JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1994, p. 1–4
0095-1137/94/$04.00+0

Vol. 32, No. 1

Analysis of Respiratory Syncytial Virus Strain Variation in Successive Epidemics in One City

PATRICIA A. CANE,* DAVID A. MATTHEWS,† AND CRAIG R. PRINGLE

Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom

Received 28 June 1993/Returned for modification 31 August 1993/Accepted 28 September 1993

The variability of respiratory syncytial virus isolates from five successive epidemics in an urban population was determined. A total of 187 isolates of respiratory syncytial virus from the southern part of Birmingham, United Kingdom, were classified into subgroups A and B and were then further assigned to genetic lineages. Allocation of isolates into lineages was achieved by reverse transcription of infected cell RNA and then PCR amplification of selected regions of the genome; PCR products were examined by restriction mapping or nucleotide sequencing of parts of the nucleoprotein gene, the small hydrophobic protein gene, and the attachment protein gene. Previous work has shown that estimations of genetic diversity by analysis of genes coding for proteins likely (attachment protein) and unlikely (nucleoprotein and small hydrophobic protein) to be under immune pressure gave concordant results. Six genetic lineages of subgroup A isolates have been defined by this procedure; these isolates differ by up to 20% in the amino acid sequences of their attachment proteins; likewise, subgroup B isolates can be divided into two categories by restriction mapping of parts of their nucleoprotein and attachment protein genes. The same genetic lineages appeared to be present worldwide during the same period. The analysis of isolates from successive epidemics showed that different lineages predominated in each epidemic and that not all lineages were present in every epidemic. Some lineages appeared to increase in numbers over several years and then decline, possibly indicating a buildup of resistance in the community to a particular genotype.

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract disease in infants, causing annual epidemics. The virus is unusual in that it can repeatedly reinfect individuals throughout life and infect babies in the presence of maternal antibody. RSV isolates can be divided into two subgroups, A and B, on the basis of their reactions with monoclonal antibodies (2, 8, 14), and the two subgroups are also distinct at the nucleotide sequence level, with the amino acid sequences of the attachment (G) proteins differing by 47% (5, 11, 12). Variability within the subgroups has been demonstrated with monoclonal antibodies, particularly against the attachment (G) protein (1), by RNase A mismatch studies of viral RNA (7, 15) and by nucleotide sequencing of parts of the genome (5, 16).

Multiple genetic lineages of both A and B subgroups have been shown to cocirculate within a single epidemic in Birmingham, United Kingdom (5). These isolates were divided into distinct lineages on the basis of three criteria: restriction mapping of part of the nucleocapsid (N) protein gene, nucleotide sequencing of part of the small hydrophobic (SH) protein gene, and nucleotide sequencing of the G protein gene. Analysis of the G protein genes of strains representing each subgroup A lineage revealed up to 20% variability of the amino acid sequence between isolates (3). Six lineages (designated SHL1 to SHL6) were discriminated among subgroup A strains, and two lineages (designated NP1 and NP3) were discriminated among subgroup B strains. In addition, it has been shown that strains very similar to those identified in Birmingham were present in other parts of the world during the same period (4).

Sequencing of amplified viral RNA obtained from nasopharyngeal aspirates and after up to 10 passages of the virus in cultured cells established that none of the variability observed was attributable to propagation of the virus isolates in vitro prior to nucleotide sequencing.

We report here the relative frequency of each of the RSV lineages over five successive epidemics in Birmingham. The data show that the predominant lineages change each year and that isolates of a particular lineage are more frequent in locations where they were rare in the preceding year.

MATERIALS AND METHODS

Virus isolation. All virus strains examined in the present study were initially isolated by culture in HEp-2 cells at the Regional Virus Laboratory, East Birmingham Hospital, Birmingham. Clinical samples were taken from babies admitted to either East Birmingham Hospital or Selly Oak Hospital and were submitted for normal routine diagnostic tests. These hospitals admit patients from much of the southern part of Birmingham. RSV-positive cultures and the original clinical specimens were stored at −70°C before further analysis. Virus isolates were either examined directly or further passaged in MRC-5 cells.

Characterization of isolates. Infected cell RNA was isolated and reverse transcribed, and then selected regions were amplified by PCR. Analysis was by restriction mapping of part of the N gene, sequencing of part of the SH gene, and sequencing and restriction mapping of part of the G gene as described previously (5, 6). In brief, the N gene was amplified between nucleotides 858 and 1135, and the PCR products were restricted with *Hin*dIII, *Pst*I, *Bgl*II, *Hae*III, and *Rsa*I. The SH gene of some subgroup A isolates was amplified between nucleotides 1 and 281, and nucleotides 22

* Corresponding author. Mailing address: Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, United Kingdom. Fax: 0203 523701. Electronic mail address: PY@dna.bio.warwick.ac.uk.

† Present address: Department of Biochemistry and Microbiology, University of St. Andrews, St. Andrews, Scotland.

TABLE 1. Classification of isolates in subgroups A and B

Epidemic	Total no. of RSV-positive samples	No. (%) of isolates		
		Total no. examined	Subgroup A	Subgroup B
1988–1989	111	4	3 (75)	1 (25)
1989–1990	76	42	26 (62)	16 (38)
1990–1991	101	37	33 (89)	4 (11)
1991–1992	155	76	70 (92)	6 (8)
1992–1993	112	28	9 (32)	19 (68)
Total	555	187	141 (75)	46 (25)

to 156 were directly sequenced. The G gene of subgroup A isolates was amplified between nucleotides 1 and 584, and the PCR products were restricted with *Alu*I, *Taq*I, *Mbo*I, and *Mse*I, or nucleotides 297 to 400 were sequenced directly. The G gene of some subgroup B isolates was amplified between nucleotides 153 and 817, and the PCR products were digested as described above for the subgroup A isolates. This analysis allowed classification of isolates into subgroups A or B and then further grouping of subgroup A isolates into the genetic lineages SHL1 to SHL6 and subgroup B isolates into NP1 or NP3 (6).

RESULTS

Effects of passaging RSV in tissue culture. The following experiments established that the observed variability of the G gene of RSV isolates was not due to in vitro culture of the virus. One isolate, RSB89-6190, was initially analyzed after one passage in HEp-2 cells and then three passages in MRC-5 cells. This isolate was then further passaged 11 times in MRC-5 cells or 11 times in BSC-1 cells. The viral RNA was then extracted and reverse transcribed, and the G gene was amplified, cloned, and sequenced. The only nucleotide difference observed between the various passages was G to A at nucleotide 30 in the virus that was passaged 11 times in BSC-1 cells; this was a noncoding change. Attempts to amplify the G gene of isolate RSB89-6190 from the original clinical specimen were unsuccessful. However, analysis of a second isolate, RSB89-6598, was successful; the G gene of this strain was found to have a sequence identical to that of RSB89-6190 after one passage in HEp-2 cells and three passages in MRC-5 cells. The sequence of this strain, as derived directly from the clinical sample, was also identical to that of RSB89-6190. Examination by restriction analysis of an additional five isolates both directly from clinical samples and after propagation in tissue culture also showed no changes as a result of in vitro propagation of the isolates. Thus, it can be concluded that the genotypic variability observed in the present study is not an artifact of the isolation and propagation methods that we used.

Epidemics analyzed. Approximately one-third of the viruses isolated by the Regional Diagnostic Laboratory from patients involved in five consecutive epidemics in the south Birmingham area were examined (Table 1). These epidemics occurred in 1988 and 1989, 1989 and 1990, 1990 and 1991, 1991 and 1992, and 1992 and 1993. Only four isolates were available from the first epidemic, but many of the isolates from the other epidemics were available. The analysis of isolates from the 1989 epidemic has been reported previously (5). The epidemic resulting in the largest number of hospitalizations was that of 1991 and 1992.

TABLE 2. Observed incidence of isolates belonging to each lineage

Epidemic	No. of isolates								
	Subgroup A						Subgroup B		
	Total	SHL1 and SHL3[a]	SHL2	SHL4	SHL5	SHL6	Total	NP1	NP3
1988–1989	3	1	0	0	2	0	1	0	1
1989–1990	26	17	6	3	0	0	16	12	4
1990–1991	33	12	13	0	1	7	4	0	4
1991–1992	70	8	38	0	0	24	6	0	6
1992–1993	9	1	6	0	0	2	19	0	19

[a] The data for SHL1 and SHL3 were combined because these have been shown to be similar in terms of G protein gene sequence (99% deduced amino acid similarity) (3).

Classification of isolates. The total numbers of RSV-positive specimens recorded from each epidemic, the relative proportions of subgroups A and B, and the numbers of isolates analyzed are given in Table 1. The proportion of subgroup B strains varied between 8% in the 1991 and 1992 epidemic and 68% in the 1992 and 1993 epidemic.

Table 2 gives the number of isolates of the different subgroup A and B lineages. Figure 1 illustrates the relative

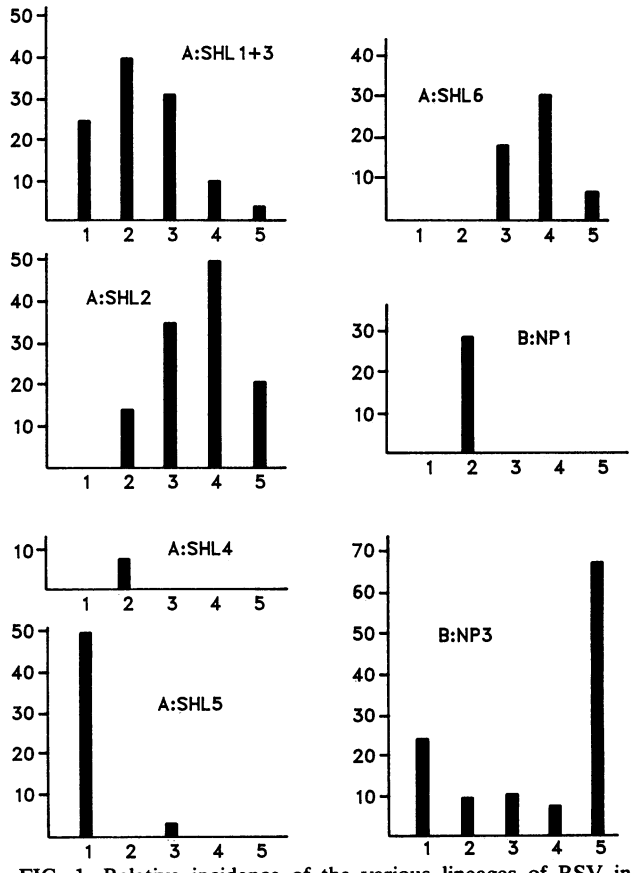

FIG. 1. Relative incidence of the various lineages of RSV in successive epidemics. The *x* axes represent isolates from the following epidemics: 1, 1988 and 1989; 2, 1989 and 1990; 3, 1990 and 1991; 4, 1991 and 1992; 5, 1992 and 1993. The *y* axes show the percent incidence of each lineage relative to those of all other isolates.

incidence of isolates from each of the lineages. The data for SHL1 and SHL3 in Table 2 and Fig. 1 were combined, because these lineages have been shown to be extremely similar in terms of G protein gene sequence (99% deduced amino acid sequence similarity), although they could be distinguished by their SH gene sequences (3, 5). This grouping was predominant among isolates from the 1989 and 1990 epidemic and then declined from 40 to 4% over the subsequent epidemics (Fig. 1).

SHL2 isolates showed a steady increase from 1989 to 1992, making up half of all isolates from the particularly large number of hospitalizations in the 1991 and 1992 epidemic. The incidence of this lineage then declined to 21% of isolates in the 1992 and 1993 epidemic (Fig. 1). SHL4 isolates were detected only in the 1989 and 1990 epidemic. With respect to SHL5, although only four isolates were examined from the 1988 and 1989 epidemic, two of these were from SHL5, so it is likely that this was the predominant lineage of isolates involved in that epidemic. Isolates of this lineage were not detected subsequently apart from one isolate in the 1990 and 1991 epidemic. Strains belonging to the SHL6 lineage were not detected until the 1990 and 1991 epidemic, during which they made up 19% of isolates, increasing to 32% in the 1991 and 1992 epidemic and then declining to 7% in the 1992 and 1993 epidemic (Fig. 1).

Subgroup B isolates belonging to NP1 made up 29% of the isolates involved in the 1989 and 1990 epidemic but were not detected subsequently (Fig. 1). Isolates belonging to NP3 caused 8 to 11% of RSV infections from the autumn of 1989 to the spring of 1992, but they then predominated (68%) in the last epidemic analyzed (1992 and 1993) (Fig. 1).

In summary, the epidemics were caused by a number of RSV strains with distinctly different genotypes, with the relative proportions of each changing each year and some declining in frequency to undetectable levels, at least in hospitalized babies.

DISCUSSION

In this report we described the relative proportions of RSV isolates belonging to different genotypes or lineages occurring in successive epidemics in Birmingham. Viruses belonging to different lineages predominated in each year, with the amino acid sequences of G proteins of isolates from different subgroup A lineages differing by up to 20% (3). These results are similar to those given by RNase A mismatch studies on a smaller number of isolates from Montevideo, Uruguay (7). It is unclear what happens when a particular lineage seems to disappear from circulation; for example, a single isolate of SHL5 was detected in the 1990 and 1991 epidemic; this was the predominant lineage in the 1988 and 1989 epidemic but was undetected in the epidemics of 1989 and 1990, 1991 and 1992, and 1992 and 1993. Since virus isolations were made only from babies admitted to hospital, it is possible that viruses belonging to this lineage were still circulating in the community but did not give rise to severe disease in the infected babies. The apparent disappearance of a particular strain of RSV may be due in part to increased levels of herd immunity to that particular strain.

The home addresses of patients were available for about one-third of cases; these were plotted in relation to the lineage of the infecting virus. It was found that although SHL2 viruses were isolated across the city in four epidemics, very few isolations were made in 1989 and 1990 and 1990 and 1991 in those areas where there was a preponderance of SHL2 in the severe epidemic of 1991 and 1992. The distribution of isolates of SHL1 and SHL3 was also analyzed; isolates of these lineages were the predominant category of subgroup A RSV present in the 1989 and 1990 epidemic, but they were concentrated in one small area. In the subsequent epidemic, isolates of this lineage were spread out across south Birmingham.

Hall et al. (10), in an extensive study of RSV strain variation using monoclonal antibodies, found that in some epidemics there is a disparity between strains isolated from hospitalized babies and those isolated from outpatients. They also suggested that there may be differences in pathogenicity between strains. In the case of the Birmingham epidemic of 1991 and 1992, which resulted in a large number of hospitalizations, strains of the SHL2 lineage predominated, particularly in the area around one hospital, suggesting that viruses belonging to this lineage can cause more severe illness. However, viruses of the same lineage were also circulating in the previous epidemics, but those epidemics were not particularly severe. It may be that there is a balance between immunity at the community level that influences the spread of a particular strain of virus and the immunity of individual mothers that modulates the severity of illness in their babies. The contribution of factors other than virus strain and immune status in influencing the severity of an epidemic remains to be determined.

Hall et al. (9) have shown that it is possible to reinfect some individuals several times with a laboratory strain of RSV. However, some people were resistant to reinfection, and the degree of illness and duration of virus shedding diminished after the first infection (9). We have observed previously (17; unpublished data) that resistance of some adult volunteers to infection by a cloned wild-type virus is more closely correlated with the serum neutralization titer to the homologous strain compared with that to a heterologous strain. It has also been found that second infections in children initially infected with subgroup A viruses tended to be with subgroup B virus (13). Therefore, it appears that although immunity to a particular strain of RSV is not always complete, such immunity can modify the result of a subsequent infection with the virus. The significance of such immunity in modifying the spread and severity of virus infection in the community remains speculative.

ACKNOWLEDGMENTS

We are indebted to U. Desselberger (now of PHLS, Cambridge, United Kingdom), R. Tudor, and A. Campbell of the Regional Virus Laboratories, East Birmingham Hospital, for collecting the RSV isolates examined in the present study. We also thank K. Tolley for technical assistance.

This work was supported by program grant PG8322715 from the Medical Research Council, United Kingdom.

REFERENCES

1. **Anderson, L. J., R. M. Hendry, L. T. Pierik, C. Tsou, and K. McIntosh.** 1991. Multicenter study of strains of respiratory syncytial virus. J. Infect. Dis. **163**:687–692.
2. **Anderson, L. J., J. C. Hierholzer, C. Tsou, R. M. Hendry, B. F. Fernie, Y. Stone, and K. McIntosh.** 1985. Antigenic characterization of respiratory syncytial virus strains with monoclonal antibodies. J. Infect. Dis. **151**:626–633.
3. **Cane, P. A., D. A. Matthews, and C. R. Pringle.** 1991. Identification of variable domains of the attachment (G) protein of subgroup A respiratory syncytial viruses. J. Gen. Virol. **72**: 2091–2096.
4. **Cane, P. A., D. A. Matthews, and C. R. Pringle.** 1992. Analysis of relatedness of subgroup A respiratory syncytial viruses

isolated worldwide. Virus Res. **25:**15–22.
5. **Cane, P. A., and C. R. Pringle.** 1991. Respiratory syncytial virus heterogeneity during an epidemic: analysis by limited nucleotide sequencing (SH gene) and restriction mapping (N gene). J. Gen. Virol. **72:**349–357.
6. **Cane, P. A., and C. R. Pringle.** 1992. Molecular epidemiology of respiratory syncytial virus: rapid identification of subgroup A lineages. J. Virol. Methods **40:**297–306.
7. **Cristina, J., A. Moya, J. Arbiza, J. Russi, M. Hortal, C. Albo, B. Garcia-Barreno, O. Garcia, J. A. Melero, and A. Portela.** 1991. Evolution of the G and P genes of human respiratory syncytial virus (subgroup A) studied by the RNase A mismatch cleavage method. Virology **184:**210–218.
8. **Gimenez, H. B., N. Hardman, H. M. Keir, and P. Cash.** 1986. Antigenic variation between human respiratory syncytial virus isolates. J. Gen. Virol. **67:**863–870.
9. **Hall, C. B., E. E. Walsh, C. E. Long, and K. C. Schnabel.** 1991. Immunity to and frequency of reinfection with respiratory syncytial virus. J. Infect. Dis. **163:**693–698.
10. **Hall, C. B., E. E. Walsh, K. C. Schnabel, C. E. Long, K. M. McConnochie, S. W. Hildreth, and L. J. Anderson.** 1990. Occurrence of groups A and B of respiratory syncytial virus over 15 years: associated epidemiologic and clinical characteristics in hospitalized and ambulatory children. J. Infect. Dis. **162:**1283–1290.
11. **Johnson, P. R., and P. L. Collins.** 1989. The 1B (NS2), 1C (NS1) and N proteins of human respiratory syncytial virus (RSV) of antigenic subgroups A and B: sequence conservation and divergence within RSV genomic RNA. J. Gen. Virol. **70:**1539–1547.
12. **Johnson, P. R., M. K. Spriggs, R. A. Olmsted, and P. L. Collins.** 1987. The G glycoprotein of human respiratory syncytial viruses of subgroups A and B: extensive sequence divergence between antigenically related proteins. Proc. Natl. Acad. Sci. USA **84:**5625–5629.
13. **Mufson, M. A., R. B. Belshe, C. Orvell, and E. Norrby.** 1987. Subgroup characteristics of respiratory syncytial virus strains recovered from children with two consecutive infections. J. Clin. Microbiol. **25:**1535–1539.
14. **Mufson, M. A., C. Orvell, B. Rafnar, and E. Norrby.** 1985. Two distinct subtypes of human respiratory syncytial virus. J. Gen. Virol. **66:**2111–2124.
15. **Storch, G. A., L. J. Anderson, C. S. Park, C. Tsou, and D. E. Dohner.** 1991. Antigenic and genomic diversity within group A respiratory syncytial virus. J. Infect. Dis. **163:**858–861.
16. **Sullender, W. M., M. A. Mufson, L. J. Anderson, and G. W. Wertz.** 1991. Genetic diversity of the attachment protein of subgroup B respiratory syncytial viruses. J. Virol. **65:**5425–5434.
17. **Watt, P. J., B. S. Robinson, C. R. Pringle, and D. A. J. Tyrrell.** 1990. Determinants of susceptibility to challenge and the antibody response of adult volunteers given experimental respiratory syncytial virus vaccines. Vaccine **8:**231–236.