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

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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.*, 1980*a, 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 (Pálsson, 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.*, 1981*a*), and multi-systemic inflammatory lesions are noted after experimental PPV infection (Oliver *et al.*, 1981*b*).

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.*, 1980*a*; 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.*, 1985*a*). 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 (Dahlberg *et al.*, 1981; Pyper *et al.* 1984; Adams *et al.*, 1985*b*) although neutralizing antisera appear to be specific for individual virus isolates (Narayan *et al.*, 1981, 1984). Dahlberg *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.*, 1980*a*). 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).

Antisera. 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 (Dahlberg *et al.*, 1981; Pyper *et al.*, 1984), except that the longer gels

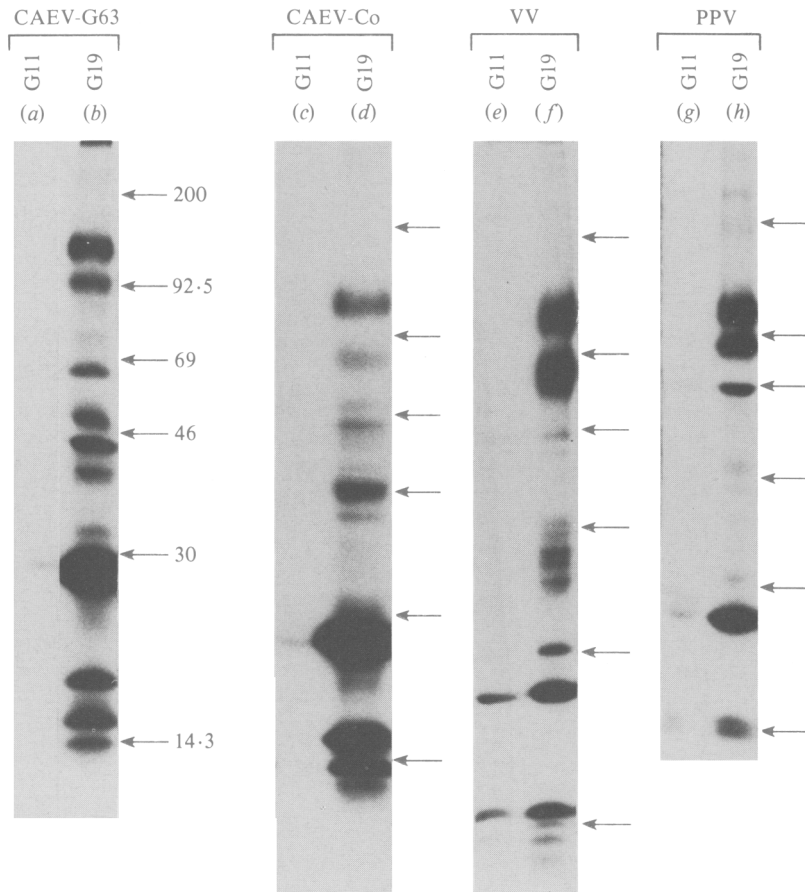


Fig. 1. Immunoprecipitation of virion-associated proteins of CAEV, VV and PPV by caprine anti-CAEV-G63 serum (G19) and serum from an uninfected specific pathogen-free goat (G11). Electrophoretic mobility of marker proteins in this and subsequent figures is shown by arrows.

employed here permitted resolution of the lowest molecular weight 14K component. The 135K, 92K, 70K and 45K components are glycosylated, as measured by [^3H]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.*, 1985*b*). 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-

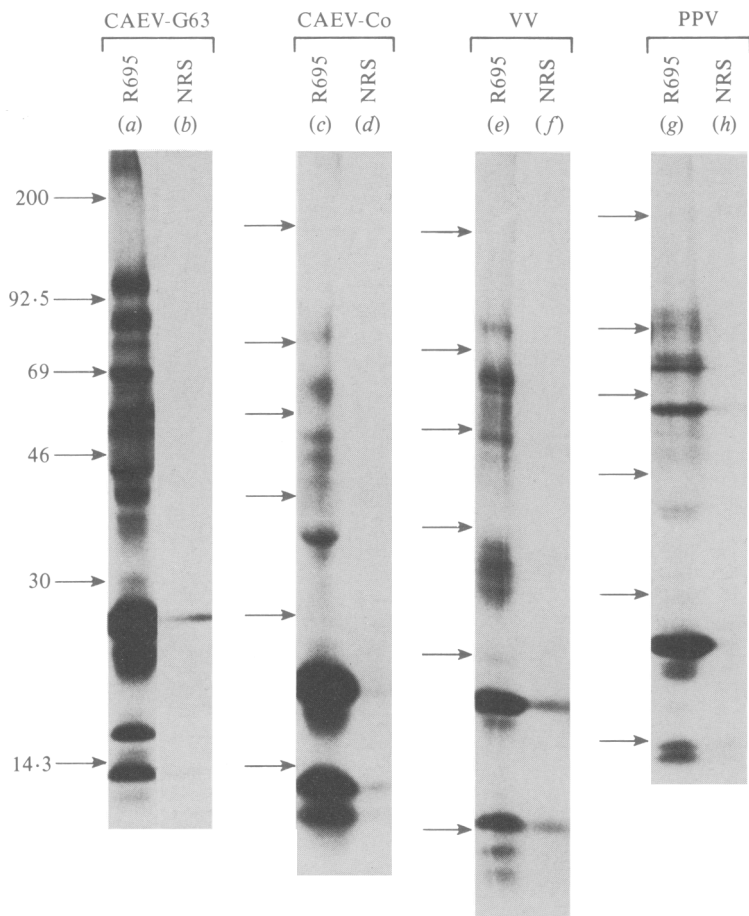


Fig. 2. Immunoprecipitation of virion-associated proteins of CAEV, VV and PPV by rabbit anti-CAEV-G63 serum (R695) and normal rabbit serum (NRS).

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. 2*a, c*) as well as the ovine VV and PPV isolates (Fig. 2*e, g*). As was evident with caprine G19 antiserum (Fig. 1*b, 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. 2*a, 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. 2*e, 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. 2*b, d, f, h*).

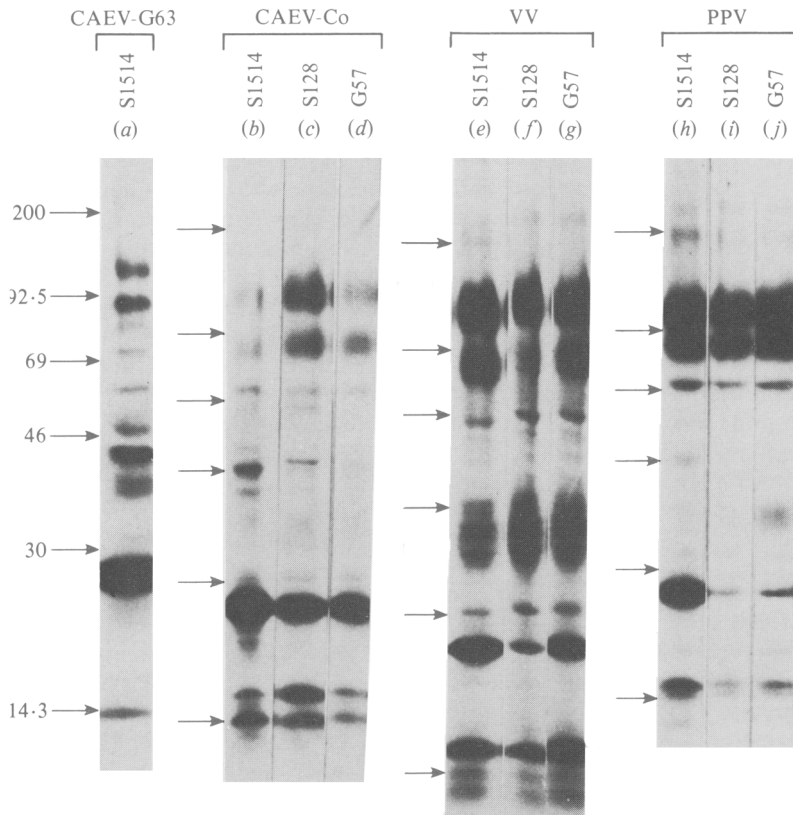


Fig. 3. Immunoprecipitation of virion-associated proteins of CAEV, VV and PPV by ovine anti-VV-1514 serum (S1514), ovine anti-CAEV-G63 serum (S128) and caprine anti-PPV serum (G57).

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 immunoprecipitate 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 homologous 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.

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