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Isolation, Identification, and Partial cDNA Cloning of Genomic RNA of Jaagsiekte Retrovirus, the Etiological Agent of Sheep Pulmonary Adenomatosis

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The genome of the jaagsiekte (JS) retrovirus (JSRV), the etiological agent of sheep pulmonary adenomatosis (jaagsiekte), has been identified, isolated, and partly cloned. The JSRV genome is ca. 8.7 kb long. cDNA of the genomic RNA was synthesized and cloned. A clone, JS 46.1, was isolated and characterized. It has an insert of 2.1 kb which hybridizes to the same 8.7-kb RNA in all the JSRV-infected sheep lung washes tested but does not hybridize to maedi-visna virus, a sheep lentivirus often found coinfecting JSRV-infected lungs. Comparison of the amino acid sequence encoded by JS 46.1 with those encoded by other retroviruses revealed that JSRV has homology to the type D and B oncoviruses and to human endogenous retrovirus.

Jaagsiekte (JS), or ovine pulmonary adenomatosis, is a naturally occurring contagious bronchioalveolar adenocarcinoma primarily affecting sheep (4, 36, 39, 43) and, to a lesser extent, goats (30, 37). The disease has morphological similarities to human bronchioalveolar carcinoma (19, 34). Although a number of agents were implicated in the etiology of JS (36, 43), it is now generally accepted that the disease is caused by a retrovirus, the JS retrovirus (JSRV) (4, 39). Serological (31), biochemical (5, 8, 40), and morphological (17) characteristics of JSRV suggest that it is more closely related to the B and D prototype oncoviruses than to the type C retroviruses. The inability to cultivate the virus in vitro has handicapped the progress of molecular studies on this retrovirus-induced pulmonary adenocarcinoma. Supportive evidence showing that a retrovirus is the causative agent includes an inverse dose relationship between reverse transcriptase activity in the infectious inoculum and the time taken for the infected sheep to reveal clinical symptoms (5, 12, 29, 40).

Biochemically, tumors and lung lavages of infected sheep were shown to contain 60S to 70S RNA and retrovirus particles that had a buoyant density in sucrose of between 1.15 and 1.20 (20, 38). However, sheep infected with JSRV are often coinfected with an ovine lentivirus (LV) (5, 16, 32). The early presence of the LV in our experimental flock was confirmed by its isolation from a tumor cell line that had been established from a sheep naturally JSRV infected (24). In fact, this cell line was the initial source of infectious inoculum used to induce JS in our experimental sheep in the early 1970s (2), thus revealing that the LV may have been present in all experimentally produced JS cases, which represented the main source of virus. Although the two viruses are morphologically distinguishable under the electron microscope (16), it was not until Sharp and Herring (31) demonstrated that serum against the group-specific antigen (p27) of Mason-Pfizer monkey virus (MPMV), the type D prototype, and mouse mammary tumor virus (MMTV), the type B prototype, reacted with particles associated with the disease that the JSRV could be monitored by a second parameter in addition to its reverse transcriptase activity. When sera against MPMV (p27) and the group-specific antigen (p30) of maedi-visna virus (MVV) and an immunoblot technique were used, the two viruses could be distinguished in coinfected lung fluid (5, 26).

In this study, the immunoblot technique was used to show that JSRV banded essentially at a slightly higher density than the LV when lung lavage fluids containing both viruses were isopycnically centrifuged through sucrose gradients. RNA from the higher-density peak contained an 8.7-kb polyadenylated RNA as opposed to a 9.4-kb RNA, which was associated with the lower-density LV region. Oligo(dT)-primed cDNA of the RNA from the high-density viral peak was cloned and shown to hybridize with the 8.7-kb JSRV RNA and not with the 9.4-kb LV RNA. In this report evidence is presented that supports our claim that we have isolated the genomic RNA of JSRV.

MATERIALS AND METHODS

Virus production and purification. JSRV was produced in newborn lambs by injecting Freon-extracted virus, obtained previously by lung lavages, intratracheally. When infected sheep showed advanced clinical symptoms, the lungs were rinsed with culture medium which was clarified and pelleted as described previously (38). After stored pellets were thawed, 2 volumes of cold fluorocarbon (Freon 113; Du Pont, Wilmington, Del.) were added, and the mixture was shaken for 3 min. After phase separation in a Beckman JA20 rotor (10 min, 10,000 rpm), the supernatant was collected and the interphase was extracted once more with 0.1 M Tris hydrochloride (pH 7.5)–0.1 M NaCl–0.001 M EDTA (TNE). The combined aqueous phase was layered onto a 2.5-ml layer of 30% sucrose–TNE and centrifuged in a Beckman SW41 rotor (1 h, 35,000 rpm). The pellet was resuspended in 1 ml of TNE and either layered onto a 20 to 48% sucrose gradient in an SW41 rotor (16 h, 25,000 rpm) for further purification or used for RNA extraction directly. Gradients were fractionated (1-ml fractions), and samples were removed for density determination (5 μl), reverse transcriptase activity (20 μl), and analysis by means of sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (15 μl).

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Protein analysis. Samples (15 μl) were taken directly from gradient fractions, suspended in 30 μl of 2× Laemmli sample loading buffer, and boiled for 5 min (10). Samples were separated in a 0.1% SDS–12.5% acrylamide-bisacrylamide (30:1) gel by using a Mini-Protein II dual slab gel apparatus (Bio-Rad Laboratories) and the protocol described in the manufacturer's instruction manual. Proteins were electrophoresed at 200 V for 30 min and then transferred to nitrocellulose membranes (Schleicher & Schuell BA83) by using a Bio-Rad semidry electrophoretic transfer cell at 5 mA/cm^2 for 18 min. Nonspecific binding sites on the nitrocellulose were blocked with 3% bovine serum albumin–phosphate-buffered saline (PBS) for 1 h. Primary antibody, consisting of goat anti-MPMV p27 serum or rabbit anti-MVV p30 serum, was used at 1:500 diluted in 3% bovine serum albumin-PBS. Incubation was for 2 to 16 h at room temperature. Filters were washed with PBS containing 0.1% Tween 20 (three times for 10 min each time). Secondary antibody was either alkaline phosphatase-conjugated anti-goat or anti-rabbit immunoglobulin G (H and L chain) (BioSys) serum used at 1:500 diluted in 3% bovine serum albumin-PBS. Incubation was for 1 h, after which the unbound conjugate was washed off as described above. Filters were soaked for 2 min in substrate buffer (0.1 M Tris hydrochloride [pH 9.5], 0.1 M NaCl, 0.5 M $MgCl_2$) before freshly prepared substrate (45 μl of Nitro Blue Tetrazolium and 35 μl of 5-bromo-4-chloro-3-indolyl phosphate [Promega] in 10 ml of substrate buffer) was added. The reaction was stopped by rinsing the filters in tap water for 5 min.

RNA extraction. Phenol-chloroform extraction was used to purify viral RNA from semipurified JSRV. Fractions selected from the Western blot (immunoblot) results were combined, diluted with 2.5 volumes of TNE, and centrifuged in a Beckman SW50.3 rotor (40 min, 40,000 rpm). The pellets were resuspended in 400 μl of TNE (final volume) and digested with 40 μg of proteinase K and 0.5% SDS (15 min, 37°C). After phenol-chloroform extraction, the RNA was precipitated with 0.15 M NaCl in 2.5 volumes of ethanol (−20°C, 16 h). After centrifugation (30 min, 10,000 × *g*, 4°C), the RNA was suspended in 500 μl of 0.5 M LiCl–10 mM Tris hydrochloride (pH 7.5)–1 mM EDTA and purified with oligo(dT)-cellulose. Nonpurified virus, pelleted through a sucrose cushion, was resuspended in 200 μl of TNE, and the RNA was extracted essentially as described previously (28). Briefly, GuSCN mixture (4 M GuSCN, 1 M Tris hydrochloride [pH 7.5], 1% 2-β-mercaptoethanol) was added to virus to a final volume of 1,215 μl. After the addition of 135 μl of 5% sodium sarcosyl, the mixture was layered onto a 5.4 M CsCl solution and centrifuged in a Beckman SW50.1 rotor (20 h, 40,000 rpm, 20°C). The RNA pellet was dissolved in 0.5 M LiCl–10 mM Tris hydrochloride (pH 7.5)–1 mM EDTA, and polyadenylated RNA was purified with an oligo(dT)-cellulose column (type 3; Collaboration Research Inc.).

Reverse transcriptase assay. Samples (20 μl) for the reverse transcriptase assay were taken directly from fractionated isopycnic gradients and added to 55 μl of an assay mix as previously described (45).

RNA analysis. RNA samples were separated in 1% agarose containing 7.2% formaldehyde (37 to 40%) and 1× MOPS (morpholinepropanesulfonic acid; 0.2 M) containing 5 mM sodium acetate and 1 mM EDTA (pH 7.0) as described elsewhere (24). RNA (100 ng) in 3 μl of this mixture was mixed with 9 μl of loading buffer, heated for 10 min at 68°C, and then loaded. Electrophoresis was for 2.5 h at 100 V/2.5 mA. *Hin*dIII-digested lambda DNA (150 ng) was dissolved in RNA loading buffer and treated the same way.

RNA samples were transferred to a nylon membrane (Amersham; Hybond N) essentially as described by Thomas (35) and fixed to the membrane by exposure to UV radiation for 5 min. Prehybridization was for 4 h at 42°C in prehybridization mix containing 5× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7])–50% formamide (vol/vol)–0.1% SDS–50 μg of denatured salmon sperm DNA per ml. Probes were added to fresh prehybridization mix containing 10% (wt/vol) dextran sulfate and hybridized for 20 h at 42°C with gentle agitation. Northern (RNA) blots were rinsed in three changes of 2× SSPE and then in the following washes: 15 min in 2× SSPE containing 0.1% SDS at 42°C repeated once, 15 min in 0.1× SSPE–0.1% SDS at 42°C, and finally 15 min in 0.1% SSPE containing 0.1% SDS at 55°C. Conditions for Southern transfers were as for Northern gels, with an additional wash in 0.1% SSPE containing 0.1% SDS at 55°C.

Filters were subsequently exposed overnight at −70°C to Kodak X-Omat S film sandwiched between two intensifying screens.

Preparation of probes. Radioactively labeled cDNA probes were prepared by reverse transcription of polyadenylated native viral RNA. Briefly, poly(A)$^+$ RNA (100 ng) was added to 20 μl of reverse transcriptase assay mix containing 10 mM magnesium acetate; 10 mM dithiothreitol; 10 mM Tris hydrochloride (pH 8.2); 50 mM KCl; 200 mM (each) dATP, dGTP, and dTTP; 10 mM dCTP; 100 ng of oligo(dT) (P-L Biochemicals); 20 U of Beard reverse transcriptase (Life Science, Inc); 0.5 U of RNase block II (Stratagene); and 60 μCi of [α-^{32}P]dCTP (3,000 Ci/mmol; Amersham International plc). Samples were incubated for 2 h at 42°C before the reaction was stopped with 2.5 μl of 20% SDS–10 μl of 0.2 M EDTA. After digestion with 10 ng of proteinase K at 42°C, the mixture was denatured with 0.25 M NaOH (46°C, 30 min), neutralized, and then extracted with an equal volume of phenol-chloroform. The cDNA was precipitated with ethanol and suspended in TNE. The final specific activity of the probe was ca. 10^7 cpm/μg.

DNA probes were prepared by random hexanucleotide-primed DNA polymerization with [α-^{32}P]dCTP (3,000 Ci/mmol) and the large fragment of the *Escherichia coli* DNA polymerase, as described elsewhere (6, 7). The final specific activity of the probes was ca. 5×10^8 cpm/μg.

Cloning of JSRV. cDNA was synthesized from high-density virus peak RNA by using a cDNA synthesis kit (Amersham) and the 1-μg-reaction procedure as described in the supplier's instruction manual. The oligo(dT)-primed cDNA (ca. 50 ng) was ligated to phosphorylated *Eco*RI linkers (Promega) digested with *Eco*RI and purified by elution through a Bio-Gel P150 packed column (1 ml). *Eco*RI-digested JSRV cDNA (10 ng) was ligated to 1 μg of *Eco*RI-predigested and phosphorylated lambda Zap II arms and packaged by using a Gigapack II packaging extract as described in the supplier's manual (Stratagene).

Positive plaques were identified by screening nylon replicas of the plated plaques with oligo(dT)-primed cDNA probes of RNA isolated from the high-density JSRV sucrose gradient peak fractions. Hybridization and washing conditions were as described above for Northern blots. Recombinant Bluescript plasmids (phagemids) were excised in vivo by using R408 as helper phage following the protocol described in the supplier's manual (Stratagene). Large-scale preparations of the plasmids were made and purified by the

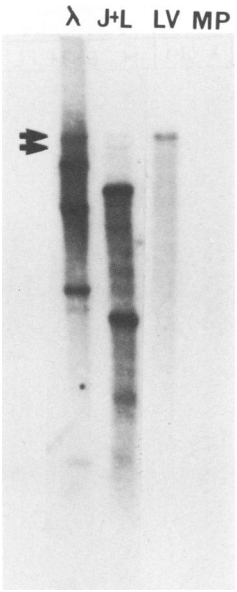

FIG. 1. Northern blot of RNA extracted from cushion-purified lung rinse pellets from JSRV-infected lungs. The pellets contained both JSRV and LV antigens. Lane J+L, oligo(dT)-primed cDNA probe of the RNA used in all three lanes; lane LV, DNA probe of SA 320, a molecular clone of SA-OMVV, which is a South African strain of MVV (23); lane MP, MPMV probe of cloned MPMV (provided by E. Hunter).

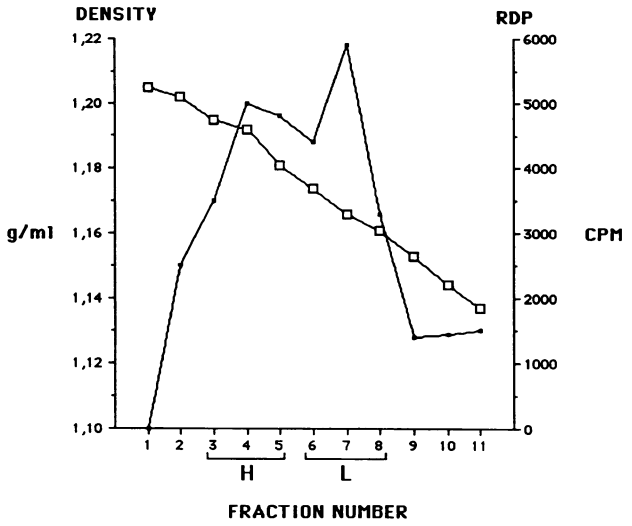

FIG. 2. Sedimentation of a lung wash concentrate containing JSRV and LV (after Freon extraction and centrifugation through a 30% sucrose cushion) through a 20 to 48% sucrose gradient to isopycnic equilibrium (SW41; 25,000 rpm, 4°C, 16 h). Samples were taken for density determination (□), RDP assay (●), and immunoblot analysis (Fig. 3) for the presence of JSRV p26 and LV p25. H, high-density peak fractions containing JSRV p26 antigen; L, low-density peak fractions containing LV p25 antigen.

alkaline lysis method followed by CsCl gradient purification as described elsewhere (28).

Nucleotide sequence accession numbers. The nucleotide sequences of JS 46.1 *env* and *pol* are deposited in GenBank under accession numbers M65013 and M65014, respectively.

RESULTS

RNA identification in JSRV- and LV-containing lung wash pellets. The main source of JSRV was the lung washes of sheep experimentally infected with JSRV, which were often found by serological assay to be simultaneously infected with LV. LV has been used in this paper to represent in a broad sense all the ovine MVV isolates. The serological assay used was a Western blot with sera against the group-specific antigens of MPMV and LV. With this technique, it was possible to identify and distinguish JSRV and LV. As reported previously (31), the anti-MPMV p27 serum reacts with a JSRV 26-kDa protein (JSRV p26). This serum does not cross-react with any LV proteins, whereas the LV anti-p30 serum reacts only with a 25-kDa LV protein (LV p25) (42) and does not cross-react with JSRV antigens.

Polyadenylated RNA was extracted from partially purified sheep lung wash pellets (Freon extracted and passed through a 30% sucrose cushion) containing both JSRV and LV and was analyzed by a Northern blot technique. To identify the RNA bands, an oligo(dT)-primed cDNA probe of the same RNA was used. Two RNA bands within the expected retrovirus genome size range, one on either side of the 9.4-kb *Hin*dIII-digested lambda marker, were observed (Fig. 1, lane J+L). Probing of the RNA in lane J+L with an LV-specific probe revealed that the upper of the two RNA bands is the LV RNA genome (Fig. 1, lane LV). However, probing of the same RNA with an MPMV-specific probe did not result in any hybridization even under low-stringency conditions (Fig. 1, lane MP). The lower intense smears present in lane J+L are the 28S and 18S rRNAs which are present in high concentrations in RNA preparations made from partially purified virus, even after oligo(dT) selection. These were the first results that led us to believe that the lower RNA band, ca. 8.7 kb, represented JSRV genomic RNA. Additional support was obtained by further purification of the viruses.

Analysis of RNA after isopycnic separation of the two viruses. It was known from previous experiments (44) that JSRV and LV have slightly different densities when separated through 20 to 50% isopycnic sucrose gradients. JSRV has a slightly higher density than LV. It was therefore anticipated that if the two viruses were isopycnically separated, there would be an associated separation of their respective RNA genomes. Figure 2 illustrates the sucrose density and RNA-dependent DNA polymerase (RDP) activity profiles when a lung wash pellet containing both JSRV and LV was isopycnically separated and selected fractions were analyzed. Samples from each fraction were also assayed for JSRV p26 and LV p25 by using a Western blot technique (Fig. 3). Two RDP peaks were consistently observed. In the one at a slightly higher density, JSRV p26 was most intense (Fig. 3A, fractions 3, 4, and 5). These fractions are referred to as the high-density region (Fig. 2). A second RDP activity peak corresponded to the fractions in which the LV p25 was most intense (Fig. 3B, fractions 6, 7, and 8). These fractions are referred to as the lower-density region (Fig. 2). When equivalent concentrations of RNA isolated from both the high- and low-density fractions were probed with an oligo(dT)-primed cDNA probe of the RNA from the high-density fractions (Fig. 4, lanes JS) and an LV-specific probe (Fig. 4, lanes LV), the high-density JSRV-containing fractions contained more 8.7-kb RNA than the low-density LV-containing fractions. The LV probe detected the 9.4-kb genomic RNA mostly in the lower-density fractions of the

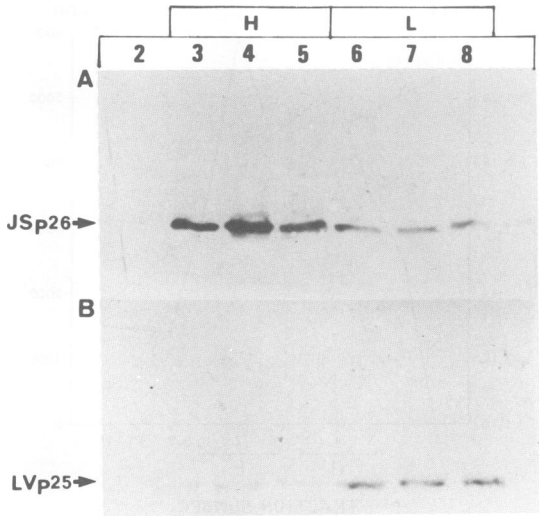

FIG. 3. Western blot analysis of gradient fractions when Freon-extracted sheep lung fluid (containing both JSRV and LV) was separated to isopycnic equilibrium (Fig. 2). (A) Anti-MPMV serum was used at a 1:500 dilution to detect JSRV p26. (B) Anti-MVV (p30) serum was used at a 1:500 dilution to detect LV p25.

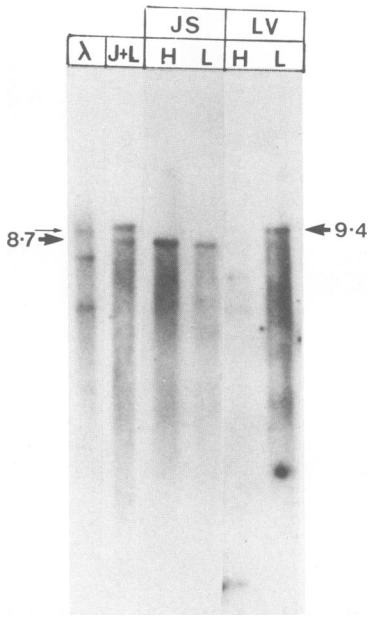

FIG. 4. Northern blot of RNA purified from the high-density (H) and low-density (L) fractions containing JSRV and LV particles. Lane J+L, poly(A)$^+$ RNA (100 ng) of lung wash pellet not isopycnically purified and containing both JSRV and LV as shown in Fig. 1; lanes H and L, poly(A)$^+$ RNA from the high- and low-density fractions, respectively, of the gradient illustrated in Fig. 2; lanes JS, oligo(dT)-primed cDNA probe of RNA from the high-density fractions was used; lanes LV, probe representative of SA 320, a molecular clone of a South African strain of MVV, was used (23). →, 9.4-kb lambda *Hin*dIII-digested DNA marker.

gradient. Since the 8.7-kb RNA is mostly associated with the higher-density reverse transcriptase activity peak, in which the JSRV p26 is most intense, we concluded that this RNA represents JSRV genomic RNA. Moreover, the high-density fractions which contain the JSRV are nearly free of LV genomic RNA.

cDNA cloning. Lung wash pellets from JSRV-infected lungs were serologically selected for their higher amounts of JSRV versus LV. Poly(A)$^+$ RNA from these pellets was shown after analysis to contain mainly the 8.7-kb RNA. Oligo(dT)-primed cDNA was made of this 8.7-kb-RNA-rich preparation [selected by oligo(dT)-cellulose from the high-density fractions] and, after *Eco*RI linker addition and digestion, cloned into the *Eco*RI site of a lambda Zap II cloning vector. Since material was very limited, the cDNA was not methylated. When the cDNA library was screened by using oligo(dT)-primed cDNA probes of this poly(A)$^+$ RNA, a number of positive clones were isolated. The clone containing the largest insert was selected for, characterized, sequenced, and found to have an insert of 2.1 kb. This clone is called JS 46.1

Radioactively labeled probes of clone JS 46.1 hybridized with the 8.7-kb RNA (Fig. 5A) as well as with RNA isolated from six different preparations of JSRV derived from experimentally infected sheep originating from the same institute in South Africa (results not presented). Material from a case of natural JS in France, which was serologically free from LV infection, contained an RNA band of identical size (Fig. 5B) which hybridized to a JS 46.1 probe, emphasizing the role of the JSRV and its 8.7-kb genomic RNA in the etiology of the disease.

Hybridization and sequence analysis of cloned cDNA. A restriction enzyme digest map of clone JS 46.1 is shown in Fig. 6. Two fragments, *Eco*RI-*Sac*I and *Eco*RI-*Xho*I, ca. 300 bp each and representing the 3′ and 5′ ends of the clone, respectively, were isolated. Probes of these two fragments hybridized to the 8.7-kb RNA, confirming that the entire clone was specific for the same RNA species (data not shown). However, sequence data of clone JS 46.1 revealed that the insert was in fact composite (Fig. 6). It consisted of a 662-bp segment which has features typical of the 3′ end of retroviruses (3), i.e., part of *env*, with a coding potential for 97 amino acids; a polypurine tract; a TATA box; and a poly(A) tail about 60 nucleotides in length. Anomalously linked to this poly(A) stretch is a 1,524-bp fragment which has homology to the *pol* gene of other retroviruses (Table 1). This homology was determined by comparing the computer-translated amino acid coding potential of the two JS 46.1 segments with those of other retroviruses. In agreement with serological findings, the JSRV *pol* gene has most homology with type B and D retroviruses. However, although the *pol* fragment of JS 46.1 is more homologous to its counterparts in type D than in type B oncoviruses, the *env* region of JS 46.1 exhibits no significant homology to MPMV or simian retrovirus type I (SRV-I) *env*. Particularly, it lacks the conserved immunosuppressive peptide which is shared by MPMV, reticuloendotheliosis-associated virus type A, and numerous other type C retroviruses (33). Instead, JS 46.1 *env* is related to MMTV (type B prototype) and human endogenous retrovirus *env* (Fig. 7).

DISCUSSION

Evidence presented in this paper suggests that the 8.7-kb RNA isolated from washes of JSRV-infected lungs is JSRV genomic RNA. Possibly the most convincing evidence in support of this claim was the demonstration that the 8.7-kb RNA was found in highest concentrations at the isopycnic density at which the MPMV-related antigen JSRV p26 is most intense. The latter is internationally accepted as a

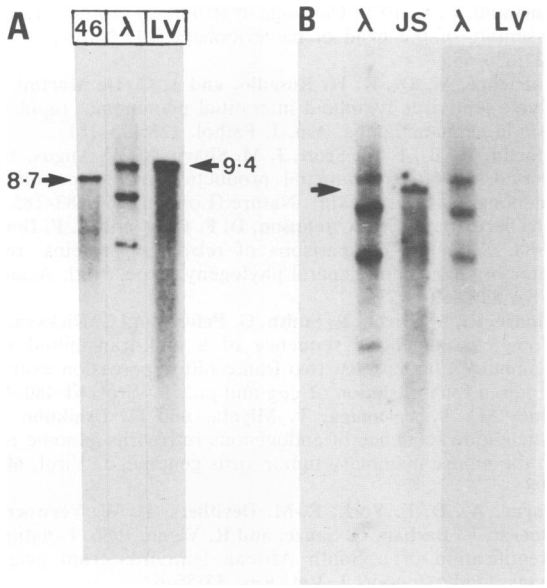

FIG. 5. (A) Northern blot of RNA from lung wash pellets (containing both JSRV and LV) from JSRV-infected lungs. The RNA was probed with labeled JS 46.1 insert (lane 46) and an LV-specific probe (lane LV). (B) RNA extracted (using a GuHCl method) from the nose fluid of a French ram naturally infected with JSRV but serologically LV free (this same RNA was separated in both lanes JS and LV). Probes were JS 46.1 (lane JS) and SA 320, a molecular clone of a South African strain of MVV (23) (lane LV). λ, lambda probe against *Hin*dIII-digested lambda DNA as DNA marker.

JSRV marker antigen (18). Particles at this density are also infectious (40). Transmission electron microscopic observations of gradient fractions in which fluid from JSRV-infected lungs containing both JSRV and LV was centrifuged to isopycnic equilibrium revealed that the higher-density fractions, in which the 8.7-kb RNA is most intense, contained retroviral particles which were identical to those seen in infectious material and JS tumors (17, 40, 44). JSRV particles are morphologically distinct from ovine LVs (16), which were present in higher concentrations at the lower density, in accord with the finding of the 9.4-kb LV RNA at the lower density and the 8.7-kb RNA in the fractions associated with the virus causing sheep pulmonary adenomatosis.

Analysis of RNA from JSRV- and LV-infected lung fluid revealed two RNA bands within the expected retroviral size range, i.e., 8.7 and 9.4 kb, whereas RNA extracted from JSRV-infected lung fluid, which was serologically LV free, revealed only the 8.7-kb band. The demonstration of the 8.7-kb RNA in all RNA extracts of JSRV-affected sheep tested but not in control or unaffected sheep adds support to our hypothesis that this RNA represents JSRV genomic RNA.

Cloning of the 8.7-kb cDNA resulted in the isolation of a

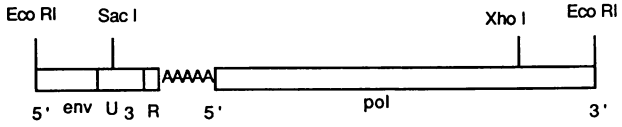

FIG. 6. Schematic representation of the genetic organization of clone JS 46.1 (not to scale) and restriction enzyme sites.

TABLE 1. Amino acid identities of Pol and Env encoded by regions of JS 46.1 with those of other retroviruses and serological relationships between *gag* antisera and JSRV p26[a]

Type	Virus[b]	Amino acid identity (%)[c]		Serologic result[d]
		Pol	Env	
D	MPMV	50.5	NA	+
D	SRV-I	50	NA	ND
D	SMRV	53.1	NA	+
B	MMTV	44	28	+
B	HERV-K	43.2	27	ND
C	BLV	24.9	NA	−
C	SA-OMVV	NA	NA	−

[a] Alignments were performed by using the Kanehisa program with the mutation data matrix of Dayhoff default parameters.

[b] Sequence references: MPMV (33), SRV-I (22), squirrel monkey retrovirus (SMRV; 1), MMTV (14), human endogenous retrovirus type K (HERV-K; 15) bovine leukemia virus (BLV; 27), South African strain of MV (SA-OMVV; 23).

[c] JSRV Pol was aligned to MPMV and MMTV Pol amino acid residues 231 and 739 and 225 and 729, respectively. JSRV Env was aligned to MMTV Env as described in the legend to Fig. 7. NA, no significant alignment.

[d] Serological data are according to Western blot results (31, 45; unpublished data for SMRV). +, positive; −, negative; ND, not done.

number of specific clones, the largest of which was characterized and sequenced. Further evidence that the 8.7-kb RNA is retroviral was the finding of classical retroviral sequence markers, i.e., a polypurine tract, a TATA box, an inverted repeat, and a poly(A) tail. It was initially disturbing to discover that the insert was a composite, consisting of part *pol* and part *env*-U3 linked anomalously to the poly(A) tail. There is no *Eco*RI or other common restriction enzyme site at the linkage between the two segments, implying a blunt ligation which probably occurred during the ligation step. The finding of "natural" *Eco*RI sites and not linker-associated *Eco*RI sequences at the 3′ and 5′ termini of the composite insert implies that there are at least two *Eco*RI sites in the JSRV genome, i.e., one in *env* and a second in *pol*. These two sites would not have been protected from *Eco*RI digestion, as the cDNA was not methylated; hence the resultant cloning of a composite sequence into the vector *Eco*RI site.

Although only 25% of the genome has been sequenced, the limited sequence information is in agreement with biochemical (8, 39) and immunological (11, 39) data that imply that JSRV is most similar to type B and D retroviruses. JSRV morphogenesis is also similar to that of type B and D retroviruses (17). However, JSRV is ultrastructurally more similar to MMTV in that both JSRV and MMTV have prominant surface projections (spikes), which are less evident on the surface of the D-type retroviruses (17). This is in accord with the sequence information which revealed that

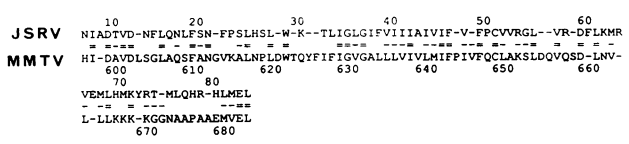

FIG. 7. Alignment of the translated *env* region of JS 46.1 insert with that of MMTV. The predicted 97-amino-acid sequence of JS 46.1 Env was aligned to the 688 residues of MMTV Env protein by using the Kanehisa program and mutation data matrix of Dayhoff (9a, 9b). =, identical residues; −, homologous residues.

JSRV *env* is more B type. The divergence of relatedness between JSRV *pol*, which is D type, and JSRV *env*, which is B type, pointed to either (i) a possible recombinational event or (ii) a divergence from the D-type phylogenetic tree before the capture of the reticuloendotheliosis-associated virus type A envelope gene which is present in MPMV, SRV-1 (13, 33), and most likely squirrel monkey retrovirus as well (9).

The role of LV in JS is uncertain, but the coexistence of both retroviruses has so often been reported that it has warranted serious investigation. Published evidence against a primary role of LV in JS includes failure to transmit JS with LV (4, 11, 16) and a lack of LV RNA in JS tumors (21). In this paper, failure to demonstrate any homology between JSRV and LV and failure to find LV in every sheep infected with JSRV, such as in the French case reported in this paper, also negate the theory that LV plays a primary role in JS. Nevertheless, the reason both viruses are so often found in the same JSRV-infected lung remains a very intriguing problem.

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REFERENCES

1. **Chiu, I., R. Callahan, S. R. Tonick, J. Schlom, and S. A. Aaronson.** 1984. Major *pol* gene progenitors in the evolution of oncoviruses. Science **223**:364–370.
2. **Coetzee, S., H. J. Els, and D. W. Verwoerd.** 1976. Transmission of jaagsiekte (ovine pulmonary adenomatosis) by means of a permanent cell line established from affected lungs. Onderstepoort J. Vet. Res. **43**:131–142.
3. **Coffin, J. M.** 1990. Retroviridae and their replication, p. 1437–1500. *In* B. N. Fields, D. M. Knipe, et al. (ed.), Virology, 2nd ed. Raven Press, New York.
4. **Demartini, J. C., R. H. Rosadio, and M. D. Lairmore.** 1988. The etiology and pathogenesis of ovine pulmonary carcinoma (sheep pulmonary adenomatosis). Vet. Microbiol. **17**:219–236.
5. **Demartini, J. C., R. H. Rosadio, J. M. Sharp, H. I. Russel, and M. D. Lairmore.** 1987. Experimental coinduction of type D retrovirus-associated pulmonary carcinoma and lentivirus associated lymphoid interstitial pneumonia. J. Natl. Cancer Inst. **79**:167–177.
6. **Feinberg, A. P., and B. Vogelstein.** 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. Anal. Biochem. **132**:6–13.
7. **Feinberg, A. P., and B. Vogelstein.** 1984. Addendum to "A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity." Anal. Biochem. **137**:266–267.
8. **Herring, A. J., J. M. Sharp, F. M. Scott, and K. W. Angus.** 1983. Further evidence for a retrovirus as the etiological agent of sheep pulmonary adenomatosis (jaagsiekte). Vet. Microbiol. **8**:237–249.
9. **Hunter, E., C. Barker, J. Bradac, S. Chatterjee, R. Desrosiers, and J. Wills.** 1986. Molecular comparisons of the D-type retrovirus. *In* P. Fumanski, J. Hager, and M. Rich (ed.), RNA tumor viruses, oncogenes, human cancer and AIDS: on the frontiers of understanding. Martinus Nijhoff, Boston.

9a. **Kanehisa, M. I.** 1982. Los Alamos sequence analysis package for nucleic acid and proteins. Nucleic Acids Res. **10**:183–196.

9b. **Kanehisa, M. I.** 1984. Use of statistical criteria for screening potential homologies in nucleic acid sequences. Nucleic Acids Res. **12**:203–215.

10. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
11. **Lairmore, M. D., R. H. Rosadio, and J. C. De Martini.** 1986. Ovine lentivirus lymphoid interstitial pneumonia: rapid induction in neonatal lambs. Am. J. Pathol. **125**:173–181.
12. **Martin, W. B., F. M. Scott, J. M. Sharp, K. W. Angus, and M. Norval.** 1976. Experimental production of sheep pulmonary adenomatosis (jaagsiekte). Nature (London) **264**:183–185.
13. **McClure, M. A., M. S. Johnson, D. F. Feng, and R. F. Doolittle.** 1988. Sequence comparisons of retroviral proteins: relative rates of change and general phylogeny. Proc. Natl. Acad. Sci. USA **85**:2469–2473.
14. **Moore, R., M. Dixon, R. Smith, G. Peters, and C. Dickson.** 1987. Complete nucleotide sequence of a milk-transmitted mouse mammary tumor virus: two frameshift suppression events are required for translation of *gag* and *pol*. J. Virol. **61**:480–490.
15. **Ono, M., T. Yasunaga, T. Miyata, and H. Ushikubo.** 1986. Nucleotide sequence of endogenous retrovirus genome related to the mouse mammary tumor virus genome. J. Virol. **60**:589–598.
16. **Payne, A., D. F. York, E.-M. Devilliers, D. W. Verwoerd, G. Querat, V. Barban, N. Sauze, and R. Vigne.** 1986. Isolation and identification of a South African lentivirus from jaagsiekte lungs. Onderstepoort J. Vet. Res. **53**:55–62.
17. **Payne, A.-L., D. W. Verwoerd, and H. M. Garnett.** 1983. The morphology and morphogenesis of jaagsiekte. Onderstepoort J. Vet. Res. **50**:317–322.
18. **Perk, K., E.-M. Devilliers, A. J. Dawson, A. J. Herring, J. M. Sharp, and J. C. Demartini.** 1985. Comparison by Western blotting of the retrovirus associated with sheep pulmonary adenomatosis (jaagsiekte), p. 345–348. *In* J. M. Sharp and R. Hoff-Jorgensen (ed.), Slow viruses in sheep, goats and cattle. Commission of the European Communities, Brussels.
19. **Perk, K., and I. Hod.** 1982. Sheep lung carcinoma: an epidemic analogue of a human neoplasm. J. Natl. Cancer Inst. **69**:747–750.
20. **Perk, K., R. Michalides, S. Spiegelman, and J. Schlom.** 1974. Biochemical and morphological evidence for the presence of an RNA tumor virus in pulmonary carcinoma of sheep (jaagsiekte). J. Natl. Cancer Inst. **53**:131–135.
21. **Perk, K., and A. Yaniv.** 1977. Lack of maedi related viral RNA in pulmonary carcinoma of sheep (jaagsiekte). Res. Vet. Sci. **24**:46–48.
22. **Power, M. D., P. A. Marx, M. L. Bryant, M. B. Gardner, P. J. Barr, and P. Luciw.** 1986. Nucleotide sequence of SRV-1, a type D simian acquired immune deficiency syndrome retrovirus. Science **231**:1567–1572.
23. **Querat, G., G. Audoly, P. Sonigo, and R. Vigne.** 1990. Nucleotide sequence analysis of SA-OMVV, a visna-related ovine lentivirus: phylogenetic history of lentivirus. Virology **175**:434–447.
24. **Querat, G., V. Barban, N. Sauze, R. Vigne, A.-L. Payne, D. F. York, E.-M. Devilliers, and D. W. Verwoerd.** 1987. Characteristics of a novel lentivirus derived from South African sheep with pulmonary adenocarcinoma (jaagsiekte). Virology **158**: 158–167.
25. **Rosadio, R. H., and J. C. De Martini.** 1986. Ovine lentivirus lymphoid interstitial pneumonia: rapid induction in neonatal lambs. Am. J. Pathol. **125**:173–181.
26. **Rosadio, R. H., J. M. Sharp, M. D. Lairmore, J. E. Dahlberg, and J. C. De Martini.** 1988. Lesions and retroviruses associated with naturally occurring ovine pulmonary carcinoma (sheep pulmonary adenomatosis). Vet. Pathol. **25**:58–66.
27. **Sagata, N., T. Yasunaga, J. Tsuzuku-Kanamura, K. Ohishi, Y. Ogana, and Y. Ikana.** 1985. Complete nucleotide sequence of the bovine leukemia virus: its evolutionary relationship to other retroviruses. Proc. Natl. Acad. Sci. USA **82**:677–681.
28. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Sharp, J. M., K. W. Angus, E. W. Gray, and F. M. Scott.** 1983. Rapid transmission of sheep pulmonary adenomatosis

(jaagsiekte) in young lambs. Arch. Virol. **78**:89–95.
30. **Sharp, J. M., K. W. Angus, F. A. Jassim, and F. M. Scott.** 1986. Experimental transmission of sheep pulmonary adenomatosis to a goat. Vet. Rec. **119**:245.
31. **Sharp, J. M., and A. J. Herring.** 1983. Sheep pulmonary adenomatosis demonstration of a protein which cross reacts with the major core proteins of Mason-Pfizer monkey virus and mouse mammary tumor virus. J. Gen. Virol. **64**:2223–2227.
32. **Snyder, S. P., J. C. Demartini, E. Ameghino, and E. Caletti.** 1983. Coexistence of pulmonary adenomatosis and progressive pneumonia in sheep in the Central Sierra of Peru. Am. J. Vet. Res. **44**:1334–1338.
33. **Sonigo, P., C. Barker, E. Hunter, and S. Wain-Hobson.** 1986. Nucleotide sequence of Mason-Pfizer monkey virus: an immunosuppressive D-type retrovirus. Cell **45**:375–385.
34. **Spencer, H.** 1985. Pathology of the lung, 4th ed., p. 892–900. Pergamon Press, Oxford.
35. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
36. **Tustin, R. C.** 1969. Ovine jaagsiekte. J. Afr. Vet. Med. Assoc. **1**:3–23.
37. **Tustin, R. C., A.-L. Williamson, D. F. York, and D. W. Verwoerd.** 1988. Experimental transmission of jaagsiekte (ovine pulmonary adenomatosis) to goats. Onderstepoort J. Vet. Res. **55**:27–32.
38. **Verwoerd, D. W., A.-L. Payne, D. F. York, and M. S. Myer.** 1983. Isolation and preliminary characterization of the jaagsiekte retrovirus (JSRV). Onderstepoort J. Vet. Res. **50**: 309–316.
39. **Verwoerd, D. W., R. C. Tustin, and A.-L. Payne.** 1985. Jaagsiekte: an infectious pulmonary adenomatosis of sheep, p. 53–76. *In* R. G. Olsen, S. Krakowka, and J. R. Blacksla (ed.), Comparative pathobiology of viral diseases. CRC Press, Inc., Boca Raton, Fla.
40. **Verwoerd, D. W., A.-L. Williamson, and E.-M. Devilliers.** 1980. Aetiology of jaagsiekte: transmission by means of subcellular fractions and evidence for the involvement of a retrovirus. Onderstepoort J. Vet. Res. **47**:275–280.
41. **Vigne, R., V. Barban, G. Querat, V. Mazarin, I. Gourdou, and N. Sauze.** 1987. Transcription of visna virus during its lytic cycle: evidence for a sequential early and late gene expression. Virology **161**:218–227.
42. **Vigne, R., P. Filippi, G. Querat, N. Sauze, C. Vitu, P. Russo, and P. Delori.** 1982. Precursor polypeptides to structural proteins of visna virus. J. Virol. **42**:1046–1056.
43. **Wandera, J. G.** 1971. Sheep pulmonary adenomatosis (jaagsiekte). Adv. Vet. Sci. **15**:251–283.
44. **York, D. F.** 1987. Ph.D. thesis. University of Natal, Pietermaritzburg, South Africa.
45. **York, D. F., A.-L. Williamson, B. J. Barnard, and D. W. Verwoerd.** 1989. Some characteristics of a retrovirus isolated from transformed bovine cells. Virology **171**:394–400.