disorder may therefore play a part in the pathogenesis of both IgA nephropathy and Buerger's disease.³ The lymphocytes of our first patient carried HLA B5 antigen, which has been associated with both Buerger's disease⁴ and, in some cases, familial IgA nephritis.⁵ The association of the two diseases in our patients may not have been coincidental.

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Subacute sclerosing panencephalitis: detection of measles virus sequences in RNA extracted from circulating lymphocytes

We have shown by an in situ hybridisation technique that measles virus ribonucleic acid (RNA) was present in the peripheral blood lymphocytes of a patient with subacute sclerosing panencephalitis,¹ as well as in the lymphoid tissue of the appendix of a patient before she developed the disease.² A few infected cells were also found in normal seropositive subjects. The large number of persistently infected mononuclear cells in the blood of patients with encephalitis prompted us to look for the presence of viral RNA with a dot blot hybridisation technique.

Patients, methods, and results

Four patients and four healthy subjects seropositive for measles virus were studied. Mononuclear cells from 5-10 ml of peripheral blood were separated on a Ficoll-Hypaque gradient¹ and stored at -80°C until required. Vero cells were infected with the Edmonston strain of measles virus for three days. Total RNA was extracted by the lithium chloride urea and phenol procedures³ and hybridised at 42°C in a solution containing a deoxyribonucleic acid (DNA) labelled with ³²P that was specific for measles virus nucleocapsid protein¹ (specific activity 5 × 10⁹ Bq/μg) in 50% formamide; 5 × standard saline citrate (SSC) (1 × standard saline citrate contains 0.15 mol/l sodium chloride and 0.015 mol/l sodium citrate); 0.1% sodium dodecyl sulphate (SDS); and 10% dextran sulphate. After hybridisation the nitrocellulose filter blots were washed four times for five minutes each in 2 × SSC and 0.1% SDS at room temperature; twice for five minutes each in 2 × SSC and 0.1% SDS at 42°C, once for eight minutes each in 0.1 × SSC and 0.1% SDS at 42°C; three times for five minutes each in 0.1 × SSC and 0.1% SDS at 50°C, and dried. Autoradiography was then carried out.

The figure shows 5 μg of total RNA then successive twofold dilutions spotted on to the filters. Only background was seen in non-infected Vero cells and in those from seropositive control subjects, whereas measles virus RNA sequences could be seen in the RNA that had been extracted from infected Vero cells and in three of the four preparations of lymphocytes from patients with encephalitis.

In cases 1 and 2 parallel in situ hybridisations indicated detection of viral RNA in over 50% of the positive cells in case 2; less than 0.1% were detected in two out of four of the control subjects.

Comment

The dot hybridisation assays that we undertook showed that RNA containing measles virus sequences could be extracted from the circulating lymphocytes of patients with subacute sclerosing panencephalitis. The

Autoradiograph of dot hybridisation for measles virus RNA in mononuclear cells. Rows 1-3: cells from patients with subacute sclerosing panencephalitis; rows 4-6: cells from seropositive control subjects; row 7: Vero cells infected with measles virus; row 8: non-infected Vero cells.

different autoradiographic signals that we saw among samples from the seropositive patients suggested that a variable amount of viral RNA was conserved in lymphocytes. Loss of the RNA during cell storage and sample handling, however, could also explain the variability in the intensity of the signal. This is suggested by the fact that in a subsequent RNA dot hybridisation analysis performed for case 2 we failed to detect any signal specific for the virus.

These findings indicate that in subacute sclerosing panencephalitis the presence of measles virus RNA in lymphocytes can be analysed by a biochemical method that confirms our previous studies of in situ hybridisation. Such a method has also permitted the detection of measles virus RNA in blood mononuclear cells during the acute and convalescent phases of measles,⁴ and in autoimmune disease associated with high antibody titres to measles virus.⁵ These data confirm our observations that viral genetic information persists in the immune system long after an attack of measles is over.¹ We are currently carrying out experiments to find out which species of molecule contain measles virus sequences.

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