Jaagsiekte retrovirus establishes a disseminated infection of the lymphoid tissues of sheep affected by pulmonary adenomatosis

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Jaagsiekte retrovirus (JSRV) is an exogenous type D-related retrovirus specifically associated with a contagious lung cancer of sheep (sheep pulmonary adenomatosis; SPA). Recently, epithelial tumour cells in the lungs of SPA-affected sheep were identified as major sites of JSRV replication by immunological techniques and RT-PCR amplification of part of JSRV gag. JSRV was not detected outside the lungs and their draining lymph nodes. However, low levels of JSRV expression in non-respiratory tissues could have been masked by co-amplification of endogenous JSRV-related sequences, which were differentiated from JSRV by the lack of a Scal restriction site in the PCR product. To further investigate the pathogenesis of SPA, an exogenous virus-specific hemi-nested PCR was developed utilizing primers in the U3 region of JSRV LTR, where major differences between endogenous and exogenous sequences exist. This technique was shown to be ≥ 10³-fold more sensitive than the previous gag PCR/Scal digestion method. Using this new assay the tissue distribution of JSRV in sheep with natural and experimentally induced SPA was analysed. Proviral DNA and JSRV transcripts were found in all tumours and lung secretions of SPA-affected sheep (n = 22) and in several lymphoid tissues. The mediastinal lymph nodes draining the lungs were consistently demonstrated to be infected by JSRV (10/10). JSRV transcripts were also detected in spleen (7/9), thymus (2/4), bone marrow (4/8) and peripheral blood mononuclear cells (3/7). Proviral DNA was also detected in these tissues although in a much lower proportion of cases. JSRV was not detected in 27 samples from unaffected control animals (n = 15).

Introduction

Retroviruses isolated from mammalian species have been invaluable to our present knowledge of cancer initiation and progression. Animal models have been established, usually in murine and avian species, and have provided a better understanding of oncogenic events, particularly in cells of the lymphoreticular system (Weiss et al., 1985; Fan, 1994). However, animal models of epithelial neoplasia associated with retroviruses have been restricted to mouse mammary tumour and the associated virus, mouse mammary tumour virus (MMTV) (Gross, 1983). Such models of epithelial neoplasia are particularly relevant as tumours arising from epithelia are the most prevalent tumours in man (Silverberg et al., 1990).

Sheep pulmonary adenomatosis (SPA; also known as jaagsiekte and ovine pulmonary carcinoma) represents a unique system because it is a naturally occurring epithelial neoplasm which is closely associated with a retrovirus (Verwoerd et al., 1985; Sharp, 1987). Moreover, SPA shares certain characteristics with human bronchiolo-alveolar carcinoma (Perk & Hod, 1982; Gazdar & Linnoila, 1988) the aetiology of which is unknown and which is increasing dramatically in prevalence (Barsky et al., 1994).

The virus associated with SPA, jaagsiekte sheep retrovirus (JSRV), cannot be cultivated in vitro. JSRV has morphological, biochemical and antigenic similarity to type D and type B retroviruses and has a genomic organization typical of a
replication-competent retrovirus, containing no sequences commonly associated with transformation (Perk et al., 1974; Sharp & Herring, 1983; Herring et al., 1983; Rosadio et al., 1986; York et al., 1992).

Current data point to the involvement of JSRV in the aetiology of SPA, in particular, the experimental transmission of the disease only with inocula that contain JSRV (Martin et al., 1976; Verwoerd et al., 1980; Sharp et al., 1983; DeMartini et al., 1987) and the constant association of JSRV with transformed epithelial cells in the lungs of both naturally and experimentally SPA-affected sheep (Palmarini et al., 1995). Recent molecular data have demonstrated that JSRV is an exogenous virus distinct from the transcriptionally active endogenous retroviral sequences present within the ovine genome [sheep endogenous retroviruses; SERVs (synonym: en[SRV]) (Palmarini et al., 1996; Bai et al., 1996; York et al., 1992). A Scal restriction site in gag has been defined as a molecular marker for the exogenous JSRV which has been consistently demonstrated in tumour and in draining lymph nodes of a proportion of SPA-affected sheep. JSRV was not detected outside the respiratory tissues of SPA-affected sheep nor in any tissue of control sheep (Palmarini et al., 1996). Nevertheless, co-amplification of endogenous and exogenous sequences could have masked the detection of low copies of exogenous JSRV due to the relatively high background formed by SERVs.

To address this question, an exogenous JSRV-specific heminested PCR which amplified a segment of the U3 region of JSRV was designed. The anatomical distribution of JSRV in tissues collected from sheep with naturally and experimentally induced SPA was then investigated.

Methods

Animals. SPA-affected sheep were defined as animals with typical clinical signs, particularly the production of an abundant sero-mucoid fluid (‘lung fluid’) from the nostrils when the rear limbs were elevated above the head. Diagnosis was then confirmed by macroscopic and histological examination of the lungs. Seventeen naturally SPA-affected sheep and six experimentally infected lambs already utilized in a preceding study (Palmarini et al., 1996) were used. Fifteen age- and breed-matched animals were selected as controls.

Preparation of samples. Samples of heparinized venous blood, lung fluid, lung, lung tumour, mediastinal lymph nodes, pre-scapular lymph nodes, mesenteric lymph nodes, spleen, femoral bone marrow, thymus, kidney and skin were collected during the post-mortem examination or (blood and lung fluid) immediately before the sheep were sacrificed. Sterile instruments were used to dissect each individual organ at post-mortem to avoid cross-contamination. Tissue samples were snap-frozen in liquid nitrogen and stored at −70°C. Lung fluid samples were clarified by centrifugation at 10,000 g for 1 h at 4°C and then stored at −70°C. Plasma (5 ml) was clarified by centrifugation at 10,000 g for 1 h at 4°C. The supernatant was then centrifuged at 100,000 g for 1 h at 4°C. The pellet was resuspended in 500 μl of solution D (4 μM-guanidine thiocyanate, 25 mM-sodium citrate pH 7.0, 0.5% sarcosyl, 0.1 M-2-mercaptoethanol) and stored at −20°C. Peripheral blood mononuclear cells (PBMC) were isolated from venous blood by centrifugation over Lymphoprep at 800 g for 30 min; aliquots of 4 × 10⁶ cells were stored at −70°C until RNA and DNA were extracted. Total RNA was extracted from 50 μl aliquots of lung fluid, 200–500 mg of lung tumour or from the plasma samples using the acid guanidium thiocyanate-phenol-chloroform extraction method (Chomczynski & Sacchi, 1987). Poly(A)+ RNA was obtained from 100 μg of all other tissues and from 4 × 10⁶ PBMC using the Microfast Track kit (Invitrogen) according to the manufacturer’s instructions. Genomic DNA was extracted from 10–25 mg of tissues and from 4 × 10⁶ PBMC using the Qiagen Tissue Amp kit following the manufacturer’s instructions.

Amplification and sequencing of exogenous LTR sequences. Sequences and nucleotide positions of the oligonucleotide primers relative to the JSRV genome (York et al., 1992) are indicated in Table 1. The 5’ LTR of a UK strain of JSRV was amplified from 500 ng of lung tumour genomic DNA isolated from a natural case of SPA using primers P-I and P-II. These formed the first primer pair of a hemi-nested LTR-gag PCR used to amplify proviral JSRV (Palmarini et al., 1996). Briefly, the forward primer (P-I) is located almost at the 5’ end of the U3 while the reverse primer anneals to gag (P-II) so that all but the first 33 bp of the JSRV 5’ LTR are amplified. Previously, primer P-I was demonstrated to be specific for the exogenous form of JSRV (Palmarini et al., 1996). The PCR products of three independent reactions were pooled and cloned in pGEM-T (Promega) as described by the manufacturer. Six selected clones (pGEM-T LTR-gag) were demonstrated to be of exogenous origin by the presence of a unique Scal site in gag (data not shown). The region corresponding to the LTR of JSRV was then sequenced in both directions by the dideoxynucleotide chain termination method, employing a Li-Cor automated sequencer.

cDNA synthesis. cDNA synthesis was carried out using total RNA derived from ultraspeed pellets of plasma (5 ml) or 50 μl of clarified lung

| Table 1. Oligonucleotide primers employed in the PCRs and hybridizations of this study |
|---------------------------------|-----------------|-----------------|
| **Primers** | **Sequence (5’ − 3’)** | **Position** |
| P-I | TGGGAGCTTCTTTGGCAGAACCC | 7210–7224 |
| P-II | ATACTGAGCAGCAGTGGGCAG | 1806–1826 |
| P-III | CACCGGATTTTTACAACAATCCCGG | 7361–7385 |
| P-IV | GCCATCAGTTCACAGAATATGAGGAATCTGATT | 7316–7350 |
| P-V | TTGTAAAGCTTCTTAAAGGCITCGGATGTGTTGCTT | 7280–7313 |
| P-VI | TGATATTTCTGTAAGCAGTGGC | 7316–7338 |

* Nucleotide positions refer to the JSRV sequence published by York et al. (1992).
fluid as template. For analysis of other samples, 2 μg of total RNA from tumour samples or 1 μg poly(A)+ RNA from other tissues and PBMC were used as template. RNA was diluted in 13.5 µl of diethylpyrocarbonate-treated distilled water and denatured at 65 °C for 10 min and cooled on ice. One microlitre (40 U) of Rnase In (Promega), 2 µl of 25 mM each deoxynucleoside triphosphate (dNTP), 2.5 µl of 100 mM-DTT, 2.5 µM random hexamers were major sequence differences between JSRV and SERVS. In this region, the JSRV U3-PCR reaction was optimized using DNA from a LTR-gag pGEM-T plasmid as template. The buffer used was 10 mM-Tris-HCl, 50 mM-KCl, 2.5 mM-MgCl₂ (pH 9.2) with 200 µM each dNTP, 6-25 pmol of each primer and 1:25 U of Taq polymerase (Boehringer) in a 50 µl reaction. Five hundred nanograms of genomic DNA or 3 µl of cDNA were used as template in the reaction. Each sample was subjected to 94 °C for 1 min followed by 35 cycles of 94 °C for 30 s, 59 °C for 1 min and 72 °C for 1 min with a final extension of 72 °C for 3 min, using a Perkin Elmer GeneAmp 2400 thermal cycler.

Total RNA extracted from 22 lung tumour and lung fluid samples from 16 different naturally acquired SPA cases and 4 experimentally induced cases was tested to verify the ability of JSRV-U3 PCR to detect different isolates of JSRV. In addition, genomic DNA extracted from lung tumour and matched kidney samples of four sheep with SPA was tested to verify that JSRV U3-PCR does not amplify SERV sequences.

To verify the specificity of the PCR products, 25 µl of the PCR product was resolved by electrophoresis in 1.5% agarose gels and transferred to nylon membranes overnight by established procedures (Sambrook et al., 1989). Membranes were then fixed, pre-hybridized and probed overnight at 42 °C with 10 pmol each of two digoxigenin (DIG) 5'-labelled oligonucleotides (P-IV and P-V; Oswel) in DIG Easy Hyb (Boehringer) hybridization buffer. The hybridized probe was then detected using the DIG Nucleic Acid Detection Kit (Boehringer) as recommended by the manufacturers.

To improve the sensitivity of the JSRV-U3 PCR, a primer internal to the PCR product, primer P-VI, was synthesized and paired with primer P-I in a second round semi-nested PCR (JSRV U3-hn PCR). One microlitre of the product of the first round of amplification was added to 49 µl of 1 x PCR buffer [1 x PCR buffer [1:25 U of Taq polymerase (Boehringer), 2.5 mM-MgCl₂, 50 mM-KCl, 10 mM-Tris-Cl, 200 µM each dNTP and 6-25 pmol of each primer]. PCR cycles employed were 94 °C for 1 min and 35 cycles of 94 °C for 30 s, 57 °C for 1 min and 72 °C for 1 min, with a final.

Fig. 1. Sequence alignment of the U3 of SERVs (Palmarini et al., 1996), of the South African strain of JSRV [JSRV(SA)] (York et al., 1992) and of a UK strain of JSRV [JSRV(UK)]. Letters indicate differences with the consensus sequence. A period (.) indicates deletions. Lack of sequence data in the JSRV(UK) sequence is indicated with (#). Positions of the primers employed in the JSRV U3-PCR and hn-U3 PCR are underlined. P-III (asterisk) appears discontinuous only in the figure due to the 50 bp deletions of JSRV sequences with respect to SERV (enJSRV).

### Table: JSRV U3 Exonogous-specific PCRs

| Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | 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Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Primer | Prime...
extension of 72 °C for 3 min. Amplified products were detected by electrophoresis of 25 μl aliquots through a 2% agarose gel containing 0.5 μg/ml of ethidium bromide.

The sensitivities of the JSRV U3-PCR and U3-hn PCR, against a background of endogenous-related sequences, were compared with each other and with the previously described JSRV gag PCR followed by ScaI digestion (Palmarini et al., 1995). ‘Reconstruction’ experiments were performed using serial dilutions (10^3 to 10^-1 copies) of clone pGemT-LTR-gag (which contains the U3 through gag of JSRV provirus) with and without the addition of 500 ng of normal sheep kidney DNA as a source of SERV sequences. In addition, 100 copies of pGem-T-LTR-gag plasmid, plus serial dilutions (10^-6 to 10^-3 copies) of SERV-LTR or SERV-gag plasmids were tested.

Contamination of PCR was minimized by the use of appropriate controls in each step of the work. Isolation of nucleic acids, preparations of PCR reactions and analysis of PCR products were conducted in separate rooms.

**Template control PCRs.** Each cDNA sample which was negative after the U3-hn PCR was tested for the presence of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) transcripts to verify the integrity of the RNA template. Primers were designed on a consensus sequence between the rat and human GAPDH (C. McInnes, Moredun Research Institute, Edinburgh, personal communication). The sense primer (5’ TCACCCATGGAAGGCCCT 3’) was based on exon 4 of the human gene and the reverse primer was based on exon 7 (5’ TTCTATTGTCATAACAGGAAA 3’) (Tokunaga et al., 1987). The buffer employed was 10 mM-Tris-HCl, 50 mM-KCl, 2.5 mM-MgCl₂ (pH 8.9) with 200 μM each dNTP, 6.25 pmol each primer and 1.25 U Taq polymerase (Boehringer). Cycles employed were 94 °C for 1 min and then 35 cycles of 94 °C for 45 s, 52 °C for 1 min and 72 °C for 1 min 30 s with a final extension of 72 °C for 5 min.

Parallel samples in which the addition of RT was omitted in the cDNA synthesis step were also tested to rule out DNA contamination. Amplified products were detected by electrophoresis of 25 μl aliquots through 1% agarose gels in 1 x TBE buffer in the presence of 0.5 μg/ml ethidium bromide. A DIG-labelled internal probe (5’ DIG-CICATGACCAAGAGCAGCACACTTGACATCCAGGCC) was used to confirm the specificity of the amplified product. Southern blotting and detection of hybridized probe were essentially as described above for U3-PCR. DNA samples which were negative after hn-U3 PCR were checked by amplification of a portion of SERV gag as previously described (Palmarini et al., 1996).

**Results**

**Sequencing of JSRV LTR and designing of JSRV exogenous-specific primers**

To better design an exogenous virus-specific PCR the LTR of a UK strain of JSRV was sequenced and compared with the sequence of a South African strain of JSRV and the LTR of SERVs (York et al., 1992; Palmarini et al., 1996). The LTR of the UK strain of JSRV (JSRV(UK)) was 89% homologous to the LTR of the South African strain (JSRV(SA)) (York et al., 1992) and 76–79% similar to SERV loci (Palmarini et al., 1995). Major sequence divergence between exogenous and endogenous viruses was confirmed in the U3 region. In particular, a 30 bp deletion in the U3 of JSRV(SA) compared to the endogenous loci was also confirmed in JSRV(UK). Primer P-III was designed across the deletion such that at the optimal annealing temperature this primer should anneal efficiently only with the exogenous sequences. The alignment of the U3 of JSRV and SERV sequences and the relative positions of the primers employed in this study are indicated in Fig. 1. The JSRV LTR sequence of the UK strain has been deposited in GenBank under accession number Z71304.

**Specificity and sensitivity of JSRV U3 and hemi-nested U3 PCRs**

Twenty-two cDNA samples of lung fluid and lung tumour were examined by PCR using primers P-I and P-III. A product of the expected size of 176 bp was obtained with all samples. Hybridization with the DIG-labelled internal oligonucleotides P-IV and P-V confirmed that the products originated from JSRV-LTR (data not shown). To demonstrate the PCR product was derived only from JSRV and not from SERV, matched genomic DNA samples of lung tumour and kidney from four SPA-affected animals were examined. An amplified product was obtained from tumour DNA and not from the corresponding kidney sample, proving that the JSRV-U3 PCR is exogenous virus specific (Fig. 2).

The sensitivity of the U3-PCR was determined by ‘reconstruction’ experiments, testing mixtures of exogenous JSRV and SERV templates described in Methods. JSRV U3 PCR successfully amplified 10^5–10^6 JSRV template copies in a background of 500 ng of normal sheep genomic DNA and 10^5 JSRV template copies in a background of 10^7 copies of SERV-LTR.

The sensitivity of the U3-PCR was increased by using primer P-VI and primer P-I in a second round PCR. A product of 133 bp was detected from the re-amplification of all the lung fluid and lung tumour PCR products but not from the kidney samples. The U3-hn PCR detected an estimated single molecule of template in a background of 500 ng of normal sheep...
Table 2. Results of the JSRV U3-hn PCR on tissues of sheep affected by SPA

Results are given as number of samples positive of the number tested. In all cases RNA refers to poly(A)+ selected RNA and DNA to genomic DNA.

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NA, Not available

JSRV RNA and proviral DNA was found consistently in the mediastinal lymph nodes of SPA-affected sheep (10/10). In addition, JSRV RNA was found in 7/9 spleens, 4/6 bone marrows and 2/4 thymuses (Fig. 3). Proviral DNA was found more rarely in these tissues. PCR products were detected in 2/8 spleens and 3/7 bone marrows. Among the non-regional lymph nodes analysed all were negative for proviral DNA, but two mesenteric lymph nodes of the five analysed were positive at the RNA level. Pre-scapular lymph nodes (n = 4) were always negative. PBMC were found to be positive for JSRV RNA in 3/7 samples but proviral DNA was not detected in any. Plasma samples were always negative.

Apart from lymphoid tissues, JSRV was detected at both the DNA and RNA level in only one kidney of nine tested and in none of four skin samples. No differences were observed in the anatomical JSRV distribution between the naturally acquired and the experimentally induced SPA cases.

Detection of proviral DNA and JSRV transcripts in non-tumour tissues of SPA-affected sheep

Following the successful development and validation of the U3-hn PCR, the anatomical distribution of JSRV in SPA-affected sheep was analysed. Table 2 summarizes the results obtained.

JSRV RNA and proviral DNA was found consistently in the mediastinal lymph nodes of SPA-affected sheep (10/10). In addition, JSRV RNA was found in 7/9 spleens, 4/6 bone marrows and 2/4 thymuses (Fig. 3). Proviral DNA was found more rarely in these tissues. PCR products were detected in 2/8 spleens and 3/7 bone marrows. Among the non-regional lymph nodes analysed all were negative for proviral DNA, but two mesenteric lymph nodes of the five analysed were positive at the RNA level. Pre-scapular lymph nodes (n = 4) were always negative. PBMC were found to be positive for JSRV RNA in 3/7 samples but proviral DNA was not detected in any. Plasma samples were always negative.

Apart from lymphoid tissues, JSRV was detected at both the DNA and RNA level in only one kidney of nine tested and in none of four skin samples. No differences were observed in the anatomical JSRV distribution between the naturally acquired and the experimentally induced SPA cases.

Fig. 3. Example of the results of JSRV U3-hn PCR in a SPA-affected animal. Lanes 1 contain the first step U3 PCR product and lanes 2 contain the second round of amplification (U3-hn PCR). Lanes A, lung tumour cDNA; lanes B, kidney cDNA; lanes C, spleen cDNA (note the faint product obtained in the first step); lanes D skin cDNA; lanes E, lung cDNA from an unaffected control animal. Lane M contains molecular mass marker IX (Boehringer). W is the negative control (water) of the hn-U3 PCR.

JSRV was not detected in 27 samples examined from 15 unaffected control animals. These samples included poly(A)+ RNA prepared from lung (n = 5), mediastinal lymph nodes (n = 7), thymus (n = 1) and PBMC (n = 4). In addition, samples from lung (n = 3), mediastinal lymph nodes (n = 1), spleen (n = 2), thymus (n = 1), bone marrow (n = 1) and kidney (n = 2) were tested for proviral DNA.

All the samples that were negative in the U3-hn PCR were positive for the amplification of GAPDH cDNA or SERV gag DNA, demonstrating that the templates were not degraded, and PCR inhibitors were not present after the nucleic acid extraction.
Discussion

Previous studies, using immunological and molecular techniques, demonstrated that JSRV is an exogenous virus, which does not arise from reactivation of an endogenous locus (Palmarini et al., 1995, 1996). These studies identified the lungs, particularly the epithelial tumour cells, as a major site of JSRV replication, although viral RNA was demonstrated in the draining mediastinal lymph nodes of a proportion of SPA-affected sheep. These observations have been extended in the present report as a result of developing a highly sensitive assay for exogenous JSRV and show that JSRV RNA and proviral DNA is present not only in the draining lymph nodes but also in several anatomically dispersed lymphoid tissues and PBMCs. This distribution of JSRV in infected animals is, therefore, similar to that reported for other type B and type D retroviruses, in which both lymphoid and non-lymphoid tissues are infected (Bentvelzen & Brinkhof, 1977; Kozma et al., 1980; Bryant et al., 1986; Lackner et al., 1988).

Although quantitative assays were not employed in the present studies, the estimated sensitivity of the different PCR assays confirmed that JSRV was expressed at much higher levels in SPA tumours than in the lymphoid tissues, particularly those at the non-local sites. Thus, there are clear parallels with the high levels of MMTV transcription associated with the development of breast carcinoma in mice (Varmus et al., 1973; Henrard & Ross, 1988; Bramblett et al., 1995). The transcriptional activity of MMTV appears to be determined by the interaction of a number of factors involving regulatory elements within the LTR, including at least one region implicated in elevated transcription in the mammary epithelium (Lefebre et al., 1991; Mink et al., 1992; Mok et al., 1992), and the absence of tissue-specific factors such as negative regulatory element binding protein (NBP) in mammary tissue (Bramblett et al., 1995). Similar putative regulatory sequences, such as NF-1- and C/EBP-binding sites, have been identified in the JSRV LTR (York et al., 1992) as well as many point mutations and large deletions compared to endogenous loci (Palmarini et al., 1996; Bai et al., 1996). Other retroviruses with LTR deletions have been reported to have a transcriptional advantage (Hsu et al., 1988) and the biological significance of these structures in JSRV awaits the development of permissive culture systems.

The interaction between type B and type D retroviruses and lymphoid cells has been demonstrated to be an important feature in the pathogenesis of these viruses. B lymphocytes have a central role in the pathogenesis of MMTV and infection of the mammary gland requires a functional immune system. MMTV initially appears to infect B lymphocytes and is then delivered to the mammary gland through T cell–B cell interactions (Tsubura et al., 1988; Golovkina et al., 1992; Held et al., 1994; Matsuzawa et al., 1995). The simian type D retrovirus, SRV1, which induces simian acquired immunodeficiency syndrome in macaques (Gardner et al., 1988), has a broad in vivo lymphoid tropism involving lymphocytes and mononuclear cells. Infection leads to both T and B lymphocyte depletion although monocyte and macrophage function appears to be unaffected (LeGrand et al., 1985; Maul et al., 1988).

The significance of infection of lymphoid tissues by JSRV in the overall pathogenesis of SPA is unknown at present. As only sheep with clinical SPA were examined it is not known whether lymphoid infection precedes, or is a sequel to, JSRV replication in the pulmonary epithelium. Further studies will be required to define which cell types are involved and at which stages during the protracted time between infection and the appearance of clinical illness. Similarly, it is not clear whether sheep infected with JSRV develop immunodeficiency, although this possibility is supported by anecdotal reports of the increased susceptibility to secondary bacterial pneumonias (Sharp & Martin, 1983; Verwoerd, 1990) and lymphopenia in SPA-affected sheep (Rosadio & Sharp, 1992). The apparent absence of circulating antibodies to JSRV in sheep with SPA has been reported (Sharp & Herring, 1983; Verwoerd, 1990) although a recent study, using a recombinant Mason-Pfizer monkey virus major capsid protein, has produced conflicting results (Kwang et al., 1995).

One of the major obstacles to progress in research on JSRV has been the lack of an in vitro culture system. The present study has clearly demonstrated permissive infection of lymphoid cells by JSRV, cell types which have been used successfully for the propagation of many retroviruses and which, therefore, could provide a permissive substrate for JSRV replication.

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