Antigenic and Structural Variation of the p28 Core Polypeptide of Goat and Sheep Retroviruses

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SUMMARY
The p28 core polypeptides of four isolates of caprine arthritis–encephalitis virus (CAEV) from goats was compared with those of visna virus (VV) and progressive pneumonia virus (PPV) from sheep. Monoclonal antibodies recognized p28 epitopes common to all six retrovirus isolates, a p28 epitope on four CAEV isolates, but not VV and PPV isolates, a p28 epitope on four CAEV isolates and VV, but not PPV and a p28 epitope unique to the CAEV isolate used for immunizing the mouse spleen donor. Comparison of two-dimensional maps of tyrosine containing tryptic peptides of p28 demonstrated that three CAEV isolates had similar maps while a fourth CAEV isolate, VV and PPV had several peptides different from the three closely related CAEV p28s and from each other.

Distinct, but related, retroviruses of the lentivirus subfamily occur in goats and sheep. In goats, caprine arthritis–encephalitis virus (CAEV) causes encephalitis in young kids (Cork et al., 1974), progressive arthritis in older goats (Crawford et al., 1980) and, in some instances, pneumonia and mastitis (Cork & Narayan, 1980; Crawford et al., 1980). Visna virus (VV) is the prototype of sheep lentiviruses and causes encephalitis (Gudnadottir, 1974). Retroviruses isolated from sheep with non-suppurative pneumonia are closely related to VV, but were designated maedi and progressive pneumonia virus (PPV) (Gudnadottir, 1974). In addition to pneumonia, PPV isolates cause lesions in the brain, mammary gland and joints (Oliver et al., 1981; Cutlip et al., 1985). These lentiviruses are not host-specific as PPV causes lesions in goats and CAEV causes lesions in sheep (Banks et al., 1983). Competitive genomic RNA/cDNA hybridizations show 15 to 30% genome sequence homology among VV, PPV and CAEV isolates (Roberson et al., 1982; Gazit et al., 1983). Heteroduplex and molecular hybridization analyses of cloned CAEV and VV demonstrate that the greatest conservation of nucleotide sequences is in the gag and pol gene regions and in two other smaller regions (Pyper et al., 1986). Antigenically, some common determinants are shared among all the proteins and glycoproteins of CAEV, VV and PPV (Gogolewski et al., 1985).

Most animals infected with CAEV make antibodies to the p28 major core polypeptide, and these antibodies are used to diagnose CAEV infection (Crawford et al., 1980; Roberson et al., 1982). Immunodiffusion and immunoprecipitation assays with sera from animals infected with CAEV, VV and PPV do not differentiate the p28s of these viruses (Weiss et al., 1977; Dahlberg et al., 1981; Roberson et al., 1982; Gogolewski et al., 1985). In this study, monoclonal antibodies (MAbs) and peptide maps were used to compare p28s from VV, PPV and four CAEV isolates.

The CAEV-63 and -89 isolates were obtained by explantation of synovial membranes from goats with arthritis (Crawford et al., 1980) and CAEV-52 was from a brain explant culture from a naturally infected goat. The CAEV-Co isolate was provided by Dr L. C. Cork, Johns Hopkins University, Baltimore, Md., VV was provided by Dr A. Haase, University of Minnesota,
Fig. 1. Immunoprecipitation of $^{35}$S-labelled goat and sheep lentiviruses with five MAbs made to CAEV-63. Lanes 1 to 6 of each panel represent MAbs 5A1, 10A1, 8B1, 13B1 and 12A1 and a control MAb respectively. Lane 7 of (b) is non-immunoprecipitated $^{35}$S-methionine-labelled CAEV-Co. (a to f) Immunoprecipitation results with $^{35}$S-methionine-labelled CAEV-63, CAEV-Co, CAEV-52, CAEV-89, VV and PPV, respectively. The lanes from each panel are cut from the same autoradiograph of the same gel. Mol. wt. values given are $\times 10^{-3}$.

Minneapolis, Mn., and PPV was provided by Dr R. C. Cutlip, U.S. Department of Agriculture, Ames, Ia., U.S.A. The CAEV isolates were grown in primary foetal goat synovial membrane cultures (Crawford et al., 1980) while VV and PPV were grown in sheep choroid plexus cells. Unlabelled and $^{35}$S-methionine-labelled viruses (Johnson et al., 1983) were purified by ultracentrifugation on 5 to 45% sucrose gradients (Cheevers et al., 1981).

BALB/c mice were immunized with p28 separated by gel filtration (P-100, Bio-Rad) from 10 mg of purified, NP40-disrupted CAEV-63. Spleen cells from these mice were fused with myeloma cells (Davis et al., 1983). Hybridoma supernatants were reacted with purified CAEV bound to flexible microtitre plates (Dynatech Laboratories, Arlington, Va., U.S.A.), washed three times with TEN buffer (20 mM-Tris–HCl, 5 mM-EDTA, 100 mM-NaCl, 5 mg/ml bovine serum albumin pH 7.4), incubated with rabbit antiserum to mouse immunoglobulin (Ig), washed three times, incubated with $^{125}$I-labelled protein A and washed three times. Individual wells were cut out and counted. Five hybridomas were cloned twice and the Ig isotype was determined to be IgG1 in double immunodiffusion with commercial antisera (Bionetics Laboratory Products Division, Charleston, S.C., U.S.A.); the Ig concentration was measured by single radial immunodiffusion (Johnson et al., 1983).
Table 1. Monoclonal antibody binding to virus-infected cells assayed by indirect immunofluorescence

<table>
<thead>
<tr>
<th>MAb, (0.1 mg/ml)</th>
<th>Virus used to infect cells</th>
<th>CAEV-63</th>
<th>CAEV-Co</th>
<th>VV</th>
<th>PPV</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>5A1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>10A1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8B1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13B1</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12A1</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

The panel of five MAbs was evaluated for binding to $[^{35}S]$methionine-labelled viral proteins in an immunoprecipitation assay (Johnson et al., 1983; Gogolewski et al., 1985). All five antibodies (5A1, 8B1, 10A1, 12A1 and 13B1) precipitated only p28 from $^{35}$S-labelled CAEV-63 proteins as determined by autoradiography of precipitates separated by SDS–PAGE (Fig. 1a). When $^{35}$S-labelled CAEV-Co was evaluated, antibody 12A1 failed to bind while the remaining four antibodies bound p28 (Fig. 1b). Results with two additional isolates of CAEV, 52 and 89, were similar to those with CAEV-Co (Fig. 1c, d). The p28 bands immunoprecipitated by antibody 13B1 in lanes 4 of Fig. 1(c) and (d) are faint, but were clearly visible on the autoradiographs. Antibodies 5A1, 8B1 and 10A1 bound $^{35}$S-labelled VV p28, while 12A1 and 13B1 did not (Fig. 1e). Antibodies 5A1 and 10A1 bound $^{35}$S-labelled PPV p28, while 12A1, 13B1 and 8B1 did not (Fig. 1f).

The reactivity of the five MAbs with virus-infected and uninfected cells was evaluated by indirect immunofluorescence (Cheevers et al., 1981) using fluorescein isothiocyanate-conjugated rabbit antibodies to mouse Ig. The reactivity of the antibody panel in immunofluorescence tests (Table 1) was the same as in immunoprecipitation tests (Fig. 1) except that antibody 10A1 failed to bind PPV in immunofluorescence tests.

The results from the MAb studies indicated that antibody 5A1 recognized a p28 epitope common to all six isolates in both immunoprecipitation and immunofluorescence assays. This antibody may be useful to detect sheep and goat retroviruses in culture and in infected tissues. Another antibody (12A1) recognized an epitope unique to CAEV-63 which was the virus isolate used for immunization of the spleen donor mice. Monoclonal antibody 13B1 bound to all CAEV isolates, but not to VV and PPV; this antibody may differentiate CAEVs. Monoclonal antibody 8B1 bound all CAEV isolates and VV, but not PPV. Demonstration of antigenic differences among the p28s from goat and sheep retrovirus isolates is similar to findings with murine retroviruses (Chuat et al., 1985).

For peptide mapping, the p28s were cut from Coomassie Brilliant Blue-stained 7.5 to 17.5% SDS–PAGE gels of purified viruses. The p28 was the predominantly stained band for each virus and was easily separated from the other proteins. Slices were washed with 25% isopropl alcohol and 10% methanol, dried and labelled with $^{125}$I (Elder et al., 1977). Labelled slices were washed, dried and digested with TPCK-treated trypsin (Elder et al., 1977). After digestion, the supernatant was removed from the gel slices, lyophilized, resuspended in electrophoresis buffer and the peptides were separated by thin-layer electrophoresis and chromatography (Whittaker & Moss, 1981) on 20 × 20 cm cellulose plates (EM Reagents, Darmstadt, F.R.G.). The plates were dried and exposed to X-Omat film.

Comparison of CAEV-63 (Fig. 2a), CAEV-Co (Fig. 2b) and CAEV-52 (Fig. 2c) revealed similar p28 peptide maps, while CAEV-89 p28 (Fig. 2d) had differences. Also, differences in p28 peptides occurred when the related p28s of CAEV (63, Co and 52; Fig. 2a, b, c) were compared with VV (Fig. 2e) or PPV (Fig. 2f), when CAEV-89 (Fig. 2d) was compared with VV (Fig. 2e) or PPV (Fig. 2f) and when VV (Fig. 2e) was compared with PPV (Fig. 2f). Structural differences provide a plausible explanation for the differential reactivity by some of the MAbs with p28s. Similar structural differences occur in the major core polypeptide of primate lymphotrophic retrovirus isolates (Jurkiewicz et al., 1986) and mouse retrovirus isolates (Gautsch et al., 1978). In contrast, equine infectious anaemia virus isolates have similar p26s (the major
core polypeptide) with the exception of a stable change in the p26 of one isolate after passage in horses (Montelaro et al., 1984).

In conclusion, the p28 peptide maps show divergence among CAEV isolates and between VV and PPV, preventing differentiation of goat from sheep lentiviruses. In contrast to the results with p28 peptide maps, one MAb differentiates goat from sheep lentiviruses.

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