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# ANALYSIS OF ANTIBODY RESPONSE TO OVINE LENTIVIRUS BY USING VIRAL GENE PRODUCTS EXPRESSED IN A PROKARYOTIC SYSTEM+

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SUMMARY: The polymerase chain reaction (PCR) was used to amplify, clone, and express eight DNA fragments encoding p25, p16, reverse transcriptase (RT) core, C'-terminal RT, N'- and C'-terminals of external (gp70), and transmembrane (gp40) envelope proteins from visna virus infectious recombinant DNA. Efforts were focused on characterizing the nature of the humoral immune response of ovine progressive pneumonia (OPP) virus infected animals and identifying the conserved and prime-reactive antigenic determinants that have potential diagnostic value. This communication reports that the N'-terminal region of gp40 appeared to be the most immunoreactive of the bacterially expressed proteins and could serve as a sensitive immunodiagnostic antigen for the detection of OPP infection.

Ovine progressive pneumonia (OPP), an ovine lentivirus (OLV), is caused by a nononcogenic, exogenous retrovirus of the lentiviridae subfamily (1,2,3). Infection with OLV occurs throughout many of the major sheep producing areas of the United States and other countries. The virus may be transmitted via colostrum, fecal-contaminated water, and inhalation (4,5,6).

Four ovine lentivirus genomes, derived from the Icelandic visna virus strain 1514, have been cloned and sequenced (7,8,9). Two of them, pLV1-1KS1 and pLV1-1KS2, were replication-competent (9). The pLV1-1KS1 infectious clones encoded a total of 10 viral structured proteins. These proteins were: p16 (matrix protein), p25 (core protein), p14, protease, reverse transcriptase, endonuclease, Q, S, gp70 (external envelope glycoprotein), and

+Proprietary or brand names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may be suitable.

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Abbreviations: PCR = polymerase chain reaction, RT = reverse transcriptase, OPP = ovine progressive pneumonia, OLV = ovine lentivirus, OPPV = ovine progressive pneumonia virus, AGID = agarose gel immunodiffusion, GST = glutathione-S-transferase.

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gp40 (transmembrane envelope glycoprotein) (9). The demonstration of specific antibodies to OPP virus (OPPV) encoded proteins is accepted as evidence of infection, but the antibodies provide no immunity against infection (10,11). Agar gel immunodiffusion (AGID) test is the most widely used method to detect antibodies in the serum of sheep infected with OPPV (12). However, the AGID test primarily detects antibodies to the env gene-encoded precursor glycoprotein-gp135. The other antigenic structures and determinants residing along the OPPV genome are yet to be determined (12,13). By using polymerase chain reaction (PCR), we amplified, cloned, and expressed eight DNA fragments encoding p25, p16, reverse transcriptase (RT) core, C'-terminal of RT, N'- and C'-terminal of both, external (gp70) and transmembrane (gp40) envelope proteins from visna virus infectious recombinant pLV1-1KS1. Efforts were focused on characterizing the nature of the humoral immune response of OPPV infected animals and identifying the conserved and prime-reactive antigenic determinants that might have potential diagnostic value. This communication describes the bacterial expression, partial purification, and immunological characterization of eight recombinant fusion proteins and the identification of a major serological marker for OPP serology among infected sheep.

## MATERIALS AND METHODS

Sheep serum samples. A panel of 12 OPP-positive sera and two OPP-negative sera, previously confirmed by AGID test, were included in this study. The 12 OPP-positive sera were from three experimentally-infected sheep (coded #1-3), six naturally-infected sheep with no OPP symptoms (coded #4-9), and three naturally-infected sheep with OPP symptoms and gross lesions (coded #10-12). The two OPP-negative sera were coded #13 and 14.

Bacterial strains, plasmids, and expression vector. Escherichia coli JM105 was used as a host strain in this study. The pLV1-1KS1 plasmid containing a complete infectious visna proviral DNA was kindly provided by Dr. Katherine Staskus (9). Plasmid pGex2T was used for the expression vector (Pharmacia, Piscataway, NJ). Procedures for restriction endonuclease digestions, purifying DNA, DNA ligations, and bacterial transformations were carried out as described (14).

Oligonucleotides. Eight sets of oligonucleotide primer pairs were synthesized on an Applied Biosystems' automated DNA synthesizer, Model 391, (Foster City, CA). The BamH I or EcoR I restriction sites were incorporated at the 5' ends of each primer to facilitate cloning. The sequence of each oligonucleotide, the region of the OLV genome (pLV1-1KS1) to which it was homologous, and the PCR amplified product are given in Table 1.

SDS-polyacrylamide gel electrophoresis and Western blotting. These procedures were performed by standard protocols (15,16).

## RESULTS AND DISCUSSION

Construction of recombinant plasmids. Eight pairs of oligonucleotide primers were designed to frame eight different regions of the pLV1-1KS1 plasmid. Through PCR amplification techniques, eight DNA fragments were amplified and each had the expected length. The PCR products were proteinase

Table 1. Summary of oligolnucleotide sequence, location at gene amplified, and length of the PCR product

| Primer designation (orientation) | Sequences(5'-3')* | Location | Size (bp) |
|---|---|---|---|
| | 5' AA GGA TCC ATG GCG AAG CAA GGC | | |
| p16 | 5' GA GGC GAA TTC TCT TTT GCT TGT | 490-738 | 249 |
| 1 (+) | | | |
| 2 (-) | | | |
| | 5' AA GGA TCC CCT ATT GTG AAT TTG | | |
| p25 | 5' CC TTC GGA TCC CAC ATC TCG ACA | 919-1569 | 651 |
| 3 (+) | | | |
| 4 (-) | | | |
| Reverse transcriptase | 5' AA GGA TCC ATA GCG CAA TGG CCT | | |
| (RT) | 5' TG GAA TTC TTT CTC CGG ATG CAA | 2178-2828 | 651 |
| 5 (+) | | | |
| 6 (-) | | | |
| | 5' AA GGA TCC GCC ATA GAA GAG GCA | | |
| C'-terminal of RT | 5' TG GAA TTC GGC CCA CTG GTC CTT | 3531-3914 | 384 |
| 7 (+) | | | |
| 8 (-) | | | |
| | 5' AA GGA TCC GAT CCT GGA GGA TTT | | |
| N'-terminal of gp70 | 5' TG GAA TTC CTT CAT GCT TTC TTC | 6311-6826 | 516 |
| 9 (+) | | | |
| 10 (-) | | | |
| | 5' AA GGA TCC ATG AAA TAC CTC AGG | | |
| C'-terminal of gp70 | 5' TT GAA TTC ACT ATC TAG ACC CCC | 6944-7777 | 833 |
| 11 (+) | | | |
| 12 (-) | | | |
| | 5' AA GGA TCC ATG GTA TAC CAA GAA | | |
| N'-terminal of gp40 | 5' TG GAA TTC TTT GAG CCA CGA GAA | 8186-8467 | 282 |
| 13 (+) | | | |
| 14 (-) | | | |
| | 5' CC GGA TCC GAA TTG GAG GAA AAA | | |
| C'-terminal of gp40 | 5' AA GAA TTC ATA GTC ATT TTC CAG | 8593-8904 | 312 |
| 15 (+) | | | |
| 16 (-) | | | |

*The sequences were derived from the published sequence of OLV 1514 strain (pLV1-1KS1).

K treated and cleaved with BamH I/EcoR I, except for the p25 fragment which was cleaved with BamH I only. Resultant fragments were gel purified and cloned into the expression vector pGex2T that had been cleaved with appropriate restriction enzymes. The pGex2T vector contains the glutathione-S-transferase (GST) gene under the control of the Tac promoter. The multiple cloning site at the 3' end of the GST gene allowed insertion of a DNA fragment fused in phase with the ATG start codon on the GST sequence, resulting in expression of a fusion protein. The resulting plasmid used for the expression of various regions of OPP viral protein is depicted in Figure 1.

Expression and partial purification of recombinant fusion proteins. All recombinant plasmids capable of expressing an OLV protein were transformed into E. coli (JM105 strain). The pGex2T was used as the control plasmid. To promote optimum production of the fusion protein, cells were grown in L broth to an optical density of 0.6 at 600 nm in the presence of ampicillin (50

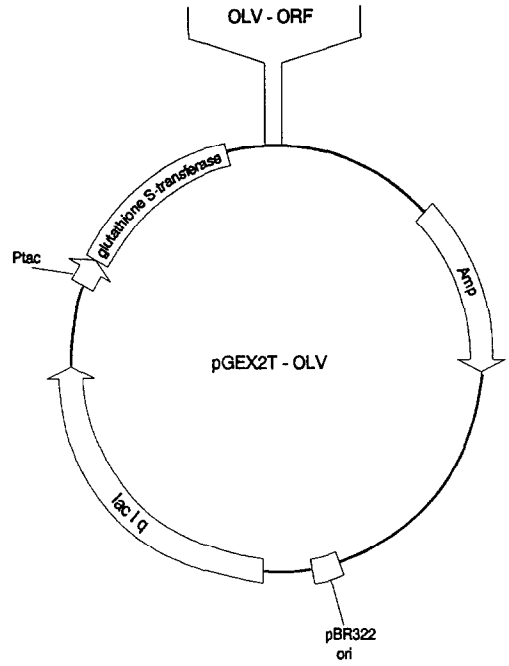


Fig. 1. pGex2T expression plasmid was used in the cloning and expresson of the OLV open reading frame (ORF). The resultant plasmid is designated pGex2T-OLV.

g/ml). The cultures were then induced with 1 mM isopropyl-ß-D-thiogalactopyranoside (IPTG) for four hours and harvested. In preparation for gel analysis of total bacterial lysates, cell pellets were sonicated and suspended in SDS-loading buffer (10% glycerol, 50 mM dithiothreitol, 3% SDS, 0.0625 M Tris-HCl, pH 8.0, and 0.2% bromophenol blue). Recombinant proteins representing 10-40% of the total protein, were readily observed (Figs. 2 and 3) in a Coomassie brilliant blue-stained sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel. The molecular size of each recombinant protein coincided with the predicted size: p25 - 50.4 Kd, p16 - 35.4 Kd, RT core - 51.3 Kd, C'-terminal RT - 40.8 Kd, N'-terminal gp70 - 46.6 Kd, C'-terminal gp70 - 57.2 Kd, N'-terminal gp40 - 37.5 Kd, C'-terminal gp40 - 38.8 Kd. To partially purify the recombinant proteins, cell pellets were sonicated and resuspended in lysis buffer (50 mM Tris-HCl, pH 8.0, 0.5 mM EDTA, 0.3M NaCl, 1 mg/ml lysozyme). Recombinant proteins were then extracted from the pelleted cell lysate by sequential urea extractions. Enriched recombinant fusion proteins, in 6 M urea or SDS buffer, were analyzed by SDS-PAGE and are shown in Figs. 2 and 3.

Immunological characterization of OPP sera with recombinant proteins. In order to identify the broad reactive antigenic determinants during OPPV infection, each of the expressed recombinant proteins were characterized by Western blot assay against a serum panel of OPP-positive and negative

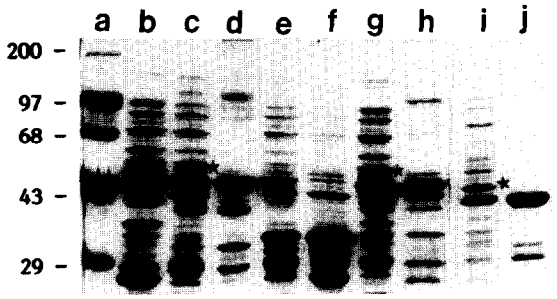


Fig. 2. Analysis of recombinant fusion proteins by Coomassie brilliant blue stained SDS-PAGE-gel. The lane designations are:
a - Molecular standard marker (in Kd)
b - pGex2T control
c - p25 total cell lysate (TCL)
d - p25 (6 M urea extraction)
e - p16 (TCL)
f - p16 (6 M urea extraction)
g - RT core (TCL)
h - RT core (SDS buffer extraction)
i - C'-RT (TCL)
j - C'-RT (SDS buffer extraction)
The * indicates the expressed recombinant proteins.

animals. The reactivity pattern of 12 AGID-positive sera are: 75% (9/12) for p16, 83% (10/12) for p25, 83% (10/12) for RT core, 33% (4/12) for C'-terminal RT, 50% (6/12) for N'-terminal of gp70, 66% (8/12) for C'-terminal of gp70, 100% (12/12) for N'-terminal of gp40, and 41% (5/12) for C'-terminal of gp40 (Table 2). Representative Western blots from sheep sera #9 (naturally infected with no symptoms) and #11 (naturally infected with OPP

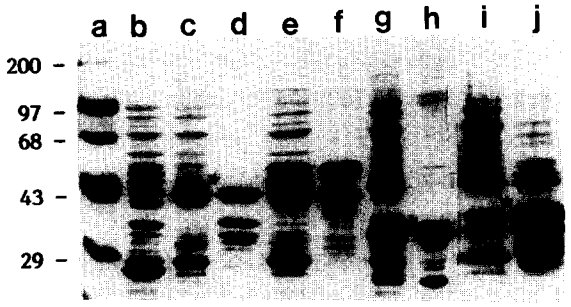


Fig. 3. Analysis of recombinant fusion proteins by Coomassie brilliant blue stained SDS-PAGE-gel. The lane designations are:
a - Molecular standard marker (in Kd)
b - pGex2T control
c - N'-gp70 (TLC)
d - N'-gp70 (6 M urea extraction)
e - C'-gp70 (TCL)
f - C'-gp70 (6 M urea extraction)
g - N'-gp40 (TCL)
h - N'-gp40 (SDS buffer extraction)
i - C'-gp40 (TCL)
j - C'-gp40 (6 M urea extraction)
The * indicates the expressed recombinant proteins.

Table 2. Reactivity of OPP-positive sera with recombinant proteins in Western blots

| Sheep serum group | Recombinant proteins | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | p25 | p16 | RT core | C'-RT | N'-gp70 | C'-gp70 | N'-gp40 | C'-gp40 |
| Experimentally infected | | | | | | | | |
| 1 | + | + | + | - | + | + | + | - |
| 2 | + | + | + | - | + | + | + | - |
| 3 | + | + | + | - | - | - | + | - |
| Naturally infected with no symptoms | | | | | | | | |
| 4 | + | + | + | - | - | + | + | - |
| 5 | - | - | + | + | + | - | + | + |
| 6 | + | + | - | - | - | - | + | - |
| 7 | - | - | + | + | + | + | + | - |
| 8 | + | + | + | - | - | - | + | + |
| 9 | + | + | - | + | + | + | + | + |
| Naturally infected with OPP lesions & symptoms | | | | | | | | |
| 10 | + | - | + | + | - | + | + | - |
| 11 | + | + | + | - | + | + | + | + |
| 12 | + | + | + | - | - | + | + | + |
| Negative control | | | | | | | | |
| 13 | - | - | - | - | - | - | - | - |
| 14 | - | - | - | - | - | - | - | - |

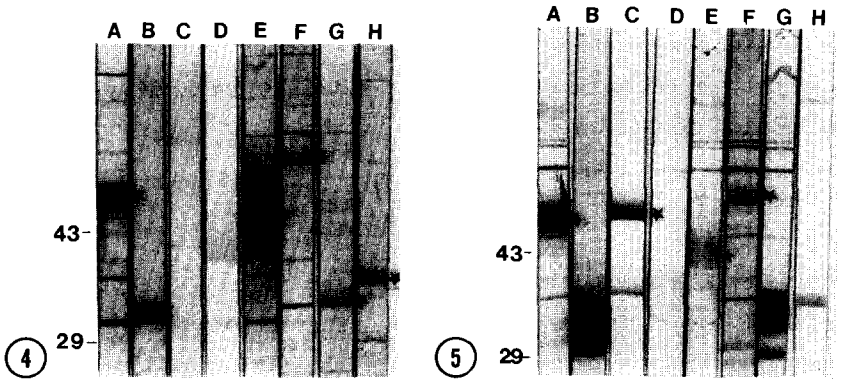


Fig. 4. Western blots from sheep serum #9 (naturally infected with OPPV with no OPP symptoms). Lane designations are:
A - p25
B - p16
C - RT core
D - C'-RT
E - N'-gp70
F - C'-gp70
G - N'-gp40
H - C'-gp40
The numbers on the left denote the molecular markers (in Kd).
The * indicates the reactive recombinant fusion protein.

Fig. 5 Western blots from sheep serum #11 (naturally infected with OPPV and showed OPP symptoms and progressive pneumonia lesions). Lane designations correspond with Fig. 4.

symptoms and progressive pneumonia lesions) are shown in Figs. 4 and 5, respectively.

The immunoreactivity of each recombinant protein was highly specific, as noted by the absence of cross-reaction of positive sera with bacterial cell extracts containing the GST moiety (data not shown). Although the recombinant proteins displayed various degrees of immunoreactivity with the serum panel, N'-terminal of gp40 region was recognized by 100% of sera tested in Western blots; thus, it is the primary immunodominant marker. The presence of antibodies to p25 and RT core proteins in 83% of the sera suggests these gene products define secondary immunodominant sites. It has been documented that antibodies to the major core protein (p25) of Human Immunodeficiency Virus (HIV) disappear with the onset of full-blown AIDS and that the absence of p25 antibody may be an indication for a pessimistic prognosis (17,18). However, the p25 antibody in OPP infected sheep may not play a similar role as in human AIDS, since sheep #10, 11, and 12 had a terminal progression of OPP disease and all had p25 antibodies.

Although N'-gp70 and C'-gp70 reacted with 50 and 66% of the positive sera, respectively, the combination of N'-gp70 and C'-gp70 resulted in detection of 75% of all positive sera tested. Apparently, there is complementary detection between N'- and C'-gp70. Thus, p25, RT, p16, and gp70 were not sufficiently reactive for the diagnosis of all OPPV infected sheep. It is concluded that the N'-terminal region of gp40 is the most immunoreactive of the bacterially expressed proteins and that this recombinant protein could be utilized as a sensitive immunodiagnostic antigen for the detection of OPP infection.

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