Enzootic nasal tumour of goats: demonstration of a type D-related retrovirus in nasal fluids and tumours

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Nasal exudate and tumour tissue from goats with enzootic nasal tumours were shown to contain a reverse transcriptase activity associated with a particle of buoyant density typical of retroviruses. The same particle contained a 25000 Mr protein that cross-reacted with the p27 of Mason-Pfizer monkey virus (MPMV) and with p25 of sheep pulmonary adenomatosis retrovirus. It also contained a low Mr protein related to p10-12 of MPMV.

Adenocarcinoma of the ethmoid turbinates of sheep and goats occurs as an enzootic disease, with clusters of cases arising in regions and individual herds (Neiberle, 1939; Cohrs, 1953; Young et al., 1961; De las Heras et al., 1985, 1986). Although the aetiology of the disease is unknown at present, several features suggest that it is infectious and that a virus could be involved. The tumour has been transmitted experimentally from sheep to sheep using filtrates of tumour homogenate, free of bacteria and cells (Cohrs, 1953). Furthermore, ultrastructural studies have revealed retrovirus-like particles in association with the epithelial tumour cells in both sheep and goats (Yonemichi et al., 1978; Njoku & Chineme, 1983; De las Heras et al., 1988). Here we present further evidence for the association of a retrovirus with nasal adenocarcinoma of goats. Certain features of the virus suggest that it is a new type D-like retrovirus.

Tumour tissue and/or exudate were obtained from four goats in which ethmoid turbinate adenocarcinoma (TIEC) was confirmed clinically and histologically. The goats were from three different Spanish herds in which ethmoid adenocarcinoma had been observed previously. The serum of each animal was negative by the agar gel immunodiffusion test for antibodies to ruminant lentivirus. The specimens were frozen immediately at -70 °C.

Briefly, putative virus was obtained from 50 ml of undiluted nasal exudate or 10% (w/v) homogenates of tumour tissue by differential centrifugation. The pelleted material was resuspended as a 200-fold concentrate in TNE buffer (0·01 M-Tris–HCl pH 7·5, 0·1 M-NaCl, 0·001 M-EDTA) and assayed for reverse transcriptase (RT) activity by measuring the incorporation of[^3H]TTP into a TCA-precipitable product in the presence of the synthetic template–primer poly(rA).oligo(dT) and poly(dA).oligo(dT) (Herring et al., 1983).

Clear evidence for RT-associated activity was demonstrated in pellets from three of three nasal exudates and in two of two tumour tissues, although much higher levels of activity were detected in two of the three nasal exudates (Fig. 1a, b). The third exudate with low RT activity had been stored at -20 °C for several weeks. The pellets from the two nasal exudates containing the highest levels of RT activity were pooled and analysed by isopycnic centrifugation on 20 to 55% (w/w) sucrose gradients (Herring et al., 1983) and each 0·5 ml fraction was examined for RT activity. As shown in Fig. 1(c), a clear peak of RT activity was detected in four fractions with densities between 1·17 and 1·19 g/ml. The pelleted material was examined further by SDS–PAGE and Western blotting (Sharp & Herring, 1983), using antisera to a variety of retroviruses and retrovirus structural proteins (bovine leukosis virus, Rauscher leukaemia virus, felin leukaemia virus (FeLV), FeLV p27, Mason–Pfizer monkey virus (MPMV), MPMV p27, MPMV p10-12, mouse mammary tumour virus (MMTV), MMTV gp32, MMTV p27, maedi–visna virus (MVV), MVV p28, equine infectious anaemia virus p26). Positive reactions were observed with only two sera. A goat antiserum to MPMV p27 (ref. 75S-148, National Cancer Institute Repository) showed a clear reaction with a single polypeptide of Mr 25000 (Fig. 2a), and another goat antiserum to MPMV p10-12 (ref. 73S-39, National Cancer Institute Repository) reacted with a low Mr protein (Fig. 2b). These two proteins were detected only in the sucrose gradient fractions containing RT activity, although the peak intensity occurred at a slightly higher density compared to the peak of RT activity.
Fig. 1. Demonstration of RT activity, which is expressed as c.p.m. obtained with poly(rA).oligo(dT) (hatched bars) compared to that obtained with poly(dA).oligo(dT) (open bars). Source of RT activity: (a) nasal exudate and (b) tumour from goats with ethmoid adenocarcinoma. AMV RT, purified RT obtained from avian myeloblastosis virus; SPA LF, purified retrovirus from SPA lung fluid; AL-1, O-20, E4, purified retrovirus from nasal exudates; AL-1, E5, purified retrovirus from ethmoid tumours. (c) RT activity from nasal exudate after isopycnic centrifugation in 25 to 55% (w/w) sucrose gradients. RT activity was assayed by using poly(rA).oligo(dT) (○) and poly(dA).oligo(dT) (▲). Density of the sucrose gradient is shown (□).

Fig. 2. Detection by Western blotting of proteins related to MPMV in the TIEC nasal exudate. (a) Isopycnic gradient fractions, corresponding to the peak of RT activity, reacted with a goat antiserum to MPMV p27. Fraction numbers are indicated above the respective lanes; (b) the same fractions reacted with a goat antiserum to MPMV p10-12.
Fig. 3. Retrovirus particles observed in TIEC tissue by transmission electron microscopy. (a) Extracellular particles (arrowheads) in proximity to the apical surface of a tumour cell (bar represents 500 nm). (b) Higher magnification of an extracellular particle (bar represents 50 nm). (c) Immature particle budding from an apical microvillus (bar represents 50 nm).

The association of the ovine and caprine retroviruses SPARV and R-TIEC with two secretory epithelial tumours offers new opportunities to investigate the pathogenesis of epithelial neoplasia.

References


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