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Characterization of Bovine Respiratory Syncytial Virus Proteins and mRNAs and Generation of cDNA Clones to the Viral mRNAs

ROBERT A. LERCH,† E. JAMES STOTT,‡ AND GAIL W. WERTZ†*

Department of Microbiology and Immunology, The University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514

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We have characterized the proteins and mRNAs of bovine respiratory syncytial (BRS) virus strain 391-2 and constructed cDNA clones corresponding to 9 of the 10 BRS virus mRNAs. The proteins of BRS virus-infected cells were compared with the proteins from human respiratory syncytial (HRS) virus-infected cells. Nine proteins specific to BRS virus-infected cells, corresponding to nine HRS virus proteins, were identified. Only a BRS virus polymerase protein remains to be identified. The BRS virus G glycoprotein showed major antigenic differences from the HRS virus G glycoprotein by immunoprecipitation and Western (immuno-) blot analysis, whereas the BRS virus F, N, M, and P proteins showed antigenic cross-reactivity with their HRS virus counterparts. Analysis of RNAs from BRS virus-infected cells showed virus-specific RNAs which had electrophoretic mobilities similar to those of mRNAs of HRS virus but which hybridized poorly or not at all with HRS virus-specific probes in Northern (RNA) blot analysis. To analyze the BRS virus RNAs further, cDNA clones to the BRS virus mRNAs were generated. Nine separate groups of clones were identified and shown to correspond to nine BRS virus mRNAs by Northern blot analysis. A 10th BRS virus large mRNA was identified by analogy with the HRS virus polymerase mRNA. These data show that like HRS virus, BRS virus has 10 genes coding for 10 mRNAs.

Bovine respiratory syncytial (BRS) virus strain 391-2 was isolated from an outbreak of respiratory syncytial virus in cattle in North Carolina during the winter of 1984 to 1985. The outbreak involved five dairy herds, a beef calf and cow operation, and a dairy and steer feeder operation (J. Fetrow, E. Henry, J. Guy, and T. Brown, North Carolina State University Agric. Extension Service Vet. Newsl., 1985).

Respiratory syncytial virus, an enveloped, single-stranded, negative-sense RNA virus (21, 23), was originally isolated from a chimpanzee (30). Subsequently, respiratory syncytial virus has been isolated from humans, cattle, sheep, and goats (9, 16, 25, 27, 33). Human respiratory syncytial (HRS) virus is a major cause of severe lower respiratory tract infections in children during their first year of life, and epidemics occur annually (37). Similarly, BRS virus causes bronchiolitis and pneumonia in cattle, and there are annual winter epidemics of economic significance to the beef industry (4, 37, 38). The highest incidence of severe BRS virus-caused disease is usually in cattle between 2 and 4.5 months old (35, 36). The outbreak of BRS virus strain 391-2 was atypical in that the majority of adult cows were affected, resulting in a 50% drop in milk production for one dairy herd and causing the death of some animals, while the young of the herds were only mildly affected (Fetrow et al., North Carolina State University Agric. Extension Service Vet. Newsl., 1985).

BRS virus was first isolated in 1970 (33), and research has focused on the clinical (39, 40) and pathological effects of the viral infection on the host (3, 7, 8) and on serological studies (2, 22, 38). The virus has not been described in molecular detail. Only one study has compared the proteins found in BRS virus-infected cells with the proteins found in HRS virus-infected cells (6). In contrast, a detailed molecular analysis of HRS virus has been undertaken. cDNA clones to the HRS virus mRNAs have been prepared and used to identify 10 virus-specific mRNAs which code for 10 unique polypeptides, and the complete nucleotide sequences for 9 of the 10 genes are available (14, 37).

Two lines of evidence suggest that HRS virus and BRS virus belong in distinct respiratory syncytial virus subgroups. First, BRS virus and HRS virus differ in their abilities to infect tissue culture cells of different species (33; unpublished observations). With one exception, studies have shown that BRS virus exhibits a narrower host range than HRS virus. Matumoto et al. (29) reported that the NMK7 strain of BRS virus has a larger host range than the Long strain of HRS virus. We and others have been unable to repeat this with other BRS virus strains (33, 34; unpublished observations). The second line of evidence indicating that BRS virus differs from HRS virus comes from the demonstration of antigenic differences in the major glycoprotein, G, of BRS virus and HRS virus (32). Studies using monoclonal antibodies have grouped HRS virus strains into two subgroups on the basis of relatedness of the G glycoprotein (1, 31). The G protein of BRS virus strains included in these studies did not react with monoclonal antibodies generated against viruses from either HRS virus subgroup (32). Work with polyclonal antibodies presented here further supports this finding.

BRS virus provides an opportunity to study the role of the major glycoprotein, G, in attachment, the possible host range restrictions of BRS virus compared with HRS virus, and the roles of the individual viral antigens necessary to elicit a protective immune response in the natural host, which is something that cannot be done easily for HRS virus at present.

We have initiated a molecular analysis of BRS virus.

* Corresponding author.
† Present address: Department of Microbiology, The University of Alabama Medical School, Birmingham, AL 35294.
‡ Present address: National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Potters Bar, Hertfordshire, United Kingdom EN6 3QG.

Virus-specific proteins and RNAs were identified and compared with the HRS virus (strain A2) proteins and RNAs by sodium dodecyl sulfate (SDS)-polyacrylamide and agarose-urea gel electrophoresis and Northern (RNA)-blot hybridizations, respectively. cDNAs corresponding to the BRS virus-specific RNAs were synthesized and used to further characterize the BRS virus RNAs.

MATERIALS AND METHODS

Virus and cells. BRS virus strain 391-2 (provided by J. Guy, North Carolina State University at Raleigh) and HRS virus strain A2(27) were grown in bovine nasal turbinate (BT) cells (provided by J. Guy; The Diagnostic Virology Laboratory, National Veterinary Services Laboratories, Ames, Ia.; and Paul C. Smith, Auburn University, Auburn, Alabama) in high glucose Dulbecco modified Eagle medium with 10% fetal bovine calf serum, and penicillin, streptomycin, and kanamycin.

Antibodies. The following antibodies were used in immunoprecipitation and Western (immuno-) blot analysis. (i) Wellcome anti-RS virus serum, which is a polyclonal antiserum generated in a calf against both the A2 HRS virus strain and the 127 BRS virus strain. (ii) Anti-G monoclonal antibody L9 (provided by E. Walsh, University of Rochester, Rochester, N.Y.) is a mouse monoclonal antibody which recognizes the G protein of both HRS virus subgroups (E. Walsh, personal communication). (iii) Anti-391-2 serum (provided by J. Guy) is convalescent serum from a calf infected with the BRS virus strain 391-2. (iv) Anti-127 serum is from a gnotobiotic calf infected with BRS virus strain 127.

Analysis of proteins and Western blots. BT cell monolayers were infected with either BRS virus (multiplicity of infection, 0.3) or HRS virus (multiplicity of infection, 1). [^{3}H]Threonine or [^{3}H]glucosamine (100 μCi/ml) was added to the medium at 21 h postinfection for HRS virus-infected cells and at 36 h postinfection for BRS virus-infected cells. After 3 h, the medium was removed, the monolayer was rinsed with phosphate-buffered saline, and the cells were harvested into lysing buffer (1% [vol/vol] Nonidet P-40, 0.4% [wt/vol] deoxycholic acid, 66 mM EDTA, 10 mM Tris hydrochloride, pH 7.4). The nuclei were removed by centrifugation, and the cytoplasmic extract was made 0.2% (wt/vol) with SDS. Proteins from uninfected cells were labeled and harvested in a similar manner. Virus-specific proteins were immunoprecipitated as previously described (41) by using Wellcome anti-RS virus serum (see above). Proteins were analyzed by SDS-polyacrylamide gel electrophoresis (24) and fluorography (15).

For Western blot analysis, proteins were harvested as described above, and after separation by nonreducing SDS-polyacrylamide gel electrophoresis were electrophoretically transferred to nitrocellulose (Micon Separations Inc.) by using a Polyblot apparatus under conditions recommended by the manufacturers. Blots were processed as reported previously (36), except that 10% (vol/vol) porcine serum was used in place of 1% (wt/vol) bovine serum albumin and 10% (vol/vol) fetal bovine calf serum in blocking and antibody solutions. Blocking occurred for 1 h at room temperature. Duplicate blots were probed with the four antisera described above. The appropriate horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin G or anti-bovine immunoglobulin G second antibody (ICN Immunobiologicals) was used as the conjugate.

Analysis of RNA and Northern blots. BT cell monolayers were infected with either BRS virus (multiplicity of infection, 0.3) or HRS virus (multiplicity of infection, 1). Actinomycin D (4 μg/ml) was added to the medium 1 h prior to labeling (21). At 19 h postinfection for HRS virus-infected cells and at 36 h postinfection for BRS virus-infected cells, [^{3}H]uridine (100 μCi/ml) was added to the medium. The medium was removed after a 3-h labeling period, and the monolayer was washed twice with HBS (0.01 M NaCl, 0.001 M $MgCl_2$, 0.01 M HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], pH 7.6). The cells were scraped off the plate in HBS and lysed by the addition of Nonidet P-40 (0.5% [vol/vol]). The nuclei were pelleted, and the supernatant was made 1% (wt/vol) with SDS. The samples were extracted twice with phenol, precipitated with ethanol, and suspended in H_2O. Samples were analyzed by agarose-urea gel electrophoresis (42) and fluorography (15). BRS virus RNA for poly(A) selection was labeled and harvested as above and then subjected to oligo(dT) column chromatography (15).

RNA from BRS or HRS virus-infected or uninfected cells to be used for Northern blot analysis was harvested as above and separated by agarose-urea gel electrophoresis (42). The RNA was transferred to diazobenzyloxymethyl paper (Schleicher & Schuell, Inc.) by capillary action (43) and probed with cDNA clones labeled with ^{32}P by nick translation (35).

cDNA synthesis and molecular cloning. BRS virus mRNA used for cDNA synthesis was harvested from infected cells grown in the presence of actinomycin D. The RNA was purified by centrifugation through a CsCl gradient (15). BRS virus RNA containing a poly(A) tail [poly(A)$^+$] was selected by oligo(dT) column chromatography (15). Poly(A)$^+$ BRS virus RNA was tested for its ability to be translated in vitro (15) before being used for cDNA synthesis. BRS virus-specific cDNAs were synthesized from poly(A)$^+$ BRS virus RNA by using the method described by Gubler and Hoffman (18). Homopolymer dCMP tails were added to the cDNAs, and they were cloned into the *Escherichia coli Pst*I-digested oligodeoxyguanylate tailed plasmid pBR322 as described previously (13).

Virus-specific cDNA clone identification. BRS virus-specific cDNA clones were identified by colony hybridization (17) by using a BRS virus-specific cDNA probe. A BRS virus-specific probe was made by synthesizing the first-strand cDNAs as indicated above except that 100 μCi of [α-^{32}P] dCTP was included and the dCTP concentration was reduced. The RNA strand was removed by treatment with NaOH (0.3M) for 1 h, the reaction was neutralized with HCl, and the single-stranded cDNAs were precipitated with ethanol. Before it was used, a 100-fold excess of cellular mRNA was added to the probe, and the mixture was boiled for 1 min and allowed to cool slowly (13).

Plasmid DNA (19) from selected BRS virus-specific clones was labeled by nick translation (35) and used to probe Northern blots of RNA from BRS virus-infected and -uninfected cells. BRS virus-specific cDNA was separated from pBR322 DNA by *Pst*I enzyme digestion and agarose gel electrophoresis (28) and then eluted from agarose and recovered by using Bethesda Research Laboratories NACS columns. Purified insert DNA was labeled by nick translation (35) and used to probe colony hybridizations (17), thus identifying clones of similar specificities.

RESULTS

Identification of BRS virus proteins. The proteins synthesized in BRS or HRS virus-infected cells and mock-infected

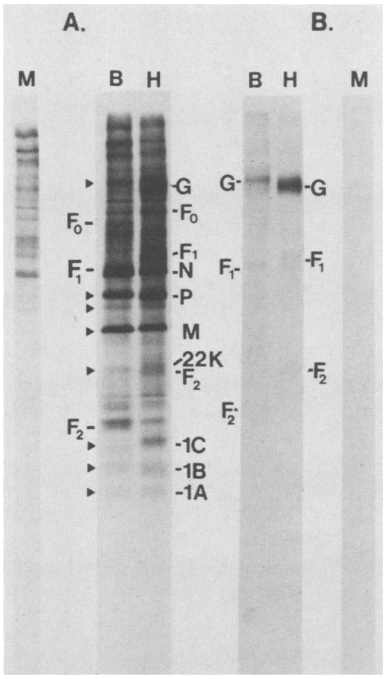

FIG. 1. Polyacrylamide gel analysis of BRS and HRS virus-specific proteins. Proteins from BT cells infected with BRS virus (lanes B) or HRS virus (lanes H) or from mock-infected BT cells (lanes M) were radioactively labeled by the incorporation of either [^{3}H]threonine (A) or [^{3}H]glucosamine (B) for 3 h and harvested by lysing the cells. Proteins were separated by electrophoresis in 15% polyacrylamide–SDS gels and fluorographed. HRS virus-specific proteins are identified. BRS virus-specific proteins which comigrate with HRS virus proteins are indicated with arrows; the F_0, F_1, and F_2 polypeptides are labeled.

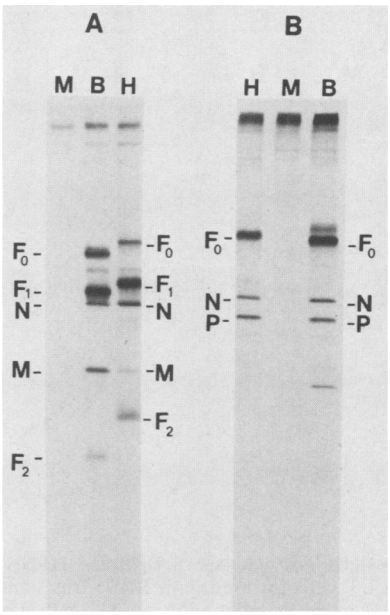

FIG. 2. Immunoprecipitation of BRS and HRS virus-specific proteins. Proteins from BT cells infected with BRS virus (lanes B) or HRS virus (lanes H) or from mock-infected BT cells (lanes M) were radioactively labeled by the incorporation of [^{3}H]threonine for 3 h and harvested by lysing the cells. Proteins were then immunoprecipitated with Wellcome anti-RS virus serum and separated on 12.5% (A) or 15% (B) polyacrylamide–SDS gels under reducing (A) or nonreducing (B) conditions. HRS virus-specific proteins are labeled, and BRS virus-specific proteins are labeled with their putative identifications.

cells were radioactively labeled with [^{3}H]threonine or [^{3}H]glucosamine and analyzed by SDS-polyacrylamide gel electrophoresis (Fig. 1). Proteins that were specific to BRS virus-infected cells and that comigrated with the HRS virus-specific proteins were observed; these were provisionally identified as the BRS virus counterparts to the previously described HRS virus proteins (20). BRS virus proteins that comigrated with the HRS virus N, P, and M proteins were observed (Fig. 1A, lane B). The putative BRS virus F, N, and M proteins were immunoprecipitated with the Wellcome anti-RS virus serum, confirming their identity as BRS virus-specific gene products (Fig. 2A, lane B).

Three BRS virus-specific polypeptides, which were shown to be glycosylated by incorporation of [^{3}H]glucosamine (Fig. 1B), were identified as corresponding to the HRS virus F_0 protein and its cleavage fragments F_1 and F_2 (Fig. 2). These BRS virus polypeptides showed differences in electrophoretic mobility compared with the HRS virus F polypeptides. This was demonstrated most clearly after immunoprecipitation of [^{3}H]threonine-labeled proteins with the Wellcome anti-RS virus serum (Fig. 2A, lane B). It was not determined whether these changes were the result of changes in carbohydrate or peptide moieties. A longer exposure of this autoradiograph showed the other immunoprecipitated proteins to be present in uninfected cells and thus to be of cellular origin (data not shown).

Under nonreducing conditions, only the BRS virus F_0 protein was observed, and the BRS virus F_1 and F_2 polypeptides were not present (Fig. 2B). Under reducing conditions, F1 and F2 were observed and the level of F_0 was greatly decreased compared with the level of F_0 under nonreducing conditions (Fig. 2A and B).

Another glycosylated BRS virus protein migrated as a broad band, with an estimated molecular mass of 80 to 90 kilodaltons, at approximately the same position as the heavily glycosylated HRS virus G protein (Fig. 1B). This putative BRS virus G protein was not observed readily in total [^{3}H]threonine-labeled BRS virus proteins (Fig. 1A) and was not immunoprecipitated by the Wellcome anti-RS virus serum (Fig. 2A).

Putative BRS virus 22K, 1A, 1B, and 1C proteins were identified by comigration with the HRS virus 22K, 1A, 1B, and 1C proteins, respectively (Fig. 1A). A putative BRS virus L protein has yet to be observed.

To further characterize the observed proteins as BRS virus specific, Western blot analysis of HRS virus and BRS virus proteins separated under nonreducing conditions was carried out by using four different anti-RS virus sera (see Materials and Methods). The anti-391-2 serum (Fig. 3A) recognized the N, P, and M proteins from both HRS and BRS virus-infected cells. Similar results were observed with the Wellcome anti-RS virus serum (Fig. 3C). The anti-127 serum (Fig. 3D) only recognized the HRS virus N protein and the putative BRS virus F, N, and M proteins.

Monoclonal antibody L9 (Fig. 3B), which recognizes the G protein of both HRS virus subgroups A and B (E. Walsh, personal communication), recognized the mature HRS virus G protein and partially glycosylated precursors (G. W. Wertz, unpublished data) but not the putative BRS virus G protein. The three polyclonal sera differed in their abilities to recognize the G proteins of BRS and HRS virus. The G protein of BRS virus-infected cells was recognized by only

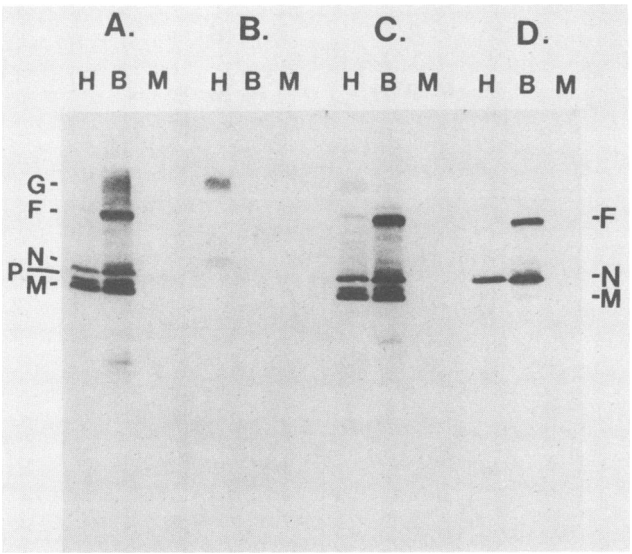

FIG. 3. Western blot analysis of BRS and HRS virus proteins. Proteins from BT cells infected with BRS virus (lanes B) or HRS virus (lanes H) or from mock-infected BT cells (lanes M) were harvested by lysing the cells when extensive syncytia were observed. The proteins were separated by electrophoresis in 15% polyacrylamide–SDS gels under nonreducing conditions and analyzed by Western blotting by using the following antisera as the first antibody: panel A, anti-BRS virus (strain 391-2) convalescent calf serum; panel B, anti-HRS virus G mouse monoclonal antibody (L9); panel C, Wellcome anti-RS virus serum (human and bovine strain 127), and panel D, anti-BRS virus (strain 127) serum. The appropriate horseradish peroxidase-conjugated anti-bovine immunoglobulin G or anti-mouse immunoglobulin G second antibody was used to identify the bound first antibody. HRS virus and BRS virus proteins are indicated.

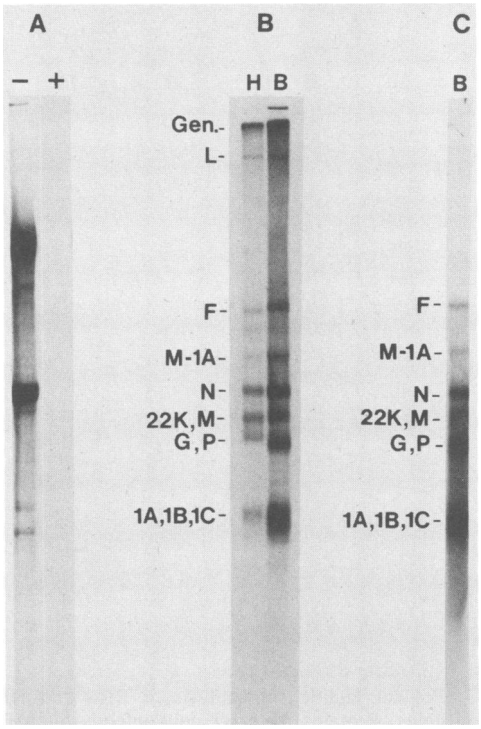

FIG. 4. Comparison of HRS and BRS virus RNAs. RNA from BT cells infected with BRS virus (lanes B) or HRS virus (lanes H) was radioactively labeled by incorporation of [^{3}H]uridine for 3 h in the presence of actinomycin D (panel B). The RNA was harvested and separated by electrophoresis in 1.75% agarose–urea gels, and the gel was fluorographed. HRS virus-specific monocistronic mRNAs, polycistronic RNAs, and genomic RNA (Gen.) are indicated. The control lanes (panel A) show the incorporation of [^{3}H]uridine in mock-infected cells with (+) and without (–) actinomycin D. BRS virus RNA, labeled as described above, was also subjected to oligo(dT) chromatography prior to electrophoresis to select for polyadenylated RNA (panel C). The BRS virus RNAs are labeled according to their putative identifications. Although not visible in panel C, repeated experiments showed that the BRS virus L RNA is also polyadenylated.

the anti-391-2 serum (Fig. 3A). The anti-127 serum (Fig. 3D) lacked specificity for either G protein. The Wellcome anti-RS virus serum (Fig. 3C) recognized only the HRS virus G protein.

In summary, the immunoprecipitation and Western blot experiments demonstrate that the G, F, N, M, and P proteins were RS virus-specific. Polyclonal antisera recognizing the L, 22K, 1A, 1B, and 1C proteins of HRS virus are not available, possibly due to the small quantities of these proteins in infected cells. However, the appearance of these proteins was specific to BRS virus-infected cells.

Analysis of BRS virus RNAs. The RNAs specific to BRS virus-infected cells were examined by metabolic labeling with [^{3}H]uridine in the presence of actinomycin D followed by electrophoretic separation in agarose-urea gels. BRS virus-specific RNAs were demonstrated by comparison with RNAs from uninfected cells labeled in the presence of actinomycin D (Fig. 4). Addition of actinomycin D to uninfected cells 1 h before addition of label effectively shut off the incorporation of [^{3}H]uridine into cellular RNAs (Fig. 4A). Eight BRS virus-specific bands were observed in BRS virus-infected cells (Fig. 4B, lane B). Comparison of these RNAs with RNAs from HRS virus-infected cells showed that the BRS virus-specific RNAs had mobilities in agarose-urea gels similar to those described previously for the HRS virus mRNAs (with the exception of a faster-migrating G and P mRNA band) and genomic RNA (11, 13, 14). Sequence analysis has shown that all 10 mRNAs of HRS virus differ in size, ranging from 400 to over 7,000 bases (14). However, in agarose-urea gels, the HRS virus G and P mRNAs comigrated, as did the 22K and M mRNAs and the 1A, 1B, and 1C mRNAs (Fig. 4B, lane H) (13). The similar patterns of RNA mobilities between HRS and BRS virus suggested that BRS virus RNA contained counterparts to all of the HRS virus mRNAs, including an L gene mRNA and some polycistronic mRNAs.

A similar BRS virus RNA pattern was observed following oligo(dT) selection, except that the largest RNA was not selected (Fig. 4C). This fact, along with the observation that synthesis of this RNA was sensitive to cycloheximide (data not shown), indicated that it was the BRS virus genomic RNA.

Analysis of BRS virus RNAs with HRS virus cDNA clones. The similarity of the HRS and BRS virus mRNA mobilities in agarose-urea gels led us to test the ability of HRS virus cDNA clones to hybridize to Northern blots of the BRS virus RNAs. HRS virus cDNA clones labeled with ^{32}P were used to probe duplicate Northern blots of RNA from BRS virus-, HRS virus-, and mock-infected cells (Fig. 5). The HRS virus cDNA probes hybridized to the appropriate HRS virus mRNAs and polycistronic RNAs as described previously (Fig. 5, panels 1 to 5, lanes H) (10, 13). The Northern blots, however, showed little cross hybridization between

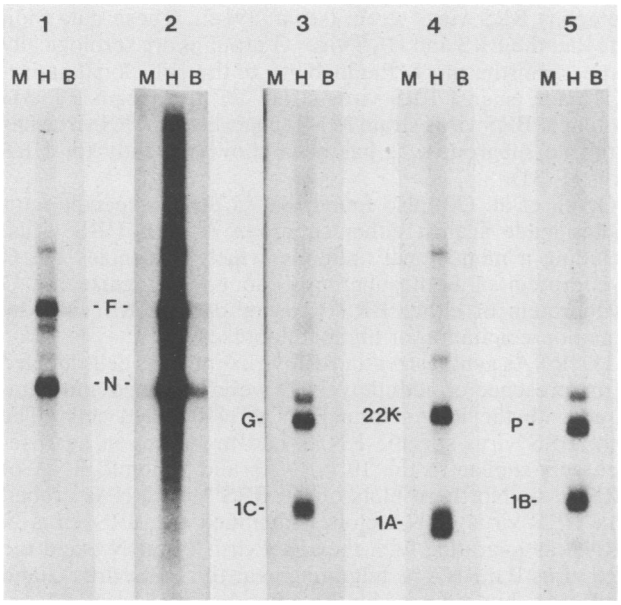

FIG. 5. Northern blot analysis of BRS and HRS virus RNAs with HRS virus cDNA clones as probes. RNA from BT cells infected with BRS virus (lanes B) or HRS virus (lanes H) or from mock-infected BT cells (lanes M) was harvested and separated by electrophoresis in 1.75% agarose–urea gels. The RNA was then transferred to diazobenzyloxymethyl paper by capillary action, and replicate sets of lanes were probed with nick-translated plasmid DNA from cDNA clones corresponding to the following HRS virus genes: panel 1, F and N; panel 3, G and 1C; panel 4, 22K and 1A; panel 5, P and 1B. Panel 2 is a longer exposure of panel 1. Bands corresponding to the HRS virus mRNAs are indicated.

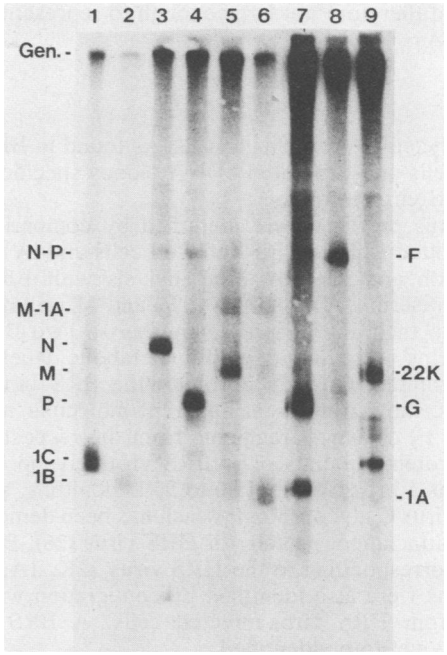

FIG. 6. Northern blot analysis of BRS virus RNAs with BRS virus cDNA clones as probes. RNA from BRS virus-infected BT cells was harvested and separated by electrophoresis in 1.75% agarose–urea gels. The RNA was then transferred to diazobenzyloxymethyl paper by capillary action, and replicate lanes were probed with nick-translated plasmid DNA from BRS virus cDNA clones of nine separate clone groups (lanes 1 to 9). The BRS virus mRNAs and polycistronic RNAs are named according to their corresponding HRS virus genes.

the HRS virus cDNA clones and BRS virus RNAs. There was slight hybridization detectable after long exposure of the HRS virus N cDNA probe to the BRS virus RNA that comigrated with the HRS virus N RNA (Fig. 5, panel 2, lane B). Northern blot analysis using different stringencies for hybridization and washes allowed hybridization of the HRS virus P cDNA probe to the BRS virus RNA that migrated slightly faster than the HRS virus G and P mRNAs (data not shown) but did not increase the specific hybridization to any other BRS virus RNAs. These data tentatively identified the BRS virus RNA which comigrated with the HRS virus N mRNA as the BRS virus N mRNA and the BRS virus RNA which migrated slightly faster than the HRS virus G and P mRNAs as containing the BRS virus P mRNA.

cDNA synthesis, cloning, and clone identification. The failure of HRS virus cDNA clones to hybridize to the BRS virus RNAs in Northern blots indicated significant differences in the sequences of HRS and BRS virus RNAs and prevented use of the HRS virus cDNA clones in further characterization of the BRS virus RNAs by Northern blot analysis.

In order to fully characterize the BRS virus RNAs and to obtain the nucleotide sequence of the RNAs, we prepared cDNA clones to the BRS virus mRNAs isolated by oligo(dT) chromatography from actinomycin D-treated infected cells. A total of 76 BRS virus-positive clones were separated into nine distinct groups which, with the exception of two clones, did not cross-hybridize by colony hybridizations. The RNA specificity of each group was determined from Northern blots of RNA from BRS virus-infected cells probed with DNA from a representative clone from each group (Fig. 6). By this method, the groups were tentatively identified as to their corresponding HRS virus gene. Groups 1 and 2 (Fig. 6, lanes 1 and 2) each recognized an RNA corresponding to the putative BRS virus 1C and 1B RNAs, respectively. Group 3 (Fig. 6, lane 3) hybridized to the BRS virus N RNA, and group 4 (Fig. 6, lane 4) hybridized to the BRS virus P RNA. Groups 5 and 6 (Fig. 6, lanes 5 and 6) annealed to the putative BRS virus M and 1A RNAs, respectively. Groups 7 and 8 (Fig. 6, lanes 7 and 8) hybridized to the putative BRS virus G and F RNAs, respectively, and group 9 (Fig. 6, lane 9) recognized the putative BRS virus 22K RNA. The two clones which hybridized to two groups by colony hybridizations recognized clones belonging to groups 5 and 6 (Fig. 6, M and 1A, respectively).

The cDNAs from all groups hybridized to the BRS virus genomic RNA, demonstrating their viral specificity. In addition, some groups hybridized to less-abundant RNA species. These probably represent polycistronic readthrough RNAs, which have been identified in HRS virus-infected cells and which can be used to map adjacent genes as described previously for HRS virus (11, 13, 14). For example, groups 3 and 4 (Fig. 6, lanes 3 and 4) both hybridized to a third RNA which migrated slightly slower than the putative BRS virus F RNA. This is where the N-P bicistronic RNA of HRS virus migrates (11, 13), and we conclude this is a BRS virus-specific N-P bicistronic readthrough RNA. Groups 5 and 6 (Fig. 6, lanes 5 and 6) recognized a third RNA which migrated between the BRS virus N and putative F RNAs and comigrated with the M-1A bicistronic RNA of HRS virus. Groups 7 and 9 (Fig. 6, lanes 7 and 9) also hybridized to additional RNAs which were the size of the 1A, 1B, and 1C

RNAs, and therefore not large enough to represent polycistronic RNAs.

DISCUSSION

We analyzed the proteins and RNAs found in BRS virus-infected cells and generated cDNA clones specific for 9 of the 10 BRS virus mRNAs.

BRS virus proteins were identified by comparison with proteins found in HRS virus-infected cells and by immunoprecipitation and Western blot analysis with BRS virus-specific antiserum. BRS virus N, P, and M proteins comigrated with their HRS virus counterparts. Two BRS virus glycoproteins were identified by metabolic labeling with [^{3}H]glucosamine. One glycoprotein, the BRS virus fusion protein, F, showed different apparent molecular masses in its F_1 and F_2 cleavage fragments from the respective HRS virus F protein subunits. The BRS virus glycoprotein, G, migrated as a broad band of 80 to 90 kilodaltons, similar to the HRS virus G glycoprotein, which has been demonstrated to be the attachment protein for HRS virus (26). BRS virus proteins corresponding to the HRS virus 22K, 1A, 1B, and 1C proteins were also identified by comigration with counterparts from HRS virus-infected cells. A BRS virus L protein has yet to be identified.

Previously, Cash et al. (6) examined the virus-specific proteins produced in BRS virus-infected cells. They reported that BRS virus had seven virus-specific proteins by comparing the proteins found in BRS virus-infected cells with the seven proteins found in HRS virus-infected cells. Further studies have shown that HRS virus has 10 virus-specific proteins (20). On the basis of apparent molecular weight, the BRS virus proteins identified by Cash et al. (6) correlate with the HRS virus proteins as follows: VP48 is F_1, VP41 is N, VP32 is P, VP27 is M, VP25 is 22K, and VP10 may represent 1A, 1B, or 1C. The seventh protein observed by Cash et al. (6), VP38, is the result of protease cleavage of VP41 (5).

The putative BRS virus N, P, and M proteins we identified were recognized in Western blot analysis by polyclonal antisera against either BRS virus or HRS virus, and the putative BRS virus F protein was serologically cross-reactive with the HRS virus F protein by both Western blot analysis and immunoprecipitation.

Another recent study tested the ability of monoclonal antibodies made against HRS virus proteins to recognize BRS virus proteins. Orvell et al. (32) included three BRS virus strains and a caprine respiratory syncytial virus in their characterization of monoclonal antibodies generated against a subgroup B HRS virus strain. Most of the monoclonal antibodies produced against the F, N, P, and M proteins of HRS virus recognized the respective proteins of BRS virus and caprine respiratory syncytial virus. In addition, Orvell et al. (32) showed that monoclonal antibodies previously generated against the F, N, and M proteins of a HRS virus subgroup A virus react with the F, N, and M proteins of both BRS virus and caprine RS virus.

We found the G glycoproteins of BRS virus and HRS virus were distinguished by both polyclonal and monoclonal antisera used in the Western blot analysis. A polyclonal antiserum made against both HRS virus (strain A2) and BRS virus (strain 127) and a monoclonal antibody which recognizes the G glycoprotein of both HRS virus subgroups (E. Walsh, personal communication) recognized the G glycoprotein of only HRS virus. The G glycoprotein of BRS virus was recognized only by a polyclonal antiserum against the homologous BRS virus strain (strain 391-2). These data indicate that the BRS and HRS virus G proteins are serologically distinct. Furthermore, the inability of the polyclonal antiserum made against BRS virus strain 127 to recognize the G protein of BRS virus strain 391-2 suggests that BRS virus has antigenic subgroups, as has been shown recently for HRS virus (1, 31).

Orvell et al. (32) also found that G protein-specific antibodies made against either subgroup A or B HRS virus, including a monoclonal antibody which recognizes the G glycoprotein of both subgroups, do not recognize the G glycoprotein of either BRS virus or caprine RS virus by immunoprecipitation or immunofluorescence.

The RNAs synthesized in BRS virus-infected cells labeled in the presence of actinomycin D were identified and compared with the RNAs from HRS virus-infected cells. The eight BRS virus-specific RNAs had mobilities in agarose-urea gels similar to the 10 mRNAs and genomic RNA of HRS virus. Northern blots of the BRS virus RNAs probed with HRS virus cDNA clones identified the BRS virus N mRNA as migrating with the HRS virus N mRNA and the BRS virus P mRNA as migrating near the HRS virus G and P mRNAs, but did not identify any other BRS virus RNAs.

BRS virus-specific cDNA clones were generated and separated into nine distinct groups on the basis of their cross hybridization patterns. Hybridization of the BRS virus cDNA clones to the BRS virus mRNAs and possible polycistronic RNAs was used to tentatively identify the groups of clones as corresponding to the following genes: group 1, 1C; group 2, 1B; group 3, N; group 4, P; group 5, M; group 6, 1A; group 7, G; group 8, F; and group 9, 22K. A clone hybridizing to the BRS virus L mRNA has yet to be identified. Identification of group 4 was confirmed by in vitro translation of hybrid-selected BRS virus RNA, which resulted in the synthesis of the BRS virus P protein (data not shown). Identification of groups 1 to 8 has been confirmed by sequence analysis (R. A. Lerch, K. Anderson, and G. W. Wertz, unpublished data).

Additional, less-abundant RNA species, with characteristics of polycistronic RNAs and to which groups 3 to 6 hybridized, were helpful in identifying groups 3 to 6 and in locating their corresponding genes on the genome relative to each other. The additional RNA species recognized by groups 7 and 9 did not help identify or locate these groups on the genome. Groups 3 and 4 hybridized to a putative N-P bicistronic RNA, and groups 5 and 6 hybridized to a putative M-1A bicistronic RNA. Although fewer polycistronic RNAs were evident for BRS virus than for HRS virus, the two that were identified suggested that the gene orders of the two viruses are similar (11, 13).

The additional RNAs of groups 7 and 9 migrated in the region of the putative BRS virus 1A, 1B, and 1C mRNAs, but were distinct from the RNA species in this region to which groups 1, 2, and 5 hybridized. The additional RNA species of group 7, although too small to be a polycistronic RNA, could have resulted from use of a polycistronic (6-7) clone as a probe. The clone used did not cross-hybridize to group 6 by colony hybridization, however, and other group 7 clones also hybridized to the additional RNA species. Two possible explanations for the smaller, third RNA species of groups 7 and 9 are as follows. (i) The BRS virus RNAs corresponding to groups 7 and 9 are unstable and are cleaved or break down to a specific size fragment(s). (ii) An internal gene start or stop signal may exist in the BRS virus RNAs corresponding to groups 7 and 9; this may be recognized by the BRS virus polymerase as observed previously for the

HRS virus 22K gene (10, 12). This would result in an RNA species which is smaller than the full gene but which is still specific for that gene. The smaller RNAs to which groups 7 and 9 hybridized will be studied to determine if the phenomenon has significance.

In summary, we have identified nine proteins specific to BRS virus-infected cells, corresponding to nine HRS virus proteins. Only a BRS virus polymerase protein remains to be identified. The BRS virus G glycoprotein showed major antigenic differences from the HRS virus G glycoprotein by immunoprecipitation and Western blot analysis, while the BRS virus F, N, M, and P proteins showed antigenic cross-reactivity with their HRS virus counterparts. BRS virus genomic RNA and 10 BRS virus-specific mRNAs were identified by analysis of RNA from BRS virus-infected cells in agarose-urea gels and Northern blots probed with BRS virus-specific cDNA clones. Further identification and characterization of the BRS virus RNAs by sequence analysis of the BRS virus cDNA clones is under way.

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