama (Birmingham, November–April); 34 isolates from the Rochester Medical Center, University of Rochester (Rochester, NY, December–March); 38 isolates from the Texas Medical Center, Baylor College of Medicine (Houston, November–April); 52 isolates from the St. Louis Children's Hospital, Washington University (St. Louis, December–April); and 53 isolates from Cadham Provincial Laboratory (Winnipeg, Manitoba, Canada, January–April). The isolates were selected to span the HRSV season in each of the communities and consisted of a systematic sampling of the entire epidemic season. In most instances,

Table 1. Median uncorrected *p* distances observed within and between human respiratory syncytial virus group A genotypes.

Genotype	GA1	GA2	GA3	GA5	GA6	GA7
GA1	**0**	.14	.16	.17	.14	.15
GA2	—	**.01**	.06	.08	.05	.05
GA3	—	—	**.02**	.09	.06	.07
GA5	—	—	—	**.01**	.07	.08
GA6	—	—	—	—	**0**	.06
GA7	—	—	—	—	—	**0**

NOTE. Median uncorrected *p* distances are the median number of pairwise nucleotide differences divided by the total number of nucleotides analyzed in the sequenced region (270 nt). Boldface numbers refer to intragenotype distances.

sequences were obtained from an aliquot of the initial cell culture material.

Population distribution. The isolates were obtained from patients admitted with lower respiratory tract illness. The median (range) age of the patients was similar among the study sites—that is, 5 months (1–38 months) in Birmingham; 3.5 months (0.7–28 months) in Rochester; 3.8 months (1 month–7.4 years) in Houston; and 6 months (1 month–4.4 years) in Winnipeg. The ages of individual patients were not available for isolates from St. Louis, but they were all from children <2 years of age.

Antigenic grouping. HRSV isolates were assigned to group A or B on the basis of their reaction pattern against a panel of monoclonal antibodies by a capture enzyme immunoassay, as described elsewhere [13].

Oligonucleotide primers. Primer sets GPA-F1 and GPB-F1 were used to amplify and sequence 270 nucleotides at the carboxy-terminal region of the G gene of group A and B isolates, as described elsewhere [22].

Reverse transcription–polymerase chain reaction (RT-PCR) analysis and nucleotide sequencing. Total RNA was extracted using a guanidinium-isothiocyanate-phenol method (RNAzol; Tel-Test, Friendswood, TX). Viral RNA was amplified in an RT-PCR procedure, as described elsewhere [22]. Briefly, RNA was annealed with the F1 primer and reverse-transcribed with avian myeloblastosis virus RT (Roche Molecular Biochemicals, Indianapolis, IN), and the cDNA was amplified with the appropriate amplification primers in a PCR assay. Both strands were sequenced by using fluorescent dye–labeled terminators on ABI 373 and 377 Sequencer models (Perkin-Elmer Applied Biosystems, Foster City, CA). The nucleotide sequences were edited with Sequencher version 3.0 (Gene Codes, Ann Arbor, MI) for Power Macintosh (Apple Computer, Inc., Cupertino, CA).

Phylogenetic analysis. Nucleotide sequences of a limited region of the G gene of A and B group viruses were aligned using Clustal W (version 1.74 for Unix) [31], applying the slow and accurate multiple alignment option. Unique sequences for both A and B group viruses were included in the phylogenetic analysis; that is, 182 group A and 38 group B sequences were summarized in 55 and 23 sequences, respectively. Phylogenetic trees for group A and B alignments were computed by maximum parsimony–, distance-, and maximum likelihood–based criteria analysis with PAUP* version 4.0.0d65 [32]. The data sets comprising A and B group sequences were also submitted to quartet maximum likelihood reconstruction (TREE-PUZZLE version 4.0) for likelihood mapping [33] (data not shown). Trees were plotted with either PAUP* or TreeView version 1.5 [34].

Pairwise nucleotide distances within and between the HRSV A and B genotypes were calculated as the proportion of differences (*p* distance)—that is, the number of pairwise nucleotide differences divided by the total number of nucleotides in the sequenced region—using the program MEGA version 1.02 [35].

Representative nucleotide sequences of HRSV group A and B genotypes were submitted to GenBank and given accession numbers AF233900–AF233933.

Results

HRSV group A and B strains in the communities: genotypes. Phylogenetic analysis of the distinct 55 group A G gene nucleotide sequences revealed 6 clusters (GA1–GA3 and GA5–GA7), displaying 70%–100% bootstrap proportions (figure 1*A*). Sequences from the prototype strain A2 and strains belonging to genotype GA4, not seen in the current study, were included to show the present data in the context of previous genotype designations [22]. The previously described genotype GA3 was split into 3 clusters, GA3, GA6, and GA7. These new clusters were identified when new sequences were added to the database: those from this study and those from various locations throughout the world, which were kindly provided by Dr. P. Cane (University of Birmingham Medical School, Birmingham, UK; data not shown).

The genetic distances computed across the group A G gene sequences (table 1) showed that the genotypes clustered in 2 main subgroups, GA1 and GA2–GA7, with the median intergenotype distances between the former and the latter genotypes ranging from 0.14 to 0.17. The median *p* distances across GA2–GA7 ranged from 0.05 to 0.06 (GA2 × GA7 through GA6 × GA7) to 0.09 (GA3 × GA5).

Group B HRSV nucleotide sequences (figure 1*B*) clustered in 2 previously described genotypes, GB3 and GB4, supported, respectively, by 97% and 100% bootstrapping values. Prototype strains 8–60 and 18537, as well as sequences belonging to the previously described genotypes GB1 (ch10) and GB2 (ch08), were included to place the current sequences in the context of our previous work [22].

The computation of the genetic distances among the group B strains indicated that the median distance between GB3 and GB4 genotypes was ~10 times greater than the intragenotype distance (table 2).

Table 2. Median uncorrected *p* distances observed within and between human respiratory syncytial virus group B genotypes.

Genotype	GB3	GB4
GB3	**.01**	.10
GB4	—	**0**

NOTE. Median uncorrected *p* distances are the median number of pairwise nucleotide differences divided by the total number of nucleotides analyzed in the sequenced region (270 nt). Boldface numbers refer to intragenotype distances.

Table 3. Distribution of human respiratory syncytial virus (HRSV) group A and B genotypes in 5 communities.

Community (no. of isolates)	HRSV isolates per genotype, no. (%)							
	GA1	GA2	GA3	GA5	GA6	GA7	GB3	GB4
Birmingham, AL (43)	30 (69.8)	3 (7)	1 (2.3)	3 (7)	1 (2.3)	—	4 (9.3)	1 (2.3)
Rochester, NY (34)	12 (35.2)	2 (5.9)	2 (5.9)	4 (11.8)	2 (5.9)	—	2 (5.9)	10 (29.4)
Houston, TX (38)	—	2 (5.3)	2 (5.3)	29 (76.3)	—	1 (2.6)	4 (10.5)	—
St. Louis, MO (52)	30 (57.7)	3 (5.8)	3 (5.8)	7 (13.5)	—	2 (3.8)	4 (7.6)	3 (5.8)
Winnipeg, CN (53)	—	7 (13.2)	5 (9.4)	16 (30.2)	—	16 (30.2)	8 (15.1)	1 (1.9)
Total	72 (32.7)	17 (7.7)	13 (5.9)	59 (26.8)	3 (1.4)	19 (8.7)	22 (10)	15 (6.8)

Distribution patterns of HRSV genotypes within communities. HRSV strain circulation patterns in the 5 communities were complex, with 5–7 genotypes cocirculating as well as a variety of distinct sequences within each genotype (table 3). Each community had 1 or 2 dominant genotypes that accounted for ⩾50% of the isolates during the HRSV season. HRSV group A predominated in 4 of 5 communities. For instance, Birmingham had 25 isolates, 83% of the predominant genotype (GA1), sharing identical sequences over the limited sequenced region of the G gene. These represented 58% of the isolates of all HRSV isolates from Birmingham (tables 3 and 4). Two additional GA1 isolates also had identical sequences that displayed only 2 nucleotide mismatches, compared with the G sequences of the aforementioned isolates. The remaining GA1 isolates had 2–6 nucleotide differences, in contrast to the 25 isolates with identical sequences. Similar observations were made regarding the isolates from the other communities (tables 3 and 4). Briefly, there were 2 predominant genotypes in Rochester (GA1 and GB4) and in Winnipeg (GA5 and GA7) and 1 predominant genotype in Houston (GA5) and in St. Louis (GA1).

Distribution patterns of HRSV genotypes between communities. Each community had a distinct pattern of circulating genotypes. Only 2 communities had the same predominant genotype (GA1): St. Louis and Birmingham. The sequences for the majority of the GA1 genotype isolates in these 2 communities were identical, and both had very similar patterns of other circulating genotypes. In most instances, isolates of the same genotype circulating within a community were less diverse than those circulating in different communities. For example, the median distance was zero for the majority of the genotypes when G gene sequences were from the same genotype within a community and was between zero (GA1) and 0.04 for G gene sequences from the same genotype among the 5 communities. There were 8 instances in which several isolates with identical sequences were seen in several communities (table 3). Most of these identical sequences were found in 1 or 2 isolates in a given community. Noteworthy was 1 GA1 sequence found in 25 isolates in Birmingham, 10 isolates in Rochester, and 28 isolates in St. Louis.

Discussion

We identified several HRSV strains in each of the 5 communities, and we also found distinct patterns of strain circulation during the same HRSV season. These findings reaffirm the complexity of HRSV strain circulation patterns in communities and extend the limited data on strains from multiple communities during the same season. Previous studies have shown some differences in the patterns of strains isolated in different communities, as well as similar or identical strains [17].

In the present study, each community had 5–7 genotypes identified and 1 or 2 dominant genotypes that included 1 predominant G gene sequence or several closely related G gene sequences. The predominant strains and overall patterns of circulating genotypes were distinct for 3 of the 5 communities; these differences in patterns between communities show that

Table 4. Human respiratory syncytial virus (HRSV) group A and B identical nucleotide sequences in 5 communities.

Genotype	HRSV isolates, by community (no. with identical sequences)				
	Birmingham, AL	Rochester, NY	Houston, TX	St. Louis, MO	Winnipeg, CN
GA1	AL19241-1 (25)	NY04 (10)	—	MO03 (28)	—
	AL19471-5 (2)	—	—	—	—
GA2	AL19365-1 (1)	NY110II (1)	—	MO40 (2)	CN749 (2)
GA5	AL19546-1 (1)	—	—	MO01 (1)	—
	—	—	TX68651 (1)	MO37 (1)	—
	—	—	TX68753 (1)	MO15 (1)	—
GA7	—	—	TX68819 (1)	MO02 (1)	—
	—	—	—	MO46 (1)	CN885 (12)
GB3	AL19794-1 (2)	—	—	—	—
	—	—	TX69958 (1)	—	CN2690 (1)

wide regional spread of 1 predominant outbreak genotype does not occur. We suspect that several strains are introduced into or circulate endemically in communities each year and that local factors, possibly including immunity induced by previous years' strains, determine which strains dominate in a given year. It is possible that a strain-specific component of the immune response affects the ability of an HRSV strain to cause disease or spread in the community. Strain-specific antibodies have been demonstrated [36, 37], but further studies are required to determine the importance of strain differences in the HRSV immune response and susceptibility to infection and disease.

In addition to the multiplicity of HRSV strains identified, strains with identical sequences were seen in several communities. Eight groups of strains with identical sequences were observed in >1 community, usually as 1 or 2 isolates per community, including a particular sequence (GA1 genotype) identified in several isolates in St. Louis (28), Birmingham (25), and Rochester (10).

The presence of isolates with identical sequences, especially those in high numbers observed in the aforementioned communities, supports the concept that some virus strains can be transmitted over broad geographic regions during a single epidemic season. However, the appearance of identical sequences does not necessarily prove that transmission of HRSV strains occurs between communities. Previous studies indicated that one must be careful when using identical sequences in selected regions of the genome to infer patterns of HRSV spread; HRSV isolates with identical G gene sequences were seen over a 3-year period in 1 community [22], and nearly identical sequences were identified over 6 years in different countries [17]. Thus, in this study, HRSV strains seen in different communities that have identical sequences in the G gene variable region could be separated by spread through several communities over several HRSV seasons. Those sequences do not necessarily indicate a direct chain of transmission during 1 season. Nevertheless, analysis of the data suggests that some HRSV strains may be particularly suited for widespread transmission during some epidemic seasons.

The genotype designation, as described elsewhere [22], was an effective tool for characterization of HRSV circulation patterns in the study communities. Phylogenetic analysis of representative G gene sequences from this work, Dr. Cane's studies, and additional published G gene sequences [16, 17, 20–22] allowed us to identify 2 additional clusters of group A sequences. The genotype assignment to phylogenetically related strains has proven to be helpful in identifying patterns of HRSV circulation in the communities, but it will likely evolve as additional sequences become available.

Interestingly, 2 distinct subgroups of group A genotypes (GA1 and GA2–GA7) were evident in the estimated trees and were also implied by the median distances between the G gene sequences. Further study is required to determine if the differences between these 2 subgroups within group A are important to the understanding of HRSV infections.

In conclusion, the patterns of HRSV strains circulating during community outbreaks are complex, consistent with the fact that outbreak strains are determined at the community level, not the regional or national levels. We suspect that local factors, such as previous strain-specific immunity to the virus and possibly the relative fitness of the circulating HRS viruses, dictate which strains (present endemically in a community or introduced from other communities) will predominate in a given season. Further study, however, is necessary to define the role that strain-specific immunity plays in the circulation patterns of HRSV outbreak strains in a community and in susceptibility to HRSV disease.

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