Sheep Pulmonary Adenomatosis: Demonstration of a Protein which Cross-reacts with the Major Core Proteins of Mason–Pfizer Monkey Virus and Mouse Mammary Tumour Virus

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SUMMARY

A retrovirus that causes pulmonary adenomatosis, a contagious lung tumour of sheep, contains a 25 000 mol. wt. polypeptide which cross-reacts with the major core protein (p27) of Mason–Pfizer monkey virus and mouse mammary tumour virus.

Sheep pulmonary adenomatosis (SPA, jaagsiekte) is a contagious lung tumour which can be transmitted experimentally with tumour homogenates or the copious secretions (SPA lung fluid) that accumulate in the respiratory tract, particularly during the terminal stages of the disease (Dungal, 1946; Wandera, 1970; Herring et al., 1983). The epithelial tumour cells have been shown to contain intracytoplasmic type A particles (Perk et al., 1971; Bucciarelli, 1973; Sharp et al., 1983) and, in close association, extracellular particles, the morphology of which has been variously described as either type C (Perk et al., 1971), type B or type D (Sharp et al., 1983). Reverse transcriptase activity has been demonstrated both in tumour homogenates and in SPA lung fluids (Perk et al., 1974; Martin et al., 1976; Herring et al., 1983). The enzyme was shown to have a preference for Mg$^{2+}$ and was associated with a particle with densities, in sucrose and CsCl, similar to those of the type B and type D retroviruses (Herring et al., 1983). Further characterization of the SPA retrovirus has been greatly handicapped by the lack of an in vitro culture system for the virus. However, we now report that an immunological cross-reaction between the 27 000 mol. wt. core protein (p27) of Mason–Pfizer monkey virus (MPMV), mouse mammary tumour virus (MMTV), and a 25 000 mol. wt. protein in SPA tumours and lung fluids can be demonstrated by the ‘Western’ blotting technique.

The antigens were first fractionated for 2-5 h at a constant current of 20 mA/cm$^2$ in a 0.75 mm-thick 10% polyacrylamide gel with a 3% stacking gel using the conditions described by Laemmli (1970). Transfer to a nitrocellulose sheet (Schleicher & Schüll; BA83) was performed as described by Burnette (1981) using an EC ‘Electroblot’ apparatus with a voltage gradient of 6-6 V/cm. The immobilized polypeptides were reacted with the various sera at a dilution of 1/40 and binding was detected using iodinated rabbit anti-sheep Fab$_2$ (sp. act. $3 \times 10^5$ ct/min $^{125}$I/μg, used at 1-4 μg/ml) and autoradiography with X-Omat S film (Kodak) and Cronex lightning-plus intensifying screens (DuPont). The detection procedures were essentially those described by Burnette (1981) with the exception that blocking of non-specific binding sites after transfer was achieved with either 50% (v/v) normal rabbit serum or 50% horse serum and all washes and serum dilutions were made with phosphate-buffered saline with the NaCl concentration raised to 0.5 M and containing 0.001 M-EDTA, and 0.5% Tween 80 (washing buffer) (Morein et al., 1983). The mol. wt. of fractionated polypeptides were determined by comparison with standards which were electrophoresed in parallel, transferred to nitrocellulose and stained with Coomassie Brilliant Blue.

Homogenates (10%, w/v) of SPA tumours and unaffected lungs, and undiluted SPA lung fluids were layered over 50% (v/v) glycerol in 0.01 M-Tris–HCl buffer pH 7.5 containing 0.1 M-NaCl and 0.001 M-EDTA (TNE), centrifuged at 100000 g for 60 min at 4°C and the pellet was resuspended as a 200-fold concentrate.

Key words: retrovirus/cross-reaction/pulmonary adenomatosis/polypeptide
Parainfluenza virus type 3 (PI3) was obtained from medium of infected cultures by centrifugation at 100000 g for 60 min at 4 °C and antigens prepared from MPMV, MMTV, maedi-visna virus (MVV), caprine herpesvirus 1, *Mycoplasma ovipneumoniae* and *M. arginini* were supplied by colleagues. Immunoglobulins were prepared from SPA serum and SPA lung fluid by chromatography using a Whatman DE52 column. Other antisera were supplied by colleagues.

The specificity of the antiserum to MPMV p27 was established by absorption with purified virus. Gradient-purified MPMV (840 μg) was pelleted, resuspended in 50 μl TNE containing 0-5% (v/v) Nonidet P40 and 1% (w/v) SDS and agitated gently for 30 min at ambient temperature. The volume was increased to 1 ml with washing buffer containing a 1/40 dilution of antiserum to MPMV p27, and agitated at 37 °C for 60 min. Antigen–antibody complexes were removed by centrifugation at 10000 g for 60 min at 4 °C and the supernatant used as absorbed serum.

The relationship of the SPA retrovirus to other members of the group was investigated by the Western blotting procedure using antisera to a variety of retroviruses. Positive reactions were observed with two sera. A goat antiserum to MPMV p27 (ref. 5-S-148) showed a clear reaction with a single polypeptide, mol. wt. 25000, present in both SPA tumour and SPA lung fluid (Fig. 1). This serum also showed a weak reaction with MMTV p27. The specificity of these reactions was verified by absorption of the antiserum with detergent-disrupted MPMV. This markedly reduced the homologous reaction and completely eliminated the reactions with the p25 in the SPA retrovirus and MMTV p27 (Fig. 2). A second antiserum, raised against whole MMTV, detected the SPA retrovirus p25 and MPMV p27, but another serum to MMTV p27 showed only a homologous reaction. Similarly, an antiserum to MVV p27 (Narayan *et al.*, 1980) did not react with the SPA retrovirus p25. All of the above antisera gave strong homologous reactions by Western blotting.
Antibodies to detergent-disrupted bovine leukaemia virus and feline leukaemia virus, and to the major internal core proteins of Rauscher leukaemia virus and feline leukaemia virus did not react with the SPA retrovirus p25 (homologous antigens were not available for testing).

The goat antiserum to MPMV p27 has detected SPA p25 in 12 of 12 SPA fluids, 4 of 4 SPA tumours and 3 of 4 early tumours experimentally induced in young lambs. The polypeptide could not be detected in lungs from five animals without the tumour or in other micro-organisms that commonly infect the ovine respiratory tract (PI3 virus, MVV, M. ovipneumoniae, M. arginini) although all of these microbial antigens reacted with homologous sera.

SPA retrovirus was prepared from two further lung fluids by isopycnic centrifugation on 20 to 55% (w/w) sucrose gradients (Herring et al., 1983) and each 1 ml fraction was examined for the presence of the 25000 mol. wt. polypeptide. As shown in Fig. 3, the polypeptide was detected in three fractions with densities of 1.15 to 1.20 g/ml, although most appeared to be mainly in the fraction with a density of 1.17 g/ml. The peptide thus has an identical distribution in sucrose gradients to reverse transcriptase activity (Herring et al., 1983).

Antibodies to the p25 in the SPA retrovirus (SPAV) could not be demonstrated in sera from seven sheep with terminal SPA or in two lambs in which the tumour had been induced. IgG prepared from a pool of sera from terminal cases, and IgA prepared from a pool of SPA lung fluids also did not detect the 25000 mol. wt. polypeptide. Sera from six sheep infected with MVV all detected MVV p25 but did not react with the polypeptide in SPA retrovirus.

These results demonstrate an immunological cross-reaction between the major internal proteins of an unclassified, and as yet uncultivated, ovine retrovirus, and MMTV and MPMV, the prototype type B and D retroviruses. Other studies have emphasized the morphological and biophysical similarities between the SPA retrovirus and type B and D retroviruses (Herring et al., 1983; Sharp et al., 1983), a classification which is supported by the antigenic relationships demonstrated in this paper. However, sensitive radioimmunoassays have revealed interspecies antigenic determinants on the internal structural proteins of many mammalian retroviruses.
Barbacid et al. (1980) have demonstrated that MPMV p27 contains at least two distinct subsets of antigenic determinants that cross-react with the corresponding internal core proteins of B and C type retroviruses. We have failed to show a reaction between the SPA retrovirus p25 and the internal proteins of several type C retroviruses, but the Western blotting technique only detects epitopes which resist SDS and mercaptoethanol denaturation and further investigations will require quantitative assays with native proteins. Such a denaturation effect may be one explanation of the failure to detect an immune response in infected animals.

Previous serological and hybridization studies (de Boer, 1970; Perk & Yaniv, 1977) have failed to demonstrate any relationship between SPA and maedi-visna. These observations are supported by the failure of this study to demonstrate any immunological cross-reaction between SPAV and MVV or between MPMV and MVV. Recently, a group in Israel have reported the isolation from an SPA tumour of a retrovirus with C-type morphology that shows 30% homology by DNA–RNA hybridization with various lentiviruses (Perk et al., 1983). Weiss et al. (1976, 1977) have reported that members of the lentiviridae may show as little as 25% genome homology, yet all possess a group-specific antigen that resides in the major internal core protein (Dahlberg et al., 1981; Roberson et al., 1982). It will be important, therefore, to determine the antigenic relationship between the Israeli virus, the known lentiviruses and the retrovirus detectable in SPA tumour and lung fluids in Scotland.

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