Antigenic heterogeneity of the attachment protein of bovine respiratory syncytial virus

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A panel of 23 monoclonal antibodies (MAbs) specific for the attachment (G) glycoprotein of bovine respiratory syncytial virus (BRS virus), recognizing seven antigenic areas on the G protein, was used to determine the antigenic heterogeneity among 19 BRS viruses isolated over a 20 year period from various parts of the world. The pattern of reactivity of the isolates, as determined by ELISA, identified two major subgroups of BRSV. This finding was confirmed by radioimmunoprecipitation of the G protein by the MAbs and was also demonstrated using polyclonal sera obtained from calves hyper-immunized with BRS virus strains from each subgroup. The subgroups could also be differentiated by differences in the apparent Mr of the fusion (F) glycoprotein and its cleavage products. The apparent Mr's of the Fα, F1 and F2 polypeptides were 73K, 46K and 17K for subgroup A strains and 77K, 46K and 23K for subgroup B strains. These studies provide evidence for two major lineages of BRS virus, similar to the situation with human RS virus.

Introduction

Bovine respiratory syncytial virus (BRS virus) is a major cause of respiratory disease in cattle (Stott & Taylor, 1985; Baker et al., 1986). The epidemiology and pathology of disease caused by BRS virus in cattle is similar to that caused by human respiratory syncytial virus (HRS virus) in children (Stott & Taylor, 1985; Baker, 1991). Thus, the greatest morbidity occurs during the first 6 months of life when maternal antibody is still present, outbreaks of RS virus infection occur annually, reinfections are common, and difficulties have been encountered with the use of both live and inactivated vaccines against RS viruses, some of which have potentiated respiratory disease (see Stott & Taylor, 1985; Kimman et al., 1989).

BRS virus and HRS virus are pneumoviruses in the family Paramyxoviridae and are antigenically related (Lerch et al., 1989). The protein composition of BRS and HRS viruses is very similar with only minor differences in Mr between corresponding proteins (Cash et al., 1977; Lerch et al., 1989). Although there is antigenic cross-reactivity of the F (fusion), N (nucleo-), M (matrix) and P (phospho-) proteins between BRS and HRS viruses, there are major antigenic differences between the attachment, G, glycoproteins (Örvell et al., 1987; Lerch et al., 1989). Furthermore, the deduced amino acid sequence of the BRS virus G polypeptide showed only 29 to 30% identity with that of the G protein of HRS virus (Lerch et al., 1990) suggesting that BRS virus belongs to a different group from HRS virus, within the pneumoviruses.

The two surface glycoproteins of RS viruses, F and G, play an important role in infectivity, replication and the immune response to the virus. Therefore antigenic variation in these proteins may have important implications in the epidemiology and pathogenesis of RS virus infections. Two major subgroups of HRS virus, designated A and B, have been identified with monoclonal antibodies (MAbs) (Anderson et al., 1985; Gimenez et al., 1986; Mufson et al., 1985) and there is antigenic diversity within each of the subgroups (Morgan et al., 1987; Örvell et al., 1987; Garcia-Barreno et al., 1989). The subgroups differ in at least four structural proteins, the G, F, N and P proteins, although the major antigenic difference was found in the G protein. In addition, there are differences in the apparent Mr of the F1 and F2 cleavage products of the F0 protein and of the P proteins between the two subgroups (Gimenez et al., 1986; Norrby et al., 1986). Epidemiological studies of HRS virus indicate that the two subgroups circulate concurrently during epidemics (Hendry et al., 1986), and there are reports that infections with subgroup A viruses are associated with more severe disease than infections with subgroup B viruses (McConnachie et al., 1990). Furthermore, there is evidence to suggest that antigenic differences between the two subgroups may be important in repeat infections (Mufson et al., 1987).
There have been few studies on the antigenic variation among isolates of BRS virus. One study failed to find antigenic differences between one caprine and three bovine strains of RS virus (Orvell et al., 1987) and another study showed variations in the Mr of the P and F proteins among nine isolates of BRS virus (Baker et al., 1990). Antigenic differences in the G protein of BRS virus have been observed with a polyclonal serum (Lerch et al., 1989). Thus, the G protein of the 391-2 strain of BRS virus was recognized by a polyclonal serum raised against the homologous strain and not by a polyclonal serum raised against the 127 strain of BRS virus. To study further the antigenic variation among strains of BRS virus we have produced and characterized MAbs specific for the G protein of BRS virus and have used them, together with previously described MAbs to other structural proteins of RS virus, to study the antigenic characteristics of 19 strains of BRS virus isolated from calves in several different countries.

**Methods**

**Cells and viruses.** The following strains of BRS virus were studied: Snook (Thomas et al., 1982); 127 (Stott et al., 1984); 901172, 9007-82, 4223-76 and 4642 were isolated by Dr E. J. Stott, (IAH, Compton, U.K.) from calves on different farms in the U.K.; 1015, 1055, 1833, 56442 and WBH were isolated from calves in different parts of the Netherlands and were obtained from Dr J. T. van Oirschot, Lelystad, The Netherlands; C88 has been described previously (Paccand & Jacquier, 1970); B097, 220-69, X11 21/72, 5/29/75 and 220/60 were obtained from Dr G. Prince, NIH, Bethesda, Md., U.S.A. B097, originally designated VC490, was isolated from a calf in the U.S.A. (Rosenquist, 1974), 220-69 was originally isolated by Dr G. Wellemons from a calf in Belgium, and the origin of strains X11 21/72, 5/29/75 and 220/60 is not known, although all were isolated prior to 1977 (Dr G. Prince, personal communication); strain 391-2 has been described previously (Lerch et al., 1989); SBAH is the SmithKline Beecham Animal Health vaccine strain 375 (SmithKline Beecham Animal Health, Lincoln, Nebr., U.S.A.), which was originally isolated by Lehmkühl et al. (1979).

The HRS virus strains A2 (subgroup A) and 8/60 (subgroup B), described previously (Lewis et al., 1961; Kennedy et al., 1988), were grown in Hep-2 cells to produce antigen for ELISA and in primary calf kidney (CK) cells for analysis by radioimmunoprecipitation. Bovine RS virus strains were grown in primary CK cells. Recombinant vaccinia virus (VV) G642, expressing the G protein from the 391-2 strain of BRS virus (Lerch et al., 1990) was grown in HTK cells.

**Antibodies.** Six murine anti-F MAbs 11, 13, 19, 20, 9 and 10, two bovine anti-F MAbs B4 and B5, two anti-NP MAbs 6 and 15, anti-P MAb 12 and anti-M2 MAb 8 have been described previously (Taylor et al., 1984, 1992). The anti-M2 MAB 1C13 was obtained from Dr G. Toms (University of Newcastle upon Tyne, U.K.) and has been described previously (Routledge et al., 1987). Anti-F bovine MAbs B13 and B14 were produced from the same RS virus-immunized calf that was used to produce bovine MAbs B1 to B6 (Kennedy et al., 1988), but the bovine lymphocytes had been stored in liquid N₂ before fusion with NSI cells. Murine MAB 29 recognizes the G protein of both HRS virus subgroups. MAbs 30 and 31 are specific for the G protein of subgroup A HRS virus and MAB 26 is specific for the G protein of subgroup B HRS virus (J. Furze et al., unpublished observations). Polyclonal serum M117 was obtained from a gnotobiotic calf hyperimmunized with the 127 strain of BRS virus. Polyclonal serum 2106 was obtained from a gnotobiotic calf hyperimmunized with the 391-2 strain of BRS virus.

MAbs 44 to 69 were prepared from specific-pathogen-free BALB/c mice, obtained from Charles Rivers. Mice were inoculated with 2 x 10⁶ p.f.u. of VV G642 by scarification. After an interval of 3 months, mice were boosted with 0.2 ml of 10-fold concentrated serum-free supernatant (199 medium containing 0.14% sodium bicarbonate, 0.01 M-HEPES, penicillin, streptomycin and mycostatin, pH 7.8) from CV-1 cells infected with BRS virus strain 391-2, administered intraperitoneally (i.p.) 4, 3 and 2 days before fusion of spleen cells with NSI cells as described previously (Taylor et al., 1984).

MAb 70 was prepared from mice immunized with 0.5 ml of 10-fold concentrated serum-free supernatant from primary CK cells infected with BRS virus strain 127, with Freund’s incomplete adjuvant, given i.p. on two occasions 9 months apart. One month after the second dose, mice were boosted intravenously with 0.2 ml serum-free supernatant (concentrated 10-fold) from Vero cells infected with the 127 strain of BRS virus. Spleen cells were fused with NSI cells 4 days later.

Culture fluids were screened for antibody to RS viruses by ELISA using BRS virus strains Snook, 391-2 and 127 grown in CK cells and HRS virus strains A2 and 8/60 grown in Hep-2 cells as antigen. Uninfected CK and Hep-2 cells were used as control antigens. Hybridomas reacting preferentially with either the 391-2, Snook or 127 strain of BRS virus and not with HRS viruses were cloned twice by the soft agar method and inoculated i.p. into pristane-primed BALB/c mice to produce ascitic fluid. The Ig isotype of MAb in hybridoma culture fluid was determined using the Serotec mouse monoclonal typing kit. The specificity of MAbs for viral polypeptides was determined by radioimmunoprecipitation assay (RIPA) using CK cells infected with the Snook or 127 strain of BRS virus and labelled with [³H]glucosamine.

**ELISA.** Antigen consisted of CK or Hep-2 cells infected with RS virus harvested when approximately 50% of the cell monolayer demonstrated c.p.e. Cells were scraped into the medium, centrifuged and resuspended in deionized water and 0.5% NP40 to yield a cell lysate. Control cell lysate was prepared in a similar way from uninfected cells. Polyvinylchloride microtitre plates (Falcon 3912) were coated with antigen diluted in deionized water, and allowed to dry overnight at 3⁰C before use. Plates were blocked with PBS containing 5% pig serum and 0.05% Tween 20 (PBS-PS-Tw) for 1 h at room temperature. Dilutions of ascitic fluid containing MAbs, made in PBS-PS-Tw, were added to wells and allowed to react at room temperature for 1 h. After five washes in PBS-Tw, horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG (Sigma), diluted 1:2000 in PBS-PS, was added to each well and allowed to react at room temperature for 1 h. After a final wash, bound conjugate was detected using 3,3',5,5'-tetramethylbenzidine (TMB) (Miles Laboratories) as substrate.

**RIPA.** Tissue culture plates (35 mm) containing control CK cells or cells infected with RS virus isolates at an m.o.i. of 0.1 were labelled with either 100 μCi/ml of [³H]glucosamine in 0.7 ml Hanks’ balanced salts containing 2% heated fetal calf serum or 100 μCi/ml of [³H]methionine. After 18 h, the medium was removed and replaced with 300 μl 0.5% NP40 in deionized water and incubated for 5 min at 4⁰C. The lysate was clarified by centrifugation at 10000 rpm for 1 min, mixed with one third of the volume of 4 x RIPA buffer (600 mm-sodium chloride, 4% sodium-deoxycholate, 4% Triton X-100, 0.4% SDS, 10 mm-Tris-HCl, pH 7.4) and stored at –70⁰C. Aliquots (100 μl) of labelled antigen were mixed with 25 μl of either undiluted hybridoma culture supernatant, ascitic fluid diluted 1:100 in PBS, purified IgG diluted 1:100 in PBS, or with a 1:10 dilution of M117 or 2106 polyclonal serum. Antigen–antibody complexes were precipitated with
10% (v/v) Protein G-Sepharose (Pharmacia) and subjected to SDS-PAGE on homogeneous 12.5% or 15% acrylamide slab gels (Laemmli, 1970). The gel was fixed in 7% (v/v) acetic acid, incubated in Amplify (Amersham), dried and exposed to X-ray film.

**Purification and labelling of antibodies.** IgG from ascitic fluid was purified using MAbTrap G (Pharmacia). Ascitic fluid (3 ml) was mixed with an equal volume of 0.02 M-sodium phosphate buffer pH 7.0 and passed through a Protein G-Sepharose 4 FF column equilibrated with the same buffer. Bound antibodies were eluted with 0.1 M-glycine-HCl pH 2.7, neutralized with 1.0 M-Tris-HCl pH 9.0 and dialysed against PBS. Purified antibodies were labelled with biotin using N-hydroxysuccinimidobiotin (NHS-biotin) (Pierce) or with $^{125}$I using chloramine T. One ml of purified IgG at 3 mg/ml was dialysed against 0.1 M-NaHCO$_3$ and incubated with 114 ml NHS-biotin, at 10 mg/ml in DMSO, with mixing at room temperature for 4 h. The biotylated MAb was dialysed against PBS and aliquots were stored at --70°C.

**Results**

**Characterization of MAbs against BRS virus**

Hybridoma cultures, prepared from the spleens of mice immunized with VV G642 were screened for antibodies by ELISA using BRS virus strains 391-2, Snook and 127 in ELISA. Since previous studies with polyclonal anti-BRS virus serum had suggested antigenic differences in the G protein of the 127 and 391-2 strains of BRS virus (Lerch et al., 1989), hybridomas secreting antibodies that reacted preferentially with either 391-2, Snook or 127 strains were selected for cloning. After cloning, culture fluids were examined for their ability to react with $^3$H]glucosamine-labelled extracts from BRS virus strain 391-2 or Snook-infected CK cells. Twenty-four of the 25 MAbs recognized a glycosylated protein of $M_r$ approximately 84K, which corresponds to the G glycoprotein of RS virus. A single antibody, MAb 51, which failed to precipitate the G protein was an IgM antibody. The ELISA titres of MAbs in ascitic fluids against the 391-2 strain of BRS virus ranged from log$_{10}$ 4.4 to 7.7 and were similar to those found using the Snook strain of BRS virus as antigen. None of the MAbs reacted in ELISA with the 127 strain of BRS virus or with the A2 and 8/60 strains of HRS virus.

Hybridoma culture fluids prepared from the spleens of mice immunized with concentrated supernatant from cells infected with the 127 strain of BRS virus were screened for antibody that reacted preferentially with the 127 strain of BRS virus. After cloning, the culture fluids were examined for their ability to react with $^3$H]glucosamine-labelled extracts from cells infected with the 127 strain of BRS virus. A single antibody, MAb 70, recognized a glycosylated protein with an $M_r$ of approximately 84K. This antibody reacted with the 127 strain of BRS virus in ELISA, and failed to react with either the 391-2 and Snook strains of BRS virus or with the A2 and 8/60 strains of HRS virus.

**Identification of antigenic areas on the G protein**

MAbs 44 to 69 recognizing the G protein of the 391-2 and Snook strains of BRS virus were purified from ascitic fluid and labelled with biotin. MAbs 65 and 68 could not be purified in sufficient amounts for labelling using Protein G-Sepharose and MAbs 53, 54, 57 and 61 had a greatly reduced ability to bind to RS virus in ELISA after biotinylation or iodination. The 19 labelled MAbs, at a dilution that gave approximately 90% of maximum binding to the Snook strain of BRS virus, were used in ELISAs either alone or in the presence of increasing amounts of unlabelled antibodies of the same protein specificity. Some of the unlabelled MAbs inhibited the binding of the labelled antibody in a dose-dependent manner, whereas others did not interfere with the binding of the test antibody. The competition profiles of MAbs 44 to 69 overlapped extensively; therefore the clustering of epitopes in antigenic areas was done on the basis of partial differences among MAbs. These studies indicated that the MAbs recognized six overlapping areas on the G protein of BRS virus (Fig. 1).

**Antigenic variation in the G protein among BRS virus isolates**

To determine the degree of variation in the G protein among isolates of BRS virus, a panel of 23 MAbs to the G protein, described above, was tested in an ELISA.
against 19 BRS viruses isolated over a 20-year period from various parts of the world. There were two main patterns of reactivity of the MAbs with the BRS virus isolates (Fig. 2). Thus, group A isolates SBAH, 1015, 1055, 1833, 4223-76, 9011T2, 56442, B097, 9007-82 and 5/29/75 were similar to 391-2 and Snook as they reacted with MAbs 44 to 69 and failed to react with MAb 70. In contrast, group B isolates C881, X11 21/72, 4642 and 220/60 were similar to 127 as they reacted with MAb 70 but failed to react with any of MAbs 44 to 69. There were two exceptions to these patterns, isolate 220-69 which appeared to be intermediate between the two groups as it reacted with all MAbs except 62, 46, 47, 57, 53, 61 and 69, and isolate WBH which failed to react with any of the MAbs to the G protein.

Reactivity of BRS virus isolates with MAbs to other structural proteins of RS virus in ELISA

None of the BRS virus isolates reacted with MAbs specific for the G protein of HRS virus (Fig. 3). All BRS virus isolates reacted with MAbs 9 and 10, which are specific for the F protein of BRS virus and also with MAbs 11, 13, 19, 20, B4, B5, B13 and B14, which are highly protective, neutralizing, fusion-inhibiting MAbs to the F protein (Fig. 3). All isolates reacted with the anti-NP MAb 6, although the reactions of two of the isolates (9011T2 and 9007-82) were weak. All isolates, except 5/29/75, also reacted with the anti-NP MAb 15, although 9007-82 was only weakly positive. Three isolates, 4223-76, 5/29/75 and 220/60, failed to react with the anti-P MAb 12. Seven of the isolates, 9011T2, 56442, B097, 9007-82, 5/29/75, 220-69 and WBH, failed to react with the anti-M2 MAb 8 and none of the isolates reacted with MAb 1C13, which is specific for the M2 protein of subgroup A strains of HRS virus (Routledge et al., 1987).

Reactions of BRS virus isolates in RIPA

The 19 BRS virus isolates were labelled with [3H]glucosamine and immunoprecipitated with M117 polyclonal serum from a calf immunized with the 127 strain of BRS virus, MAb 57 (and 44 for strain 220-69) and MAb 70. Eleven of the isolates were also immunoprecipitated with 2106 polyclonal serum from a calf immunized with the 391-2 strain of BRS virus. The results from the RIPA confirmed the findings shown in Fig. 2. Thus, MAb 57 precipitated only the G protein of isolates (group A) that reacted with MAb 57 in ELISA. Similarly, MAb 70 precipitated the G protein of isolates (group B) that reacted with MAb 70 in ELISA. The results for 13 of the isolates are shown in Fig. 4(a and b). Polyclonal serum M117 precipitated the G protein only of isolates that reacted with MAb 70 and were like the 127 strain of BRS virus (Fig. 4a and b). Polyclonal serum 2106 precipitated the G protein of all the group A strains studied and reacted only weakly, if at all, with the group B strains (Fig. 5). The G protein of strain 220-69 was precipitated
The apparent $M_r$ of the F glycoprotein and its cleavage products differed among the various BRS virus isolates. The apparent $M_r$ values of $F_0$, $F_1$, and $F_2$ were 73K, 46K and 17K, respectively, for group A isolates (Figs. 4a, b and Fig. 6), and 77K, 46K and 23K, respectively, for group B isolates (Fig. 4a, b and Fig. 6). The $M_r$ of the $F_0$, $F_1$, and $F_2$ proteins of the intermediate strain 220-69, which reacted with MAb 70 as well as some of MAbs 44 to 69, were similar to those of group B isolates. The apparent $M_r$ values of the $F_0$, $F_1$, and $F_2$ proteins of WBH, which failed to react with any of the anti-G MAbs, were 73K, 46K and 17K which are the same as those of group A isolates. These results are summarized in Fig. 2.

To determine whether there were differences in the apparent $M_r$ of the P protein amongst strains of BRS virus, 10 (including four from group A and four from group B) of the strains (391-2, SBAH, 4223-76, 5/29/75, 127, C881, X11 21/72, 220-60, WBH and 220-69) were labelled with $[^35]S$-methionine and immunoprecipitated with MAb 12 to the P protein. Although strains 4223-76, 5/29/75 and 220-60 failed to react with MAb 12 in ELISA (Fig. 3), MAb 12 reacted with the P protein of these strains in RIPA. The $M_r$ of the P protein of the SBAH strain was 36K compared with 38K for the other nine strains studied (results not shown).
Discussion

The development of MAbs to the G protein of BRS virus was greatly facilitated by the use of recombinant VV G642 expressing the G protein of the 391-2 strain of BRS virus. Twenty-four MAbs, which reacted with the 391-2 and Snook but not the 127 strain of BRS virus nor with HRS virus, were produced from the spleens of two mice given recombinant VV. In contrast, only one MAb to the G protein of the 127 strain of BRS virus was obtained from four mice immunized with concentrated culture supernatant from infected cells, which contains the secreted form of the G protein (Hendricks et al., 1987). None of the MAbs reacted with either of the HRS virus strains A2 or 8/60 (subgroups A and B respectively), confirming the findings obtained using MAbs to the G protein of HRS virus that there are major antigenic differences between the G proteins of human and bovine strains of RS virus (Örvell et al., 1987).

A panel of 23 MAbs to the G protein of the 391-2 strain and one MAb to the 127 strain were used to determine the extent of antigenic heterogeneity in the G protein among 19 strains of BRS virus isolated over a period of 20 years from different parts of the world. The MAbs categorized the isolates into two main groups by ELISA and by RIPA, with 12 of the strains (subgroup A) reacting with MAbs 44 to 69 and not with MAb 70, and five of the strains (subgroup B) reacting only with MAb 70. One strain, 220-69, appeared to be intermediate as it shared epitopes with both of the subgroups. Thus, MAbs 62, 46, 47 and 57, mapping to antigenic areas C and D, failed to react with strain 220-69, whereas other MAbs mapping to these antigenic areas and MAb 70 recognized the 220-69 strain. This finding suggests that there are distinct epitopes within antigenic areas C and D, which can be distinguished by their differing reactivity in the 220-69 strain. Another strain, WBH, which failed to react with any of the MAbs to the G protein of BRS virus was confirmed as a bovine strain by its failure to react with MAbs to the G protein of HRS virus and also by its ability to react with MAbs 9 and 10, which are specific for the F protein of BRS virus (Taylor et al., 1984).

The antigenic variation in the G protein determined with MAbs was also detected using polyclonal hyperimmune serum and this indicates that there is little antigenic cross-reactivity in the G protein between viruses in the two subgroups. This may have important implications for vaccine development. It may be that the G protein from the 220-69 strain will be useful in
inducing cross-reactive anti-G immune responses. Nevertheless, despite the variation in the G protein, the eight neutralizing, fusion-inhibiting anti-F MAbs that are highly protective in vivo (Taylor et al., 1984, 1992) reacted with all the strains of BRS virus studied. There were some differences in reactivity of BRS virus strains and MAbs to the N and P proteins in ELISA. However, these were not confirmed by RIPA (results not shown) and did not correlate with antigenic differences in the G protein.

There were distinct differences in the apparent $M_r$ of the F protein, and its cleavage products, amongst strains of BRS virus, which appeared to correlate with reactivity to MAb 70. Thus, the $M_r$ of $F_0$ and $F_2$ of those isolates which failed to react with MAb 70 (subgroup A viruses), were smaller than those of isolates that did react with MAb 70 (subgroup B viruses). The differences observed in the electrophoretic mobility of the $F_2$ polypeptides of the BRS virus strains are probably due to differences in the extent of glycosylation, as has been shown previously for the $F_2$ polypeptides of the 391-2 strain of BRS and the A2 strain of HRS virus (Lerch et al., 1991).

Baker et al. (1992) also observed differences in the apparent $M_r$ of the $F_0$, $F_1$ and $F_2$ polypeptides of nine BRS virus isolates. Thus, the $M_r$s of the $F_0$, $F_1$ and $F_2$ of the SBAH strain were 68K, 48K and 21K, respectively, which are similar to those of HRS virus. In contrast, for the other eight isolates, which included the 375 strain (which was the origin of the SBAH strain), the $M_r$s values were 63K, 47K and 17K. Baker et al. (1992) suggested that the differences in the $M_r$ of the $F_0$ protein and its cleavage products between the SBAH strain and 375 could be related to passage level in tissue culture. However, in our study, the $M_r$ of the $F_0$ protein of the SBAH strain was similar to those of other subgroup A BRS viruses and lower than that of subgroup B viruses.

Distinct differences in the $M_r$ of the P protein have been observed between subgroup A and B HRS viruses (Åkerlind et al., 1988) and also amongst strains of BRS virus (Baker et al., 1992). Thus, the $M_r$ of the P protein for three strains of BRS virus (SBAH, 375 and MN) was 36K and for six other strains was 38K. In our study, the P protein of the SBAH strain was unique in having an $M_r$ of 36K, whereas the molecular size of the P protein of nine other strains was 38K.

Several lines of evidence suggest that the antigenic differences in the G protein seen in this study appear to be stable characteristics of the strains. Thus, the reaction patterns were not changed by multiple passages in tissue culture. In particular, the pattern of reactivity of MAbs with the 220-69 strain, which underwent more than 90 passages in tissue culture to produce the SBAH ‘Rispoval’ vaccine (Dr N. Zygraich, SBAH, personal communication) was exactly the same as that of ‘Rispoval’ vaccine virus (unpublished observations). Further, for each subgroup the $M_r$s of the $F_0$ and $F_2$ proteins were characteristic for that subgroup. In addition, strains belonging to the same group included strains isolated from different continents and separated by more than 10 years.

These results represent the first demonstration of different subgroups of BRS virus based on antigenic differences in the G protein. Studies are underway to determine the molecular basis for this antigenic heterogeneity. It is not clear from this limited study of BRS virus strains whether the two subgroups of BRS virus circulate concurrently, as is observed for HRS virus (Hendry et al., 1986). Subgroup A BRS viruses have been present in Europe since at least 1977 and in the U.S.A. since at least 1972 and all of the subgroup B strains appear to have been isolated prior to 1976, suggesting that the subgroup B strains identified in this study may have been replaced by subgroup A strains. Detailed studies on the biological properties, cell tropism and pathogenicity of viruses that differ in the G protein together with the characterization of the G protein of more strains of BRS virus will provide information on the clinical and epidemiological significance of strain variation in BRS virus and its importance in vaccine development.

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References


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