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Infection of lung epithelial cells and induction of pulmonary adenocarcinoma is not the most common outcome of naturally occurring JSRV infection during the commercial lifespan of sheep

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Abstract

Jaagsiekte sheep retrovirus (JSRV) is the causative agent of ovine pulmonary adenocarcinoma (OPA). In this study, we followed over a 31-month period the natural transmission of JSRV in adult sheep and in their offspring. We established groups derived from flocks with either a high or low incidence of OPA and monitored virus transmission, clinical disease and macroscopic/microscopic lung lesions at necropsy. Results obtained show that (i) JSRV infection can occur perinatally or in the first few months of life in lambs and in adult sheep; (ii) only a minority of JSRV-infected animals develop clinical disease during their commercial lifespan; and (iii) JSRV is more readily detectable in peripheral blood leucocytes and lymphoid organs than in the lungs. These data support a model of opportunistic JSRV infection and tumorigenic conversion of type II pneumocytes/Clara cells in the lungs, while lymphoreticular cells serve as the principal virus reservoir.

Keywords: Ovine pulmonary adenocarcinoma; OPA; Jaagsiekte sheep retrovirus; JSRV; JSRV transmission; Retroviruses; Retrovirus pathogenesis; Sheep

Introduction

Ovine pulmonary adenocarcinoma (OPA) is one of the major infectious diseases of sheep in many regions of the world and is considered a large animal model for lung carcinogenesis (Fan et al., 2003; Palmarini and Fan, 2001; Palmarini et al., 1997). OPA is caused by a retrovirus known as Jaagsiekte sheep retrovirus (JSRV) (DeMartini et al., 2001; Palmarini et al., 1999). The disease is clinically characterised by a chronic respiratory syndrome resulting from the development of lung adenocarcinoma and often secondary bacterial infections (Sharp and DeMartini, 2003).

In OPA-affected sheep, JSRV antigens are readily detectable by immunohistochemistry or ELISA only in lung tumour cells and in lung secretions ('lung fluid') that accumulate in the airways of most animals with classical clinical signs. The neoplasm originates from type II pneumocytes and Clara cells of the lungs of OPA sheep (De las Heras et al., 2003; Palmarini and Fan, 2001; Palmarini et al., 1995, 1996a, 1997; Platt et al., 2002). Interestingly, by immunohistochemistry, normal type II pneumocytes or Clara cells surrounding the tumour in OPA lesions do not show JSRV antigens. In OPA-affected sheep, JSRV is also detectable in cells of the lymphoreticular system, but only by employing highly sensitive PCR assays (Gonzalez et al., 2001; Holland et al., 1999; Palmarini et al., 1996b); only occasionally, lymphoid cells were revealed to contain JSRV antigens by immunohisto-

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chemistry in mediastinal lymph nodes draining lung tumours (Holland et al., 1999). Thus, the target cells for JSRV-induced cell transformation are also those that appear to produce the greatest amount of virus.

The incidence of clinical disease in an affected flock is generally around 2–5% with most of the clinical cases occurring in sheep between 2 and 4 years of age (Sharp and DeMartini, 2003).

JSRV is unique among oncogenic retroviruses as its envelope protein (Env) behaves in vitro as a dominant oncoprotein (Allen et al., 2002; Maeda et al., 2001; Rai et al., 2001). In addition, mice inoculated with an adeno-associated virus vector expressing the JSRV Env developed OPA-like lesions in as little as 8 weeks (Wootton et al., 2005). Thus, JSRV behaves essentially as a rapidly transforming retrovirus in vitro, in the experimental lamb model in vivo and in an experimental mouse model. However, the disease is believed to have a very long incubation period in naturally occurring OPA (Sharp and DeMartini, 2003). Indeed, OPA was included, along with scrapie, paratuberculosis and Maedi-Visna, among the 'slow' diseases of sheep originally described by Sigurdsson in Iceland in the 1950s (Sigurdsson, 1954, 1958). In old transmission studies using adult sheep, OPA was reproduced after an incubation period of several months to years (Dungal, 1946). However, the disease is reproducible in as little as 3 to 4 weeks when concentrated lung secretions from an affected animal are inoculated into neonatal new-born lambs (Salvatori et al., 2004; Sharp et al., 1983; Verwoerd et al., 1980).

The majority of virus transmission studies in the JSRV/OPA system have been done using the experimental lamb model. Data on the natural transmission of JSRV-infection are scarce. The difficulty of monitoring virus transmission studies in the JSRV/OPA system is due to the combination of two factors: sheep infected with JSRV (with or without clinical disease) do not show a detectable immune response towards the virus (Ortin et al., 1998; Spencer et al., 2003; Summers et al., 2002) and a very low proviral burden is present in the blood of infected animals (Gonzalez et al., 2001; Holland et al., 1999; Palmarini et al., 1996b). Sheep are probably immunotolerant to JSRV due to the expression of JSRV-related endogenous retroviruses during fetal development (Spencer et al., 2003). Only very sensitive PCR assays, based on the described hemi-nested LTR PCR (hnLTR-PCR) on PBLs of infected animals, can be employed to detect JSRV during the pre-clinical stages of OPA (Palmarini et al., 1996b). To increase the sensitivity of the assays, various investigators have modified the assay above by employing more than 5 or 6 replicates of DNA (~500 ng each) per sample (Gonzalez et al., 2001; Holland et al., 1999). A single step LTR-PCR using three replicates of genomic DNA has also been used with comparable sensitivity (Salvatori et al., 2004) although in our hands the hnLTR-PCR is more sensitive than a single step PCR assay (results not shown; De las Heras et al., in press).

In this study, the objectives were to follow (i) the natural transmission of JSRV infection, (ii) development of clinical disease and (iii) histopathological lesions in adult sheep and their offspring over a 31-month period. Our data indicate that JSRV infection can spread readily both in lambs and adult animals but that the onset of clinical disease and tumor lesions during the commercial lifespan of sheep occurs in a minority of JSRV-infected animals after a long incubation period. This study introduces new concepts in the pathogenesis of OPA.

Results

Sensitivity and specificity of the PCR procedure used in this study

In this study, we followed the natural transmission of JSRV infection and clinical/histopathological lesions among adult sheep and lambs. We used the hnLTR-PCR already described (Gonzalez et al., 2001; Holland et al., 1999; Palmarini et al., 1996b) to detect JSRV in PBLs, mediastinal lymph nodes, spleen and lungs of sheep and used three replicates of 500 ng of DNA per sample to reach an optimal compromise between sensitivity (e.g., the probability to detect a JSRV-infected animal) and specificity (e.g., the probability that a sample tested positive is not a false positive) of the test given the high numbers of samples analysed.

The sensitivity and specificity of a single hnLTR-PCR reaction to detect JSRV in PBLs of a JSRV-infected sheep depends on the number of replicates performed in each single test. Within a single replicate per sample, the hnLTR-PCR sensitivity is 6.1% and specificity is 99.4% (see also Materials and methods). With 3 replicates in a single test, the theoretical sensitivity is 17.5% (the observed one was 16.2%) and the theoretical specificity is 98.3% (the observed one was 98.8%).

Sensitivity and specificity of the entire procedure based on repeated testing of the same animal depends on both the number of replicates performed in each test and on the number of repeated tests performed on the same animal. Thus, a minimal acceptable value for the sensitivity (i.e., around 50–60%) would result from 4 to 5 repeated tests with 3 replicates per each test. The specificity corresponding to these sensitivity values is between 91% and 93%. Given the difficulty in defining with certainty an OPA-free flock in this study, case and control animal definitions were designed to be as conservative as possible.

Given the high level of sensitivity of the PCR test (Holland et al., 1999; Palmarini et al., 1996b), great care was taken to avoid, and control for, PCR contaminations. We tested a total of 835 intercalated extractions and DNA controls for a total of 1106 samples, excluding the common reaction mixture controls included in each PCR reaction (see also Materials and methods).