Characterization of a New York ovine lentivirus isolate

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A lentivirus has been isolated from a Finnish ewe with ovine progressive pneumonia in a closed upstate New York flock. We demonstrated that the virus, designated ovine lentivirus strain CU1 (OLV-CU1), is biologically, biochemically and molecularly related to, but distinct from, previously described sheep and goat lentiviruses. Nine of 32 ewes (from the affected flock) with precipitating antibodies for ovine lentivirus also produced antibodies that were able to neutralize the infectivity of OLV-CU1. The virus replicated in cultured sheep fibroblasts and caused the formation of large multinucleated cells. OLV-CU1-specific RNA transcripts found in infected cells and virion antigenic proteins were similar to those of other small ruminant lentiviruses. However, the virus was distinguished from other isolates at the DNA level by nucleic acid hybridization, restriction endonuclease mapping and partial sequencing of the virus genome.

Introduction

Ovine progressive pneumonia (OPP) was first described during the 1920s in the U.S.A. (Marsh, 1923) as a specific disease syndrome affecting sheep. Infection of North American sheep is widespread (seroprevalence ranges from 0 to 98% in flocks) and is recognized as a serious problem in flocks throughout the U.S.A. (Smith, 1992). Classical OPP is characterized by wasting accompanied by progressive depletion of pulmonary function. Death usually occurs within 6 to 12 months following the onset of clinical signs. The loss of pulmonary function is the result of a slowly developing interstitial pneumonitis. The syndrome can also include encephalitis, mastitis and/or arthritis.

The virus responsible for ovine progressive pneumonia, OLV, is a member of the lentivirus subfamily of retroviruses. By most physical and biochemical criteria, OLV isolates from North America are indistinguishable from maedi–visna virus, which was first described as a ‘slow virus’ by Sigurdsson (1954), after an outbreak of disease in Iceland (Petursson & Hoff-Jørgensen, 1990). However, at least one North American strain of OLV differs from visna virus in nucleic acid sequence by at least 20% (Weiss et al., 1976). Most biochemical and molecular analyses of small ruminant lentiviruses have been performed with visna virus (Sonigo et al., 1985; Braun et al., 1987; Staskus et al., 1991) or the goat lentivirus, caprine arthritis–encephalitis virus (CAEV) (Jackson et al., 1991; Saltarelli et al., 1990). South African and British ovine lentivirus isolates have been described recently (SA-OMVV and EV-1, respectively) (Sargan et al., 1991; Querat et al., 1990). These isolates are genetically and biologically related to both maedi–visna virus and CAEV. The diseases caused by these recently described viruses are very similar to OPP. These four ovine/caprine lentivirus genomes have been cloned and sequenced (Braun et al., 1987; Staskus et al., 1991; Sargan et al., 1991; Saltarelli et al., 1990; Jackson et al., 1991). The nucleotide sequences of the three ovine lentiviruses share approximately 80% identity with each other, whereas CAEV has only about 60% identity overall with the ovine lentiviruses. The amino acid identities among the three ovine lentiviruses range from 65% in the rev open reading frame (ORF) product to 90% in the gag ORF product (Sargan et al., 1991). However, visna virus (as well as the other ovine lentiviruses) and the goat lentivirus, CAEV, are distinguished at the amino acid level with only approximately 60% amino acid identity in their Env proteins (Saltarelli et al., 1990).

The ovine/caprine lentiviruses productively infect cells of the monocyte/macrophage lineage in their respective hosts (Narayan, 1983; Narayan & Clements, 1989; Gendelman et al., 1986). The outcome of lentivirus infections in sheep and goats is similar: there is an inflammatory response in specific tissues (usually the...
brain, lung, joints or mammary gland) due to virus replication in macrophages in the affected organ (Lehmkuhl et al., 1985; Ellis, 1983). The pattern of disease production is slowly progressive, with a low level of virus replication followed by steadily increasing production of virus. This progression from low to high levels of lentivirus replication is probably due to a combination of virus-specific and cell-specific regulatory factors (Haase et al., 1982; Narayan et al., 1982, 1983).

One of the regulatory factors produced by lentiviruses is a positive transcriptional trans-activator, called Tat (Narayan & Clements, 1989; Cullen, 1991; Garcia et al., 1988). Human immunodeficiency virus (HIV) Tat interacts with cellular transcriptional machinery by binding to a stem-loop structure (TAR) in the leader region of the mRNA. TAR occurs immediately downstream of the HIV transcriptional start site (Colvin & Garcia-Blanco, 1992). Tat becomes anchored close to the virus transcriptional start site, and is thought to interact with proteins bound in the U3 region of the HIV LTR to increase the rates of transcriptional initiation and/or elongation (Berkhout et al., 1990; Sharp & Marciniak, 1989; Kato et al., 1992). The functional regions of HIV Tat include the activation domain (N-terminal, cysteine-rich and core regions) and the RNA-binding and nucleolar localization domains (basic region) (Cullen, 1991; Colvin & Garcia-Blanco, 1992).

Two regions are highly conserved between visna virus and CAEV Tat proteins: a hydrophobic domain and a cysteine-rich domain (Saltarelli et al., 1990). The functional significance of these domains has not been determined. The sheep and goat lentivirus Tat proteins do not have a core region similar to HIV Tat and a TAR stem–loop structure has not been identified in the visna virus or CAEV LTRs. Therefore, Tat may function differently in the transcriptional regulation of sheep and goat lentiviruses compared to HIV Tat.

Most ovine lentivirus research in the U.S.A. has been done with either the isolate obtained by Cutlip & Laird (1976), designated WLC-1, or the lytic (H/24 and 85/34) and non-lytic (84/28 and 85/14) strains described by Lairmore et al. (1987). These studies have demonstrated the multi-systemic nature of North American OLV infection, with lesions present in the lungs, brain, joints and mammary glands. The diversity of disease syndromes seen in ovine lentivirus-infected sheep is probably due, in part, to the genetic background of the host, but may also be related to genetic variations among strains of ovine lentiviruses (Cutlip et al., 1986; Narayan & Cork, 1985; Houwers, 1989). Lairmore et al. (1988) have demonstrated differences in the histological lesions following infection of sheep by different strains of ovine lentiviruses. However, no combined biochemical and molecular data have yet been published on a North American isolate. Such information would allow comparison with the Icelandic, South African and British ovine lentiviruses.

This paper describes the isolation and characterization of a virus from an infected sheep in an upstate New York flock. The isolate was compared, where appropriate, with other ovine lentivirus strains, including the WLC-1 isolate from Ames, Iowa, U.S.A., kindly provided by Dr R. C. Cutlip. Data are also presented on the initial molecular characterization of the New York OLV isolate (OLV-CU1). A portion of the OLV-CU1 genome was sequenced and found to correspond (by comparison with the sequenced ovine and caprine lentiviruses) to parts of the pol and env ORFs, and to the entire Q (vif) and S (tat) ORFs.

Methods

Animals. The sheep used in this work were part of a 500-ewe closed flock of Dorset and Finnish Landrace sheep and their crosses, located at the Cornell University Teaching and Research Center in Harford, N.Y., U.S.A.

Cocultivation of infected peripheral blood leukocytes with tissue culture cells. Ovine leukocytes from heparinized blood were collected either by isolation from buffy coat preparations or by Ficoll–Hypaque centrifugation (Thompson, 1989). The cell concentration was adjusted to 10⁶ cells/ml in Leibovitz L-15 medium containing 10% lamb serum and gentamicin (10 μg/ml). The cell suspension was then layered onto a subconfluent monolayer of primary foetal ovine choroid plexus (OCP), ovine tracheal (OT) or ovine foetal lung (OFL) cells, and incubated at 37 °C. Medium was replaced after 24 h and again at 4 to 5 day intervals.

Reverse transcriptase (RT) assay. Virus-specific RT activity was measured essentially as described by Heine et al. (1980) but with the following modification: the 50 μl reaction cocktail comprised 50 mM-Tris–HCl pH 7.8, 60 mM-KCl, 6 mM-MgCl₂ (or 0.6 mM-MnCl₂), 2 mM-dithiothreitol, 1 μg poly(rA) as template, 0.017 units oligo(dT) as primer and 5 μCi [3H]dCTP (83.2 Ci/mmol).

Virus growth and purification. OLV-CU1 was harvested from foetal ovine cells that were acutely [4 to 5 days post-infection (p.i.)] infected with the virus produced from the leukocyte–fibroblast cocultures. Harvested medium was pooled, infectious virus was titrated using an endpoint dilution assay and the medium was stored at –70 °C until required.

Virus was purified from infected cells by isopycnic centrifugation of virions (pelleted from cell culture medium) through a 30 to 60% sucrose gradient. Gradient fractions were collected and assayed for RT activity. Fractions containing RT activity were pooled and virus was pelleted by ultracentrifugation.

Serological assays. Virus neutralization titres were determined by incubating serial dilutions of heat-inactivated antisera with 100 TCID₅₀ of virus. Subconfluent OCP cells were then infected with the virus–antisemur mixture. The virus neutralization titre was calculated as the reciprocal of the highest dilution of antisemur that protected 50% of the monolayers from the typical cytopathic changes seen with this virus isolate ( multinucleated cells) after 2 weeks incubation.

Western immunoblot analysis. Pelleted, purified virus was disrupted and subjected to SDS-PAGE for Western blot analysis (Cutlip &
Laird, 1976; Towbin et al., 1979). Blots were developed using polyclonal sheep antiserum, rabbit anti-sheep IgG-peroxidase conjugate (Kirkegaard & Perry) and 4-chloro-1-naphthol as the substrate.

DNA and RNA isolation and restriction enzyme analysis. Cultured foetal ovine bone marrow (FOBM) fibroblasts were infected with plaque-purified OLV-CU1 at an m.o.i. of 1 to 5. DNA was isolated from either infected or mock-infected cells 4 days p.i. using a modified high salt procedure to enrich for unintegrated DNA (Chinsky & Soeiro, 1981), the prevalent form of viral DNA in lentivirus-infected cells (Narayan & Clements, 1989). The DNA was then digested with BamHI, HindIII, SacI or XbaI, singly or in combinations, and the resulting DNA fragments were electrophoresed through a 1% agarose gel (Sambrook et al., 1989). The DNA was transferred to a nylon membrane and hybridized at 42 °C in the presence of 100 µg/ml calf thymus RNA, using radioactively labelled cDNA probes derived from sucrose gradient-purified virion RNA. Briefly, medium was collected from OLV-CU1-infected foetal OCP (FOCP) cells every 4 h for 2 days. The virus was purified as described above by sucrose gradient centrifugation. RT peak fractions were pooled and dialysed against TNE overnight at 4 °C. The samples were then treated with proteinase K (Boehringer Mannheim) and SDS, followed by extraction with phenol–chloroform isomyl alcohol and precipitation of the RNA with ethanol. Radiolabelled cDNA was synthesized in a reaction mixture containing 1 µg of OLV-CU1 RNA, 18.5 µg random hexamers (Pharmacia), 0.6 mM each of dGTP, dATP and dTTP, 100 µCi dCTP (New England Nuclear; specific activity 3000 Ci/mmol), 10 mM-DTT, 1 x MuLv RT buffer (BRL) and 200 units MuLv RT (BRL). The mixture was incubated for 3 h at 37 °C and unincorporated nucleotides were removed using a Sephadex G-50 spin column (Boehringer Mannheim).

RNA was isolated from either uninfected FOCP cells or FOCP cells at 48 or 96 h p.i. using guanidine isothiocyanate followed by phenol–chloroform extraction and ethanol precipitation (Chomczynski & Sacchi, 1987). Poly(A)+RNA was selected by passage through an oligo(dT)-cellulose column.

Electrophoresis and Northern blotting were performed as described previously (Sambrook et al., 1989). The hybridization probe was generated from a visna virus long terminal repeat (LTR) M18039 fragment from the plasmid pVISLTR-CAT (Hess et al., 1989; kindly supplied by J. Clemets, Johns Hopkins University, Baltimore, Md., U.S.A.) by random-primed radioactive labelling (USB). This probe hybridizes to the 5' leader sequence in all OLV-CU1-specific transcripts. Hybridization and hybridization washes were carried out under moderately stringent conditions (hybridized at 42 °C with 50% formamide, washed twice at 42 °C with 1 x SCC, 0.1% SDS).

Molecular cloning. We initially wanted to obtain full-length clones of OLV-CU1, but were unsuccessful. An alternative approach was to generate an OLV-CU1 phage library from a ligation of SacI-digested unintegrated DNA (see above) and SacI-digested J2111. The library was screened by hybridization overnight at 42 °C with 5 x 10⁶ c.p.m./ml of a radiolabelled fragment of the visna virus LTR that was generated as described above. The filters were washed under moderately stringent conditions (see above).

1 DNA from positive plaques purified to homogeneity was isolated using a plate lysate protocol (Sambrook et al., 1989). The expected sizes of clones were 8-0 kb and a 0.5 kb doublet (Southern blot analysis); however, only two sizes of clones were obtained, 0.5 and 5.5 kb. These two J2111 clones were subcloned into a pUC19 vector and the termini were sequenced using a commercially available kit (Sequenase, USB). These sequences were aligned with visna virus sequences using the IBI Pustell sequence analysis program.

Virus neutralization. Homologous serum from the ewe from which the virus isolate was obtained neutralized OLV-CU1 infectivity (Table 1). Sera from nine of 32 additional seropositive (by AGID assay) animals from the same flock also neutralized the CU1 isolate.

Antigenicity of virion proteins. OLV-CU1 shared antigenic determinants with the WLC-1 isolate as determined by AGID assay. Also, sera from sheep infected with the WLC virus reacted positively.
Fig. 1. (a) RT activity of fractions (O) obtained after sucrose gradient centrifugation of OLV purified from infected cells. Density of the fractions (●) is shown on the right-hand axis. (b) RT activity of feline leukaemia virus (FELV), OLV, WLC-1 and medium from uninfected ovine turbinate (OTr) cells in the presence of either 6 mM-magnesium (shaded bars) or 0.6 mM-manganese (solid bars).

Table 1. Neutralizing antibodies to OLV-CU1

<table>
<thead>
<tr>
<th>Number tested</th>
<th>Number positive</th>
<th>Number negative</th>
<th>AGID positive</th>
</tr>
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<tr>
<td>32*</td>
<td>9</td>
<td>23</td>
<td>32</td>
</tr>
<tr>
<td>10†</td>
<td>-</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>1‡</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>4§</td>
<td>-</td>
<td>4</td>
<td>-</td>
</tr>
</tbody>
</table>

* Serum from 32 seropositive adults.
† Serum from 10 seronegative adults.
‡ Control serum specific for the WLC isolate.
§ Serum from colostrum-deprived newborn lambs.

when OLV-CU1 was used as antigen in an ELISA (data not shown).

A Western blot was performed using OLV-CU1 purified from sucrose gradients (Fig. 2). The antiserum used was taken at different times p.i. from a lamb experimentally infected with OLV-CU1. Prominent among the proteins detected using this method were proteins with apparent M, of 130K, 90K, 72K, 42K and 27K. Identical bands were detected by Western blotting using the WLC-1 isolate as antigen (data not shown).

Northern blot analysis

The sizes of transcripts produced (or predicted, based on sequence information) from visna virus and CAEV are very similar (Davis et al., 1987; Sargan & Bennett, 1989; Hess et al., 1986). Northern blotting was used to characterize the intracellular RNA species produced in cells infected by the Cornell isolate. The hybridization probe used corresponded to the visna virus LTR. As shown in Fig. 3(a), OLV-CU1 RNAs were similar in size to those of visna virus (Fig. 3b). The OLV-CU1 transcripts consisted of a full-length RNA of approximately 9.4 kb, and subgenomic RNAs of 5.0 kb and 1.5 to 1.8 kb. The visna virus-specific RNAs were identical in size to those previously reported, of 9.4, 5.0, 4.3, 4.0, 1.8 and 1.5 kb (Davis et al., 1987). Small RNAs of 1.8 and 1.5 kb appeared first in both OLV-CU1 and visna virus-infected cells (Fig. 3, lanes 2 and 5). Background bands representing the 18S and 28S rRNAs were detected in the uninfected total cellular RNA (Fig. 3a, lane 1). However, these bands were not seen in any of the other lanes.

Restriction enzyme mapping

Restriction analysis of OLV-CU1 DNA was performed to compare the OLV-CU1 genome to the visna virus, CAEV and SA-OMVV genomes (Sonigo et al., 1985; Braun et al., 1987; Staskus et al., 1991; Querat et al., 1987) (Fig. 4). The Southern blot was probed with
New York ovine lentivirus isolate

Fig. 3. Northern blots of uninfected total RNA (a, lane 1) and poly(A)+ RNA (b, lane 4), OLV-CU1 total RNA at 48 or 96 h p.i. (a, lanes 2 and 3), and visna virus poly(A)+ RNA at 48 or 96 h p.i. (b, lanes 5 and 6).

Fig. 4. Southern blot of uninfected extrachromosomal FOBM DNA (lane 1) or OLV-CU1-infected extrachromosomal DNA (lanes 2 to 9). OLV-CU1 DNA (5 µg) was either undigested (lane 2) or digested with HindIII (lane 3), SacI (lane 4), BamHI (lane 5), XbaI (lane 6), SacI and BamHI (lane 7), SacI and XbaI (lane 8), or BamHI and XbaI (lane 9).

radiolabelled cDNA generated from virion RNA. All of the enzymes tested except HindIII cut the OLV-CU1 DNA at least once. Non-specific bands appeared in lanes 4, 5, 7, 8 and 9 (restriction digests with either BamHI or SacI) at approximately 0.8 kb. These bands probably represented abundant DNA because they were detected by ethidium bromide staining (data not shown) and were probably derived from repetitive host DNA sequences that code for rRNA. The cDNA hybridization probe used had the potential to hybridize to repetitive sequences because it had been generated from total virion RNA, which may contain up to 20% cellular RNA (Brahic & Haase, 1978).

The deduced restriction map of OLV-CU1 is shown in Fig. 5 and is compared to the restriction maps of the other small ruminant lentiviruses listed above. The most striking difference observed in the maps was the lack of HindIII sites in OLV-CU1 DNA, whereas the other viruses contained at least two sites. OLV-CU1 DNA had one SacI site in the LTR in the same location as the visna virus genome. The XbaI and SacI sites of OLV-CU1, CAEV and SA-OMVV were located in similar positions in the gag region of all three viruses. All the other restriction sites of OLV-CU1 and the other sheep and goat lentiviruses were different. The orientation of the OLV-CU1 map was confirmed by sequence analysis of several regions of the genome (see next section).

**Molecular cloning and sequence analysis**

PCR was used to amplify the region from the middle of the pol ORF to the end of the env ORF. The sequence of OLV-CU1 DNA corresponding to the first 2000 bases of the 4.2 kb PCR fragment was determined (Fig. 6). The positions of the ORFs (by comparison with the visna virus genome) are indicated. The OLV-CU1 sequence was aligned individually with the corresponding genomic regions of either visna virus, SA-OMVV or CAEV to determine the percentage identity between OLV-CU1 and the small ruminant group (Table 2). The pol regions of visna virus, SA-OMVV and CAEV were the most similar to OLV-CU1, whereas the 5' portion of the env ORF was the most heterogeneous. The ovine lentiviruses, visna and SA-OMVV, were more similar to OLV-CU1 than was CAEV (74 to 81% for the ovine lentiviruses compared to 54 to 72% for CAEV).

The deduced amino acid sequence of the ORFs from the four viruses were compared (Table 2). In general, the
Fig. 6. DNA sequence of 2000 bp of the ORF products. Perfect matches are indicated by an asterisk, conserved amino acids by a point. Sequences were aligned using the CLUSTAL V software program (see Methods).

Fig. 7. Amino acid alignments of ovine and caprine lentivirus 3' ORFs are presented in Table 1. The OLV-CU1 sequence is presented in Fig. 3. Other lentivirus ORFs and their nucleic acid and amino acid similarities between OLV-CU1 and visna virus were consistent. In contrast, the percentage identity decreased when the amino acid sequences of OLV-CU1 were compared with SA-OMVV or CAEV. However, this decrease was much smaller with SA-OMVV than with CAEV. The amino acid similarities indicated that OLV-CU1 was more related to the ovine lentiviruses than to CAEV.

Table 2. Nucleic acid/amino acid sequence comparisons between OLV-CU1 and other lentivirus ORFs and their products

<table>
<thead>
<tr>
<th>ORF</th>
<th>pol/Pol</th>
<th>vd/Vd</th>
<th>tat/Tat</th>
<th>env/Env</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAEV</td>
<td>72/59</td>
<td>59/48</td>
<td>58/48</td>
<td>54/38</td>
</tr>
<tr>
<td>SA-OMVV</td>
<td>81/79</td>
<td>77/67</td>
<td>75/75</td>
<td>74/69</td>
</tr>
<tr>
<td>Visna virus</td>
<td>81/84</td>
<td>77/76</td>
<td>78/74</td>
<td>74/78</td>
</tr>
</tbody>
</table>

* The OLV-CU1 sequence is presented in Fig. 3. Other lentivirus sequences were obtained from GenBank (see Methods).
The pol region of OLV-CU1 was aligned with the visna virus, SA-OMVV and CAEV pol ORFs (Fig. 7a). There were no significant regions of heterogeneity. In contrast to the pol identities, the 5' portion of the env gene had a large region of dissimilarity (Fig. 7b). The dissimilar region corresponded to the beginning of env, where the first rev exon is located. The full Rev message has been shown to vary as much as 35% even within the ovine lentivirus subgroup (Sargan et al., 1991). There is, however, a conserved stretch of amino acids in Env from residues 109 to 217 (the end of the OLV-CU1 sequence). Highly variable regions were identified immediately following this conserved region in the visna virus, SA-OMVV and CAEV env ORFs (Saltarelli et al., 1990).

OLV-CU1 ORF Q (vif) potentially encodes a 230 amino acid protein with a calculated Mr of 28.3K. The putative Vif protein is approximately 80% hydrophilic and positively charged throughout (data not shown). OLV-CU1 vif is approximately 70% identical to both visna virus and SA-OMVV at the amino acid level, whereas it is only 48% identical to the CAEV vif ORF.

The OLV-CU1 S (tat) ORF codes for a potential Tat protein, analogous to the visna virus Tat protein. The protein has a calculated Mr of 11.4K and more than 12% of the residues are arginine. The putative Tat protein also shares limited stretches of amino acid homology with the other ovine and caprine lentivirus Tat proteins (Fig. 7c). There are two regions of identity among the members of the subgroup, as previously characterized (Saltarelli et al., 1990). The first, LQRWLAML, is very hydrophobic and the second, located near the carboxyl termini (CGCRLCNPGWG), is very cysteine-rich. A similar cysteine-rich domain in HIV Tat is essential for trans-activation (Ruben et al., 1989). There is one other region in OLV-CU1 that is similar in amino acid composition to HIV: a highly acidic region, located from amino acids 15 to 30 (data not shown). The acidic region near the amino-terminal portion of HIV Tat is essential for trans-activation (Ruben et al., 1989).

**Discussion**

Sheep and goat lentiviruses are a range of viruses that are biologically, antigenically and genetically related, but these viruses have significant differences at all three levels. At the biological and serological levels, differences have been observed in organ tropisms and virus neutralization patterns. The isolates can be distinguished by their effects on cultured cells since some are highly cytopathic and others are not. These isolates are also different when proviral DNAs are compared by sequence analysis and by restriction mapping.

We have isolated a virus from peripheral blood leukocytes obtained from an OLV seropositive ewe with clinical signs and histopathological lesions diagnostic for OPP. In culture, this virus replicated only in sheep cell lines, where it induced syncytium formation, with cells often containing 15 to 20 nuclei. Similar target cell restriction and cytopathicity have been described for visna virus (Harter & Choppin, 1967). Syncytium formation is also induced by other North American OLV isolates (Kennedy et al., 1968; Cutlip & Laird, 1976) by Zwoegerziekte isolates (de Boer, 1975) and to a lesser extent by CAEV isolates (Clements et al., 1980).

The failure of many infected animals to produce any neutralizing antibodies is in accord with the findings of Klein et al. (1985) who detected neutralizing antibodies in only 28% of animals naturally infected with OLV isolates. Similarly, Sheffield et al. (1980) found that when animals were experimentally infected with OLV they produced little or no neutralizing antibody. These results are similar to those found with CAEV, but contrary to observations with visna virus where most infected sheep produce neutralizing antibodies (Narayan et al., 1987).

The antigenic proteins of OLV-CU1 defined by Western blotting are of apparent Mr's consistent with those reported for other small ruminant lentiviruses (McGuire et al., 1987; Gogolewski et al., 1985; Lehmkuhl et al., 1985; Cutlip et al., 1978). It was determined by AGID and ELISA analyses that this isolate shares antigenic determinants with the prototypic North American OLV isolate, WLC-1.

The results of the restriction enzyme analysis and partial sequence analysis suggest that OLV-CU1 is genetically a separate and distinct member of the sheep lentivirus family. The isolate has some restriction sites in common with visna virus, CAEV and SA-OMVV, but other sites are clearly different (Sonigo et al., 1985; Braun et al., 1987; Staskus et al., 1991; Querat et al., 1987). One interesting finding is the similarity between the restriction sites of the visna virus and OLV-CU1 LTRs, and the OLV-CU1 and CAEV gag regions.

We initially chose visna virus or CAEV DNA representing most of the virus genome for the production of hybridization probes, but were unsuccessful in detecting OLV-CU1-specific DNA using these probes, even under moderately stringent conditions. Only the visna virus LTR (pVISLTR-CAT) was similar enough to use under the conditions necessary to avoid background signals. However, the LTR would be a poor choice to use for restriction map analysis because it represents only a fraction of the genome. We found that cDNA derived from purified virions could be successfully used as a probe to detect internal fragments with only a minimal amount of background.

The OLV-CU1 sequence presented here was derived from clones generated from PCR products. The error rate of Taq polymerase is approximately 1 in 1500 to 1 in
4000 bases (Keohavong & Thilly, 1989). However, retroviruses are replicated by an error-prone RT, and exist inside cells as a cohort of quasi-species (Narayan & Clements, 1989; Balfe et al., 1990). It has been suggested that the sequence changes introduced by PCR are fewer than those which occur in a natural population (Sargent et al., 1991; Weber & Weiss, 1988). Indeed, whole sections of other lentiviruses have been cloned using PCR, and a PCR-λ chimeric clone of simian immunodeficiency virus is infectious (Hirsch et al., 1989). Thus, the sequence of PCR-derived clones of OLV-CU1 is a valid indication of the actual population existing within OLV-CU1-infected cells.

The sequence of a subgenomic portion of OLV-CU1 was compared to the well characterized visna virus, SA-OMVV and CAEV genomes. The OLV-CU1 sequence is approximately 78% similar overall to other ovine lentiviruses, visna virus and SA-OMVV, whereas the similarity drops to approximately 60% with the same regions of the goat lentivirus, CAEV.

The vif and env ORFs are the most dissimilar within the small ruminant lentiviruses. The function of the putative Vif protein is unknown, although it is thought to be analogous to the HIV Vif protein. Vif is necessary for HIV infectivity, and functions in budding and release of infectious HIV virions (Cullen, 1991). The envelope glycoprotein is the most heterogeneous among the ovine and caprine lentiviruses. This protein plays a direct role in cell tropism and variants are selected because of their ability to escape neutralization by the host immune system. However, the 5′ region of OLV-CU1 env contains two subregions, an amino-terminal region that is highly variable, and a downstream region that is well conserved among the ovine and caprine lentiviruses. The variable region of the 5′ portion of env also contains the first exon of rev, which varies more than any other ovine lentivirus ORF (Sargent et al., 1991).

The role of the trans-activator, Tat, from sheep and goat lentiviruses has not been clearly defined (Jackson et al., 1991; Davis & Clements, 1989; Hess et al., 1986). Visna virus and CAEV Tat proteins are marginally trans-activating (up to fivefold) in transient transfections of sheep and goat fibroblasts, and, more recently, an even greater trans-activational effect of Tat on the visna virus LTR has been demonstrated in Vero cells (approximately 50-fold induction) (Gdovin & Clements, 1992). However, experiments to determine the regions of visna virus Tat that are required for trans-activation have not been performed.

By comparison with HIV Tat, the amino-terminal acidic regions of visna virus and OLV-CU1 Tat may be responsible for the trans-activating effect of the protein. However, a stem–loop structure similar to that found in the HIV genome (TAR) has not been identified in the visna virus LTR. Therefore, visna virus (and OLV-CU1) Tat may function differently from HIV Tat. Further studies of the ovine lentivirus Tat protein, including mutational analysis, are necessary to identify the regions responsible for trans-activation.

The results from the restriction mapping of OLV-CU1, along with our preliminary hybridization and sequence data comparing visna virus, CAEV and OLV-CU1, support our conclusion that the New York isolate is a separate ovine lentivirus. In addition, the transcripts produced by this virus are identical in size to those of visna virus as well as CAEV (Davis et al., 1987; Sargent & Bennet, 1989; Hess et al., 1986).

We have described the isolation and characterization of a North American ovine lentivirus. This virus can be grouped biologically and molecularly with CAEV, visna virus and SA-OMVV. We have found it to be more closely related in some respects to visna virus (disease produced, replication in cell culture, nucleic acid hybridization and sequence analysis), whereas it shares with CAEV an apparent inability to be neutralized by antiserum from most infected animals. Further molecular characterization of OLV-CU1 will allow a more detailed analysis of its relationship to the other small ruminant lentiviruses. In addition, the fact that the ovine lentiviruses are at least 20% dissimilar in most regions of the genome but cause very similar diseases indicates the need to sequence other ovine lentivirus isolates (American and others) at least partially. The additional sequence information would also allow a more detailed study of the evolution of ovine lentiviruses.

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References


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