Unique Virus-related DNA Sequences in Sheep Progressive Pneumonia Lung

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SUMMARY

Hybridization studies with [3H]-cDNA of progressive pneumonia, maedi and visna viruses demonstrate that lung DNA from sheep afflicted with progressive interstitial pneumonia possesses virus-related sequences not present in normal sheep lung DNA.

Progressive pneumonia virus (PPV), maedi and visna are ‘slow’ viruses of sheep that cause chronic interstitial pneumonia and/or meningoencephalitis (Marsh, 1923; Sigurdsson, Grimsson & Pallson, 1952; Sigurdsson, Pallson & Grimsson, 1957). These agents resemble RNA tumour viruses in their dimensions, sensitivity to physico-chemical agents, morphogenesis in cell culture, number and size of protein components, and possession of DNA polymerase activities associated with a major 60 to 70S single-stranded RNA component (Stone, Takemoto & Martin, 1971; Takemoto et al. 1971; Harter & Coward, 1974; Thormar, Lin & Trowbridge, 1974). Although infection of cell cultures with these viruses leads to polykaryocyte formation and cytolysis, they have been reported capable of transforming AL/N and Balb/c mouse cells (Takemoto & Stone, 1971).

Visna virus appears to replicate by way of a DNA intermediate. This was initially suggested by the observation that actinomycin D and BUdR inhibit virus multiplication (Thormar, 1965) and supported by the subsequent demonstration of virus-specific DNA sequences in infected cells that were, in part, covalently linked to host cell DNA (Haase & Varmus, 1973). We here report that lung from sheep afflicted with progressive interstitial pneumonia contains virus-related DNA sequences that are not detected in normal sheep lung.

The 6484 isolate of PPV cultured from experimentally-induced interstitial pneumonia sheep lung (Lopez, Eklund & Hadlow, 1971), M88 strain of maedi virus and K485 strain of visna virus (Harter et al. 1973) were used. Lung tissue was obtained from Montana sheep with either naturally-occurring or experimentally produced viral progressive pneumonia. Inoculating a susceptible cell monolayer with a 10% suspension of lung tissue from naturally or experimentally produced viral progressive pneumonia results in prominent c.p.e. and active virus production with a yield of 10^3 to 10^5 TCD_{50}/g tissue. Progressive pneumonia was induced in normal sheep by inoculation of a cell-free filtrate prepared from naturally-occurring diseased sheep lung. Lung from healthy unafflicted sheep was used as control.

PPV, maedi and visna viruses were harvested from infected sheep choroid plexus (SCP) cells, concentrated by ammonium sulphate precipitation and purified by two cycles of equilibrium sedimentation in potassium tartrate density gradients (Friedmann et al. 1974). Viral 60 to 70S RNA and DNA from sheep lung were extracted and isolated by procedures previously described (Goodman et al. 1973; Harter et al. 1973). To obtain DNA from virus-infected and control sheep choroid plexus cells, 100 confluent SCP plates were infected with virus; another 100 mock-infected plates served as controls. Cells were harvested 96 h after infection when prominent cytopathic changes were seen in virus-inoculated plates. Total cell DNA was isolated according to Britten, Pavich & Smith (1970) and sheared by sonication to a size approx. 6 to 8S.
Viral [ZH]-cDNA was synthesized from NP40-disrupted virions with tritiated TTP (50 Ci/mmol) in the presence of actinomycin D (100 mcg/ml) (Harter et al. 1973). The specificity of the viral probes was demonstrated by their ability to hybridize to homologous viral RNAs, but not to unrelated RNA. As assayed by hydroxylapatite chromatography, visna, maedi and PPV cDNAs annealed 80 % or more to homologous RNA and 3 % or less to 70S RNA from avian myeloblastosis virus.

DNA-DNA hybridization mixtures in a final vol. of 0.02 ml containing 0.8 M-phosphate buffer (pH 6.8), 20 mM-EDTA, 0.1 % SDS, 5 to 20 mg/ml cellular DNA, and 2.5 × 10⁻³ g/ml viral [3H]-cDNA (4 × 10⁵ ct/min/µg), were denatured by heating to 105 °C for 2 min and incubated at 68 °C. Hybrid formation was assayed by thermal elution hydroxylapatite chromatography at 10 °C intervals from 60 ° to 100 °C as previously described (Sweet et al. 1974). Single-stranded DNA was eluted at 60 °C with 0.12 M-phosphate buffer (pH 6.8) containing 0.4 % SDS; DNA eluting above 60 °C represents hybrid structures (Kohne & Britten, 1966). Recovery of acid-precipitable radioactivity was greater than 90 % in all experiments.

We first determined whether or not virus-specific DNA could be detected in infected tissue culture cells. For maximum sensitivity in these experiments, we prepared ‘recycled’ virus probes (Baxt & Spiegelman, 1972) to eliminate viral cDNA sequences homologous with normal sheep lung DNA as well as intrinsically double-stranded viral cDNA. PPV, maedi and visna [3H]-cDNAs were first hybridized to normal sheep lung DNA in vast cellular DNA excess to a Cₜ value of 2 × 10⁴ mol-s/1, and then passed through a hydroxylapatite column equilibrated at 60 °C in 0.12 M-phosphate buffer. Less than 10 % of each probe annealed to normal lung DNA. [3H]-cDNA eluting at 60 °C represents a ‘recycled’ virus-specific probe that is unable to hybridize to normal lung DNA. Visna virus [3H]-cDNA was annealed with DNA isolated from uninfected and visna-infected SCP cells in vast cellular DNA excess. The visna [3H]-cDNA hybridized to a maximum of 96 % with infected cell DNA, but not significantly with DNA from control cells (Fig. 2 a). The hybrid between visna cDNA and visna-infected cell DNA had a Tₛₑ of approx. 85 °C indicating the formation of well-matched duplexes (Fig. 1 b). Identical results were obtained when maedi virus [3H]-cDNA was substituted for visna [3H]-cDNA (Fig. 1 b). Similarly, PPV [3H]-cDNA hybridized to 76 % with DNA from PPV-infected SCP cells (Tₛₑ 87 °C) and did not anneal appreciably with control cell DNA (Fig. 1 b).

The ability to detect virus-specific sequences in infected cell cultures using these virus probes led us to determine whether similar methods could be used to demonstrate virus-related DNA sequences in lung tissue from sheep afflicted with progressive interstitial pneumonia. Recent studies have shown that the viral genomes of maedi and visna virus are virtually identical and possess considerable homology with that of PPV (manuscript in preparation). Therefore, cDNA prepared from any of these viruses can be used as a probe for virus-related sequences in diseased tissue.

Recycled [3H]-cDNA prepared from PPV, maedi and visna agents was hybridized to DNA extracted from diseased and normal sheep lung to a Cₜ value of 3 × 10⁴ mol-s/1, in large tissue DNA excess. PPV, maedi and visna virus [3H]-cDNA probes hybridized 39, 25 and 27 % respectively to lung DNA from experimentally infected sheep and 12, 24 and 17 % respectively to different samples of lung DNA from naturally diseased animals. A low level of hybridization (4 to 7 %) was observed with normal lung DNA; however, similar values were also obtained in annealing reactions between the three probes and unrelated normal rabbit, cow, chicken and human DNAs. Increasing the ratio of naturally infected lung DNA to visna [3H]-cDNA fivefold did not alter the extent of hybridization at a Cₜ of
either $3 \times 10^8$ or $1 \times 10^4$ mol-s/l, establishing that cell DNA was in excess in the above experiments.

Although maedi and visna [H]-cDNA probes can be used to detect virus-related sequences in diseased tissue, thermal analysis of the hybrids formed between PPV, maedi and visna [H]-cDNA probes and DNA from experimentally inoculated sheep discloses that the $T_{ma}$ of the hybrids using the visna and maedi probes is 75 °C as compared with 84 °C obtained using the homologous PPV probe. This finding is consistent with the extent of genomic relatedness observed between PPV, maedi and visna virus as measured by competition hybridization with $[\beta^35]I$-labelled viral RNA (manuscript in preparation). In contrast, hybridization of the three probes to DNA extracted from naturally diseased animals resulted in hybrids with a decreased thermal stability of 4 to 5 °C.

$C_{dt}$ analysis of the reaction between recycled PPV [H]-cDNA and lung DNA from experimentally inoculated sheep indicated that the virus-related sequences were in the non-repeated fraction of DNA from diseased tissue (Fig. 2); similar annealing kinetics were observed with DNA from naturally infected animals. From Fig. 2, we estimate that if virus-related sequences are present at all in normal sheep lung, they are at least 25-fold more dilute than those detected in progressive pneumonia lung.

Our inability to detect more than 40 % hybridization in the homologous reaction between PPV [H]-cDNA, and DNA from experimentally infected sheep may mean that only 40 % of the information expressed in our probes is actually present in the DNA from experimentally infected lung. Several possibilities could explain the greater extent of hybridization found using lung from experimentally inoculated sheep compared with lung from naturally infected animals. Different samples of lung tissue may contain varying proportions of infected cells. Alternatively, it is possible that under conditions of natural infection, diseased lung possesses qualitatively less of the information expressed in our probes than lung from animals inoculated experimentally. The reduced melting temperature of the hybrids observed with naturally infected, as compared with experimentally infected, tissues suggests that the virus-related sequences in these tissues are not identical.
Fig. 2. Kinetics of annealing of PPV [3H]-cDNA to lung DNA from sheep with experimentally induced progressive pneumonia (●—●) and from normal sheep (○——○). Reassociation kinetics of total spleen DNA from normal sheep (×—×) were analyzed by hydroxyapatite chromatography. Samples were collected at 60 °C and 100 °C and the extinction at 260 nm of each fraction recorded. C50 values are uncorrected for salt concentration and temperature.

These experiments indicate that virus-related sequences can be detected in lung tissue from progressive pneumonia sheep by hybridization to viral DNA probes. The histologic changes found in certain human interstitial pneumonias and neurological conditions mimic those of sheep progressive pneumonia and visna (Harter & Coward, 1974). One would anticipate that certain human diseases are caused by viral agents resembling those of the PPV maedi-visna complex. Use of hybridization techniques similar to those employed in this study may demonstrate that similar agents are involved in the aetiology of human chronic inflammatory diseases.

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Short communications

Departments of Microbiology, Neurology, Human Genetics and Development
Institute of Cancer Research
College of Physicians and Surgeons
Columbia University, New York, N.Y. 10032
Sloan-Kettering Institute for Cancer Research
410 East 68th Street
New York, N.Y., U.S.A.

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