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Prevalence of Respiratory Syncytial Virus Subgroups A and B in France from 1982 to 1990

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A fluorescence antibody test with monoclonal antibodies was used to determine the subgroup (A or B) of respiratory syncytial virus from infants hospitalized in Caen, France, over eight consecutive epidemics from 1982 to 1990. From 1982 to 1985, 27 (30%) out of 90 frozen nasal slides were classified as subgroup A strains and 63 (70%) were classified as subgroup B. B strains predominated over A in 1983–1984 and 1984–1985. From 1985 to 1990, 284 respiratory syncytial virus field strains were reisolated from frozen materials; 115 (40.5%) were typed as subgroup A and 169 (59.5%) were typed as subgroup B. In 1985–1986, 1986–1987, and 1988–1989, both subgroups were present in almost equal numbers; subgroup A (88.3%) predominated in 1987–1988, and subgroup B (84.5%) predominated in 1989–1990. In conclusion, both subgroups occur together each year, and one subgroup rarely predominates, e.g., subgroup A in 1987–1988 and subgroup B in 1983–1984 and 1989–1990. Therefore, there is a gradual change of the predominant subgroup into another over a period of about 5 years; the relative frequency of subgroup A strains increased from 1983 to 1988, whereas the percentage of subgroup B decreased during the same period.

Winter outbreaks of respiratory syncytial virus (RSV) infections are the cause of serious acute lower respiratory disease in infants (5). Two major groups of RSV strains with antigenic differences on the N, F, and G proteins have been identified with panels of monoclonal antibodies (MAbs): subgroup A, represented by the Long strain, and subgroup B, represented by the 18537 strain (1, 3, 6, 9, 13). Several surveys of the prevalence of the RSV subgroups have been published (8, 11, 12, 18, 19). This study compares the results of those studies with the results obtained in Caen, Normandy, France, during winter epidemics of 1982–1983 through 1989–1990.

The patients were infants from southern Normandy hospitalized with acute lower respiratory tract infection, bronchiolitis, or pneumonia in the University Hospital of Caen. Most of the children were less than 1 year old, and 50% were under 3 months of age. Viral diagnosis was made on nasal secretions by a fluorescence antibody test and isolation in cell culture. Nasal aspirates were collected by hospital staff nurses; transported to the Virus Laboratory, usually within 3 h; and resuspended in 5 ml of transport medium. Sample suspensions (2 ml) were used for the fluorescence antibody test. The cells were separated by centrifugation, washed in phosphate-buffered saline, deposited on microscope slides, and fixed in acetone. The test used either the bovine polyclonal antibody (Wellcome Reagents Ltd.) and a rabbit anti-bovine fluorescein isothiocyanate conjugate (Nordic, Tilburg, The Netherlands) at dilutions of 1/10 and 1/30, respectively, or an RSV MAb (MONOFLUOKIT VRS; Diagnostics Pasteur, Marnes, France) (16) and a anti-mouse fluorescein isothiocyanate conjugate (Diagnostics Pasteur) at a dilution of 1/100. All incubations were carried out at 37°C for 30 min, and slides were washed two times in phosphate-buffered saline. No counterstain was used. For isolation, cultures of MRC-5 human embryonic lung fibroblasts in 25-cm^2 flasks were inoculated with 0.2 ml of the resuspended specimens. In cultures exhibiting typical RSV cytopathic effect, infected cells were harvested, pelleted by centrifugation, deposited on microscope slides, fixed in acetone, and identified by the fluorescence antibody test. MRC-5 cells were kept 4 weeks before a culture was considered negative. Blind passages were not done routinely, and no other cell lines were used.

Subgroups of RSV were identified by a fluorescence antibody test. A panel of mouse MAbs was supplied by E.N. (Karolinska Institute, Stockholm, Sweden). These included one anti-G specific antibody (clone C793), one anti-F specific antibody (clone B151), and one anti-NP specific antibody (clone B90) (13). One other anti-F specific antibody (clone 7858) has been developed by C. Orvell in this laboratory (2). Another anti-F specific MAb (clone 2B8) was supplied by P.P. (Centre Hospitalier et Universitaire, Dijon, France). All MAbs were used at a dilution of 1/40, and an anti-mouse fluorescein isothiocyanate conjugate (Diagnostics Pasteur) was employed at a dilution of 1/100. All incubations were carried out at 37°C for 30 min, and slides were washed two times in phosphate-buffered saline. No counterstain was used. The first part of the study was made on slides of nasal aspirates kept frozen at −20°C, over three consecutive outbreaks, from 1982 to 1984. Attempts were made to test a total of 102 slides, but 16 specimens were unsuitable because of insufficient numbers of exfoliated cells or cross-contamination of reagents on the slides. We examined 28 slides from winter 1982–1983, 29 from winter 1983–1984, and 33 from winter 1984–1985. The second part of the study was made on RSV isolated from the five following outbreaks. Vials of frozen strains were rapidly thawed and inoculated into cultures of MRC-5 cells. Of 337 RSV field strains, 284 (82%) were successfully recovered from frozen materials. The reisolation was not repeated for the 53 strains which failed to grow. Forty-five strains were reisolated from the 1985–1986 outbreak, 37 from 1986–1987, 43 from 1987–1988, 37 from 1988–1989, and 122 from 1989–1990. In the two parts of the

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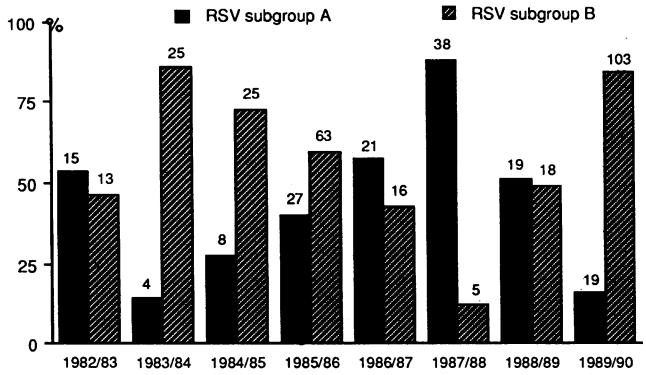

FIG. 1. Temporal distribution of RSV subgroups A and B in Caen on frozen nasal slides (1982–1985) and reisolated viral strains (1985–1990). Numbers above bars indicate the total number of cases studied each year. The horizontal axis shows winter periods from 1982 to 1990.

study, no correlation was established for the same patient between the subtype from frozen nasal slides and that from the reisolated strains. The slides and the reisolated RSV were selected as possible at the start (25%), at the end (25%), and at the peak (50%) of the outbreak.

During the eight winter epidemics studied, from November 1982 to February 1990, 1,298 RSV infections were identified in the hospitalized infants. Outbreaks started during October or November, had often a sharp peak of illness for 1 month in December or January, and lasted about six months (5). The number of infected infants was approximately the same each year and varied from 156 in 1985–1986 to 217 in 1988–1989.

For the identification of subgroups of RSV, two MAbs reacted with both A and B strains, one G-specific MAb (clone C793) and one F-specific MAb (clone B151), and three antibodies were used to distinguish A and B strains, one anti-NP MAb (clone 7858) specific for B strains and two MAbs specific for A strains, one anti-NP (clone B90) and one anti-F (clone 2B8) (2, 12, 13). This clone detected subgroup A variants of RSV (15). Cells infected by subgroup A strains reacted with MAbs C793, B151, and B90, and by contrast cells infected by subgroup B strains reacted with MAbs C793, B151, and 7858. In the epidemics of 1982–1983, 1983–1984, and 1984–1985, 27 (30%) out of the 90 slides tested were classified as subgroup A strains and 63 (70%) were classified as subgroup B. Subgroup B predominated over subgroup A in 1983–1984 and 1984–1985, and the proportion of subgroup A to subgroup B was 2.5:1 (Fig. 1). Through the five following winter epidemics, i.e., 1985–1986, 1986–1987, 1987–1988, 1988–1989, and 1989–1990, 284 RSV strains were typed as subgroup A or B by using the MAbs. Eight strains initially typed as intermediate were clearly subgroup A or B when reisolated and retested. A total of 115 strains (40.5%) were categorized as subgroup A and 169 (59.5%) were categorized as subgroup B. Overall, the subgroup B predominated over subgroup A, and the proportion of subgroup A to subgroup B was 1.5:1 (Fig. 1). During three outbreaks, 1985–1986, 1986–1987, and 1988–1989, both subgroups were present in almost equal numbers. In one year, 1987–1988, subgroup A strains predominated (88.3%), and subgroup B strains predominated in the last winter, 1989–1990 (84.5%).

So, from 1982 to 1990 in Caen, both subgroups occurred each year, but one subgroup predominated in some years, i.e., subgroup A in 1987–1988 and subgroup B in 1983–1984 or 1989–1990. Therefore, we observed a gradual change of the predominant subgroup to another over a period of about 3 to 5 years: the relative frequency of subgroup A strains isolated from patients increased from 1983 to 1988, whereas the percentage of subgroup B strains decreased during the same period of time. The pattern was the opposite from 1987 to 1990.

In Caen, the peak of each outbreak occurs regularly in December or January. This RSV epidemic does not tend to follow the pattern first described in Washington, D.C. (10), with a peak occurrence alternating early and late in successive years, and is more similar to the one observed in northeast England (17). The subgroup A and B strains from the eight annual outbreaks have reacted in a consistent manner with the five MAbs used in this study. No intermediate or indeterminate strains were isolated, supporting the concept that the two subgroups of RSV have been antigenically stable for over at least 25 years (11–13). Recently, by using other MAbs, subgroup A strains of RSV have been classified into four patterns: Long-like, A2-like, Bernett-like, and "Massachusetts (M)/85-like"; the last is the predominant strain in four epidemics from 1983 to 1987 (8). Two major variants of subgroup B strains have also been differentiated (14).

The comparison of our results with previous studies of RSV subgroup prevalence in Huntington, W.Va. (12), Boston, Mass. (8), St. Louis, Mo. (18), and Sapporo, Japan (19), indicates that temporal distribution of the two subgroups of RSV is not worldwide and varies in geographic localization, from North America to Asia. Among the five epidemics that were studied in the United States from 1981 to 1986, subgroup A strains predominated in 1981–1982, 1982–1983, and 1985–1986, in Huntington as well as in St. Louis or in Boston, and only in the first two places during winter 1983–1984. A different pattern was reported in Sapporo: the strains of the 1982–1983, 1984–1985, and 1985–1986 epidemics were predominantly subgroup B strains. In Caen, France, subgroup B of RSV also predominated during the winters of 1983–1984, 1984–1985, and 1985–1986.

The major antigenic differences between the two subgroups A and B of RSV are linked to the F and G glycoproteins that play a major role in the acquisition of immunity. The weak immunogenicity of RSV and the progressive immunization against a subgroup after several epidemics of this virus can exclude one strain and favor the spread of the other to occupy an almost exclusive place (7, 8). This pattern was observed in Caen, where subgroup B strains decreased slowly from 1983 to 1988 whereas subgroup A strains increased progressively in the same time. As the clinical illness associated with RSV is most common between 2 and 4 months of age, and as a new repository of susceptible infants will be available for each annual epidemic, then children older than 1 year must be important for allowing epidemic circulation of the subgroups of RSV and for the gradual change of the predominant subgroup.

RSV is a major cause of bronchiolitis and pneumonia during the first year of life. Mufson et al. (12) reported that clinical characteristics of the disease are different between subgroups A and B of RSV because fewer children develop bronchiolitis in subgroup B infections than in A infections. Monto et al. (11) observed no differences in illness characteristics between the subgroups. The rate of hospitalization of infants for RSV disease is estimated at between 1 in 100 and 1 in 200 (4, 10). Our study and most of those on the

distribution of subgroup A and B strains of RSV have been conducted with hospitalized children. This indicates the importance of doing further studies of the clinical characteristics of the diseases associated with subgroup A and B strains occurring in nonhospitalized children.

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REFERENCES

1. **Akerlind, B., and E. Norrby.** 1986. Occurrence of respiratory syncytial virus subtypes A and B in Sweden. J. Med. Virol. **19**:241–247.
2. **Akerlind, B., E. Norrby, C. Orvell, and M. A. Mufson.** 1988. Respiratory syncytial virus: heterogeneity of subgroup B strains. J. Gen. Virol. **69**:2145–2154.
3. **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.
4. **Clarke, S. K. R., P. S. Gardner, P. M. Poole, H. Simpson, and J. O. H. Tobin.** 1978. Respiratory syncytial virus infection: admissions to hospital in industrial, urban, and rural areas: report to the Medical Research Council subcommittee on respiratory syncytial virus vaccine. Br. Med. J. **2**:796–798.
5. **Freymuth, F., C. Boucher, M. Lemarinel, F. Daon, M. Larchet, J. F. Duhamel, P. Boutard, J. Guihard, P. Charbonneau, C. Lecacheux, and C. Bazin.** 1983. Contribution du diagnostic direct en immunofluorescence à l'épidémiologie des infections respiratoires virales aigues du jeune enfant. Ann. Pediatr. **30**:433–437.
6. **Gimenez, H. B., N. Hardman, H. M. Keir, and P. Cash.** 1986. Antigenic variation between human respiratory syncytial virus. J. Gen. Virol. **67**:863–870.
7. **Henderson, F. W., A. M. Collier, W. A. Clyde, and F. W. Denny.** 1979. Respiratory syncytial virus infections, reinfections and immunity: a prospective longitudinal study in young children. N. Engl. J. Med. **300**:530–534.
8. **Hendry, R. M., L. T. Pierik, and K. McIntosh.** 1989. Prevalence of respiratory syncytial virus subgroups over six consecutive outbreaks. J. Infect. Dis. **160**:185–190.
9. **Hendry, R. M., A. L. Talis, E. Godfrey, L. J. Anderson, B. F. Fernie, and K. McIntosh.** 1986. Concurrent circulation of antigenically distinct strains of respiratory syncytial virus during community outbreaks. J. Infect. Dis. **153**:291–297.
10. **Kim, H. W., J. O. Arrobio, C. D. Brandt, B. C. Jeffries, G. Pyles, J. L. Reid, R. M. Chanock, and R. H. Parrott.** 1973. Epidemiology of respiratory syncytial virus infection in Washington, D.C. I. Importance of the virus in different tract disease syndromes and temporal distribution of infection. Am. J. Epidemiol. **98**:216–225.
11. **Monto, A. S., and S. Ohmit.** Respiratory syncytial virus in a community population: circulation of subgroups A and B since 1965. J. Infect. Dis. **161**:781–783.
12. **Mufson, M. A., R. B. Belshe, C. Orvell, and E. Norrby.** 1988. Respiratory syncytial virus epidemics: variable dominance of subgroups A and B among children. J. Infect. Dis. **157**:143–148.
13. **Mufson, M. A., C. Orvell, B. Rafnar, and E. Norrby.** 1985. Two distinct subtypes of respiratory syncytial virus. J. Gen. Virol. **66**:2111–2124.
14. **Orvell, C., E. Norrby, and M. A. Mufson.** 1987. Preparation and characterization of monoclonal antibodies directed against five structural components of human RSV subgroup B. J. Gen. Virol. **68**:3125–3135.
15. **Pothier, P.** Personal communication.
16. **Pothier, P., J. C. Nicolas, G. Prudhomme de Saint Maur, S. Ghim, A. Kazmierczak, and F. Bricout.** 1985. Monoclonal antibodies against respiratory syncytial virus and their use for rapid detection of virus in nasopharyngeal secretions. J. Clin. Microbiol. **21**:286–287.
17. **Sims, D. G., M. A. P. S. Dowham, J. McQuillin, and P. S. Gardner.** 1976. Respiratory syncytial virus infection in northeast England. Br. Med. J. **2**:1095–1098.
18. **Storch, G. H., and C. S. Park.** 1987. Monoclonal antibodies demonstrate heterogeneity in the G glycoprotein of prototype strains and clinical isolates of respiratory syncytial virus. J. Med. Virol. **22**:345–356.
19. **Tsutsumi, H., M. Onuma, K. Suga, T. Honjo, T. Chiba, S. Chiba, and P. L. Ogra.** 1988. Occurrence of respiratory syncytial virus subgroup A and B strains in Japan. J. Clin. Microbiol. **26**:1171–1174.