Antigenic Cross-reactivity between Caprine Arthritis–Encephalitis, Visna and Progressive Pneumonia Viruses Involves All Virion-associated Proteins and Glycoproteins

By RUBY P. GOGOLEWSKI, D. SCOTT ADAMS, TRAVIS C. MCGUIRE, KEITH L. BANKS AND WILLIAM P. CHEEVERS*

Department of Veterinary Microbiology and Pathology and 1 U.S. Department of Agriculture, Animal Disease Research Unit, Washington State University, Pullman, Washington 99164, U.S.A.

(Accepted 1 February 1985)

SUMMARY

Antigenic relatedness between the virion-associated proteins of caprine arthritis–encephalitis, visna and progressive pneumonia viruses was examined. Antigenic cross-reactivity was assessed by immunoprecipitation of disrupted, radiolabelled virus with goat, sheep and rabbit antisera, followed by resolution of the immunoprecipitation products by SDS-polyacrylamide gel electrophoresis. The results indicate that antigenic cross-reactivity between the caprine and ovine virus isolates involves all of the major virion-associated proteins and glycoproteins. The common antigenic determinants exhibited by virion structural proteins are immunogenic in goats, sheep and rabbits.

INTRODUCTION

The lentiviruses, caprine arthritis–encephalitis virus (CAEV), visna virus (VV) and progressive pneumonia virus (PPV), cause complex multi-systemic disease processes in sheep and goats. The CAE syndrome is primarily characterized by leukoencephalomyelitis in young goats, occasional interstitial pneumonia and, predominantly, progressive arthritis in older animals (Cork et al., 1974; Cork & Narayan, 1980; Crawford et al., 1980a, b). VV causes progressive demyelinating leukoencephalomyelitis in sheep, particularly the Icelandic breed; the same or a closely related virus causes maedi or Icelandic progressive pneumonia (Pålsson, 1976; Pétursson et al., 1976). PPV (North American maedi virus) is commonly isolated from cases of ovine progressive pneumonia (Cutlip & Laird, 1976). Lesions of meningoencephalitis, chronic arthritis and mammary lymphoid proliferation also occur in sheep seropositive for PPV (Cutlip et al., 1979; Oliver et al., 1981a), and multi-systemic inflammatory lesions are noted after experimental PPV infection (Oliver et al., 1981b).

Our understanding of the genetic and antigenic relationships among CAEV, VV and PPV isolates is incomplete. At least six strains of CAEV have been described (Crawford et al., 1980a; Narayan et al., 1980; Dahlberg et al., 1981; Sundquist, 1981; Oliver et al., 1982; Ellis et al., 1983), and various strains of all of these lentiviruses probably occur worldwide (Perk, 1982; Adams et al., 1985a). At the genetic level, CAEV, VV and PPV share only 15 to 30% genomic sequence homology under stringent hybridization conditions (Weiss et al., 1976; Roberson et al., 1982; Gazit et al., 1983). The genetic divergence indicated by these results is also reflected by the dissimilar restriction endonuclease cleavage maps of CAEV and VV provirus (Clements & Narayan, 1981; Harris et al., 1981; Roberson & Cheevers, 1984; Pyper et al., 1984; Querat et al., 1984). Under conditions of low stringency hybridization, however, CAEV and VV appear more closely related than previously indicated (Pyper et al., 1984; Cheevers et al., 1984). In this regard, group-specific epitopes associated with the major structural protein of the ovine viruses (Stowring et al., 1979) are exhibited by the p28 of all CAEV isolates so far examined. Antigenic
cross-reactivity between the 135K surface glycoproteins of caprine and ovine viruses has also
been observed (Dahlgren et al., 1981; Pyper et al., 1984; Adams et al., 1985) although
neutralizing antisera appear to be specific for individual virus isolates (Narayan et al., 1981,
1984). Dahlgren et al. (1981) reported that PPV core proteins other than p28 are not
immunoreactive with goat anti-CAEV serum.

In the present study, we have qualitatively analysed antigenic relatedness between all of the
virion structural proteins of CAEV, VV and PPV isolates. Purified radiolabelled virus was
disrupted and immunoprecipitated with antisera, and the immunoprecipitation products were
resolved by SDS–PAGE. The results show that the caprine and ovine lentiviruses exhibit shared
antigenic determinants on all major structural protein and glycoprotein components and that
these common determinants are immunogenic in goats, sheep and rabbits.

METHODS

Viruses. CAEV, strain 75-G63, was isolated by explantation of synovial membrane from an arthritic adult dairy
goat (Crawford et al., 1980a). CAEV, strain Co, was isolated from a thymus explant of a goat inoculated with a
brain suspension from a case of caprine leukoencephalomyelitis (Narayan et al., 1980). CAEV-G63 and CAEV-Co
were propagated in foetal caprine synovial membrane cultures (Klevjer-Anderson & Cheevers, 1981). VV, strain
1514, was isolated from a case of Icelandic visna (Pétursson et al., 1976), and the North American Cutlip strain of
PPV was isolated from a case of ovine progressive pneumonia (Cutlip & Laird, 1976). VV and PPV were
propagated in primary ovine choroid plexus cells (Roberson et al., 1982).

Radiolabelling of viruses. Viral proteins were labelled by exposure of infected cultures to [35S]methionine as
previously described (Johnson et al., 1983). [3H]Glucosamine (10 μCi/ml, sp. act. 60 Ci/mmol) was also added to
label the glycosyl residues of virion-associated glycoproteins. Labelled viruses were purified from culture media by
differential and isopycnic density gradient centrifugation (Cheevers et al., 1981).

Antiserum. The following antisera were used in this study: (i) G19, G20 and G40 from goats hyperimmunized with
CAEV-G63 (Klevjer-Anderson & McGuire, 1982); (ii) G40, G54, G57, G59, G60, G62 and G67 from goats orally
infected with milk containing CAEV-G63 (Adams et al., 1983); (iii) R692, R693, R694 and R695 from rabbits
hyperimmunized with CAEV-G63 (Klevjer-Anderson & McGuire, 1982); (iv) S126, S127 and S128 from sheep
experimentally infected with CAEV-G63 (Banks et al., 1983); (v) S1514 from a sheep immunized with VV-1514;
(vi) G56, G57, G58 and G59 from goats experimentally infected with PPV (Banks et al., 1983).

Immunoprecipitation and SDS–PAGE. The radiolabelled viral proteins recognized by the various antisera were
determined by SDS–PAGE evaluation of immunoprecipitates isolated with formalin-treated
Staphylococcus aureus bearing Protein A (Pansorbin; Calbiochem-Behring). Briefly, purified radiolabelled virus (600000 c.p.m.
per reaction, determined as 5% TCA-insoluble radioactivity) was disrupted and immunoprecipitated using the
methods of Shapiro & August (1976) as previously modified (Johnson et al., 1983), except that rabbit anti-goat
serum was not included. Non-specific precipitation was assessed in reactions containing (i) antigen, (ii) antigen
and Pansorbin or (iii) antigen, Pansorbin and normal goat or rabbit serum. Immunoprecipitates were washed and
processed as described (Johnson et al., 1983) and analysed by SDS–PAGE using a 7.5 to 17.5% polyacrylamide
gradient slab gel containing 0.1% SDS, employing a V16-2 vertical electrophoresis unit (Bethesda Research
Laboratories). Radioactive proteins were detected by fluorography (Johnson et al., 1983). Molecular weight
markers were 14C-methylated proteins consisting of myosin (200K), phosphorylase b (92.5K), bovine serum
albumin (69K), ovalbumin (46K), carbonic anhydrase (30K) and lysozyme (14.3K) (Amersham).

RESULTS AND DISCUSSION

Fig. 1 shows the distribution after SDS–PAGE of CAEV-G63 proteins (lane b) and CAEV-Co
proteins (lane d) immunoprecipitated by G19 goat anti-CAEV-G63 hyperimmune serum.
Eight major protein bands were evident with apparent molecular weights of 135K, 92K, 70K,
45K, 28K, 19K, 16K and 14K. Similar results were obtained using G20 and G40 antisera, as well
as antisera from goats orally infected with CAEV-G63-containing milk (data not shown).
Seronegative control serum (G11) did not specifically precipitate labelled CAEV proteins; the
faint p28 background in Fig. 1(a, c) was also evident in the absence of serum.

Results analogous to Fig. 1(b, d) were obtained for labelled CAEV in the absence of serum as
well as unlabelled virus visualized by staining with Coomassie Brilliant Blue or silver (data not
shown). With minor variations in molecular weight estimates, these results are comparable to
our previous data on the protein composition of CAEV (Cheevers et al., 1981; Johnson et al.,
1983) and those of others (Dahlgren et al., 1981; Pyper et al., 1984), except that the longer gels
Fig. 1. Immunoprecipitation of virion-associated proteins of CAEV, VV and PPV by caprine anti-CAEV-G63 serum (G19) and serum from an uninfected specific pathogen-free goat (G11). Electrophoretic mobility of marker proteins in this and subsequent figures is shown by arrows.

employed here permitted resolution of the lowest molecular weight 14K component. The 135K, 92K, 70K and 45K components are glycosylated, as measured by $[^3]H$glucosamine incorporation (data not shown) and are labelled by lactoperoxidase-catalysed surface iodination of intact purified virus (Johnson et al., 1983).

CAEV is therefore apparently composed of eight major protein components, including four surface glycoproteins and four core proteins. The gp135 of VV induces neutralizing antibody (Scott et al., 1979). The nature of the additional glycoproteins is unknown. It is unlikely that they are degradation products of gp135, since they are not immunoreactive with monospecific anti-gp135 serum (Adams et al., 1985b). They may be cellular components packaged into virions, as suggested for VV on the basis of tryptic peptide mapping (Vigne et al., 1982). In any case, antisera from outbred goats immunized or orally infected with CAEV recognize antigenic determinants exhibited by all of the virion-associated structural proteins. Two different isolates of CAEV, G63 and Co, share antigenic determinants of all structural proteins that are recognized by goat anti-G63 sera.

To approach the question of antigenic cross-reactivity between caprine and ovine lentivirus proteins, labelled VV and PPV were immunoprecipitated with caprine anti-CAEV-G63 sera. Fig. 1 shows representative gels of VV and PPV proteins reacted with antiserum G19 (lanes f and h) and with control serum (G11) (lanes e and g). G19 antiserum precipitated eight major VV-
associated proteins (lane f) which corresponded to labelled VV and PPV proteins in the absence of serum and unlabelled virus detected by staining (data not shown). This distribution of ovine lentivirus-associated proteins is similar to that described previously (Dahlberg et al., 1981; Vigne et al., 1982), except that four rather than three low molecular weight components were resolved. The extra 12K PPV protein is not evident in Fig. 1 (h) but was detected in other experiments. Thus, all of the major virion-associated proteins of both ovine viruses are immunoreactive with caprine anti-CAEV sera, although the reaction with ovine virus core proteins is weaker than in homologous reactions with CAEV.

The extent of antigenic cross-reactivity between labelled lentivirus proteins was also investigated by immunoprecipitation with rabbit anti-CAEV-G63 sera. Like caprine anti-CAEV sera (Fig. 1), R695 antiserum precipitated all the major virion-associated proteins of both the G63 and Co isolates of CAEV (Fig. 2a, c) as well as the ovine VV and PPV isolates (Fig. 2e, g). As was evident with caprine G19 antiserum (Fig. 1b, d), the fluorographic signal given by the reaction between R695 antiserum and labelled CAEV-Co glycoproteins was weaker than in the homologous reaction with CAEV-G63 (Fig. 2a, c). Unlike caprine G19 antiserum, however, this less intense reaction was also noted with VV and PPV glycoproteins using the rabbit anti-CAEV serum (Fig. 2e, g). Similar results were obtained with three other rabbit anti-CAEV-G63 sera. Reactions with normal rabbit serum (NRS) were limited to a low amount of non-specific precipitation (Fig. 2b, d, f, h).
Radiolabelled CAEV-G63, CAEV-Co, VV and PPV proteins were immunoprecipitated with ovine anti-VV-1514 serum (S1514). As expected, S1514 antiserum precipitated all major VV-1514 structural proteins (Fig. 3e). All the major structural proteins of CAEV-G63 and CAEV-Co were also precipitated by S1514 antiserum (Fig. 3a, b) as were the PPV proteins (Fig. 3h). The intensity of the glycoprotein bands with both CAEV isolates was much weaker with sheep anti-VV-1514 serum (Fig. 3a, b) than in the homologous reaction with VV (Fig. 3e) or with PPV (Fig. 3h). Precipitation of CAEV-G63 19K and 14K core proteins by S1514 antiserum was not apparent in this experiment (Fig. 3a); however, these bands were easily visualized after a longer exposure.

Ovine anti-CAEV-G63 serum (S128) recognized all the major structural proteins of CAEV (Fig. 3c), VV (Fig. 3f) and PPV (Fig. 3i). The reactivity of PPV core proteins with this antiserum was much less than that of CAEV or VV. Similar results were obtained with two other sheep anti-CAEV sera.

Labelled caprine and ovine viruses were immunoprecipitated with four caprine anti-PPV sera. Representative SDS–PAGE gels obtained with one of these antisera (G57) are shown in Fig. 3. These antisera were strongly reactive with virion-associated glycoproteins and weakly reactive with core proteins in homologous reactions with PPV (Fig. 3j). With VV, all virion-associated proteins were precipitated, and core protein reactivity was much stronger than with PPV (Fig. 3g). With CAEV, however, reactivity with glycoproteins was weaker than with either PPV or VV (Fig. 3d).

This study shows that the caprine retrovirus CAEV and the ovine retroviruses VV and PPV are principally composed of eight structural proteins and that antigenic cross-reactivity between
the virus isolates involves all of these structural components. The common antigenic
determinants are recognized in heterologous immunoprecipitation reactions with goat, rabbit
and sheep antisera. Although variations were noted in the apparent amount of immunoprecipi-
tate formed with individual labelled proteins and various sera, no distinct virus-specific antigens
could be detected with the polyclonal antisera employed.

The data confirm and extend the observations of Clements et al. (1980) and Dahlberg et al.
(1981) that the molecular weight distribution of the ovine virus core proteins is different from
that of CAEV. In the present work, four associated core proteins were resolved for both ovine
and caprine virus isolates. The apparent molecular weights of these proteins are 28K, 17K, 15K
and 12K for VV and PPV as compared to 28K, 19K, 16K and 14K for CAEV.

Querat et al. (1984) described a virus isolate from a field case of ovine progressive pneumonia
in France which has a protein composition similar to CAEV rather than the ovine prototype
viruses VV-1514 or North American PPV. These authors classified the ovine and caprine
lentiviruses on the basis of structural, genetic and replication properties into two types: (i)
viruses with protein compositions similar to VV which are lytic in cell culture and (ii) viruses
with protein compositions similar to CAEV which are persistent in cell culture. It was suggested
that these viruses may be differentiated by type-specific antigenic determinants associated with
one or more low molecular weight core proteins. Dahlberg et al. (1981) support this view in that
immunoreactivity of PPV proteins other than p28 was not detectable with goat anti-CAEV
serum.

The present data do not support the possibility of the presence of lentivirus type-specific
antigens detectable by polyclonal antisera. Although somewhat more weakly than the homo-
logous reaction with CAEV, anti-CAEV sera from goats, sheep and rabbits precipitated all of
the core proteins of both VV and PPV. Heterologous immunoprecipitations between CAEV
proteins and anti-VV or anti-PPV sera were also positive.

One explanation for these differing results may be that the apparent amount of labelled
immunoprecipitate is influenced by several factors other than antigenic cross-reactivity. The
most important of these are the specific activity of labelled antigens and the concentration of
specific antibodies, i.e. variability of the immune response in different animals. Regarding the
latter, the response of goats to immunization with CAEV and PPV is strongly biased towards
glycoprotein antigens whereas rabbits respond more uniformly to glycoproteins and core
proteins. This is apparent from the data presented here but was more obvious in
immunoprecipitations employing serial dilutions of antisera. In addition, we have observed
substantial variability in the concentration of anti-p28 and anti-gp135 antibodies among serum
samples from several hundred CAEV-positive goats (D. S. Adams et al., unpublished results).
Further studies on antigenic typing and characterization of surface glycoproteins of sheep and
goat lentiviruses using monoclonal antibodies are in progress.

We thank Opendra Narayan for CAEV, strain Co, and ovine anti-VV-1514 serum, A. T. Haase for VV, strain
1514, and R. C. Cutlip for PPV. The technical assistance of Alberta Brassfield, Louise Leendersten, Chewelah
Nett and Deta Stem is gratefully acknowledged. This work was supported by grant AM 27680 from the National
Institutes of Health and grants 84-CRSR-2-2442 and 58-9AHZ-2-679 from the U.S. Department of Agriculture.

REFERENCES


ADAMS, D. S., OLIVER, R. E., AMEGHINO, E., DEMARTINI, J. C., VERWOERD, D. W., HOUWERS, D. J., WEGHELA, S., GORHAM,
encephalitis virus infection in eleven of fourteen countries tested. Veterinary Record (in press).

1143.

arthritis–encephalitis virus and goats by progressive pneumonia virus. American Journal of Veterinary
Research 44, 2307–2311.

Retrovirus antigenic cross-reactivity


(Received 21 November 1984)