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Human Cytomegalovirus DNA in the Temporal Cortex of a Schizophrenic Patient

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Summary. Using highly sensitive nucleic acids hybridization techniques, which allow the detection of 0.1–0.5 single copy gene equivalents per cell, DNA from the temporal cortex of seven definite schizophrenics, five persons with schizophrenia-like psychoses, three patients with Huntington's chorea and nine mentally normal individuals were probed with human cytomegalovirus (HCMV) DNA. A clear hybridization signal was obtained with DNA from the temporal lobe of a young schizophrenic patient, whereas DNA from the temporal cortex of controls did not hybridize to the HCMV probe. This finding is in agreement with the cytomegalovirus hypothesis of schizophrenia and hints at the possibility that viral infection of the temporal cortex may in some sporadic cases be a contributing factor to the development of schizophrenic psychoses. There is no indication, however, that infection of the central nervous system with HCMV is an aetiological factor in the great majority of schizophrenic disorders. Clearly further studies, preferably in situ hybridizations of whole brains, are needed to prove or disprove the cytomegalovirus hypothesis of schizophrenia.

Key words: Schizophrenia – Cytomegaloviruses – Virus diseases – Recombinant DNA

Introduction

It has been suspected for several years that viruses – particularly human cytomegalovirus (HCMV) – may play a role in the aetiology of schizophrenic psychoses (Torrey 1973; Torrey et al. 1983; Crow 1983). Serum, blood, cerebrospinal fluid and postmortem brain tissues have been investigated for traces of HCMV infection.

Gotlieb-Stematzky et al. (1981) described elevated levels of HCMV antibodies in the serum of schizophrenic patients; other groups were unable to confirm this finding (Torrey 1973; Lacke et al. 1974; Schindler et al. 1986). Some studies indicated that cerebrospinal fluids contained raised antibody levels against HCMV (Albrecht et al. 1980; Torrey et al. 1982; Kaufmann et al. 1983); others could not support these findings (Gotlieb-Stematsky et al. 1981; Rimon 1985; Shrikhande et al. 1985).

Furthermore, the search for HCMV in brain autopsy materials from schizophrenic patients has remained unsuccessful (Aulakh et al. 1981; Stevens et al. 1984; Taylor et al. 1985; Taylor and Crow 1986).

In search of HCMV DNA in brain tissue, the aim of our study was to use highly sensitive Southern blot hybridization techniques allowing the detection of 0.1–0.5 single copy gene equivalents per cell by employing a series of viral DNA fragments without virus-human DNA homologies cloned in plasmid and cosmid vectors.

Materials and Methods

Brain tissue from seven definite schizophrenics, five persons with schizophrenia-like psychoses, three patients with Huntington's chorea and nine mentally normal individuals (Table 1) were obtained under standard conditions soon after death, collected in the Cambridge Brain Bank Laboratory and kept frozen at −70°C until the isolation of the DNA. About 250 mg of mixed temporal cortex tissue, mainly from Brodmann Area 38 temporal pole, was processed and DNA was prepared as described by Saldanha et al. (1984). Previous experiments had shown that five regions of the HCMV genome contain DNA sequences that hybridize with intermediate repetitive DNA of

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Table 1. Schizophrenia, Huntington's chorea and control post-mortem temporal cortex samples used in hybridization studies

	Age (years)	Sex	Post-mortem delay (months)	Cause of death
Schizophrenia				
1	63	M	26	Coronary atherosclerosis Acute pyelitis
2	23	M	64	Asphyxia – hanging
3	27	F	28	Drowning
4	53	M	34	Congestive heart failure
5	62	F	35	Bronchopneumonia Cancer of oesophagus
6	56	F	23	Cardiac arrest Myocardial infarction
7	65	F	78	Myocardial fibrosis
8	62	F	26	Probable Gram negative Bacteraemia
9	80	M	54	Bronchopneumonia
10	77	F	43	Pelvic tumour Pulmonary embolism
11	56	M	NK	Myocardial infarction
12	23	F	48	Overdose
Huntington's chorea				
1	46	M	36	Bronchial asthma Coronary atherosclerosis
2	25	M	NK	Bronchopneumonia
3	42	F	23	Bronchopneumonia, sarcoidosis
Controls				
1	65	M	19	Lobar pneumonia
2	42	M	46	Acute heart failure
3	38	M	13	Coronary thrombosis
4	81	M	63	Not known
5	75	M	46	Not known
6	60	M	51	Acute ventricular dysrhythmia
7	60	F	36	Inhalation of faeculent vomit
8	59	M	50	Acute left ventricular Failure
9	60	M	71	Acute heart failure Coronary atherosclerosis

NK = Not known

normal human cells (Rüger et al. 1984). A pool of cosmid and plasmid clones (pCM3-5018, pGHS2-4, pRR3-7) without virus-cell homologies (Rüger and Fleckenstein 1985) was used as radioactive labelled probes.

^{32}P-labelled HCMV probes (>600 Ci/mmol) (New England Nuclear) were prepared by the nick-repair method (Rigby et al. 1977). Cellular DNA was digested with *Eco*RI and the restriction fragments separated on 0.8% agarose gels. To achieve an optimal transfer, the cellular DNA was fragmented by exposure to UV light and treatment with 0.3 N HCL. After alkali denaturation, the cellular DNA fragments were transferred to nitrocellulose filters in 20 × SSC (SSC = 0.15 *M* NaCl, 0.015 *M* sodium citrate) by Southern blotting (Southern 1975). The filters were dried at 80°C for 4 h. They were preincubated twice with 10 × SSC and 5 × SSC, respectively. Overnight incubation of the filters with nick-repair labelled HCMV DNA and washing were carried out as described previously (Rüger et al. 1984). The hybridization temperatures were chosen 18.0°–21.5°C below average Tm of HCMV DNA (Ebeling et al. 1983; Rüger and Fleckenstein 1985). These conditions allow detection of 0.1–0.5 single copy gene equivalents per cell. Experiments were performed blindly with regard to clinical diagnoses.

Results

Figure 2 shows the results of a Southern blot experiment with DNA from six brains of schizophrenic patients and seven samples of the various other groups. The DNA from the temporal cortex of controls did not hybridize to the HCMV probe. However, a clear hybridization signal was detected with DNA from the temporal cortex of a young man with the full picture of schizophrenia; he committed suicide at the age of 23 years (hanging). It is notable that this patient was first hospitalized at the age of 20 years with an acute psychotic disturbance which was difficult to control with neuroleptic treatment. No history of herpes infection, glandular fever, or other severe viral diseases was known for this patient, nor was a family history of psychiatric disorders recorded in the case notes, although such a possibility cannot be excluded. The intensity of the autoradiogram suggested approximately one genome copy per average diploid brain cell. The restriction pattern was distinct from DNA of the HCMV laboratory strain Ad169, and appreciable genetic complexity of hybridizing viral DNA fragments was lower than the molecular weight of DNA probes, indicating substantial sequence divergence. The brain DNA sample did not hybridize with the pure prokaryotic vector. None of the other brain DNA specimens appreciably hybridized with cloned HCMV DNA.

Discussion

Human cytomegalovirus DNA was found in the temporal lobe of a young schizophrenic patient. This finding could be the result of (1) chance, (2) activation of latent virus infection by immunosuppression, or (3) a causal relationship between viral infection and schizophrenic psychosis.

Chance could play a role in the detection of HMCV DNA in the brains of patients and controls. In line with this interpretation would be the fact that Taylor and Crow (1986) found HCMV sequences in

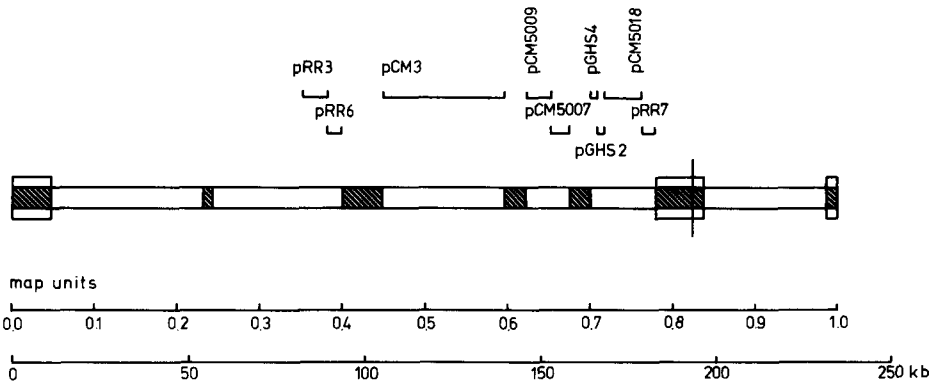

Fig. 1. Schematic drawing of the linear double stranded genome of human cytomegalovirus (HCMV). The virus cell homologous regions are shown as *hatched boxes*. *Horizontal brackets* indicate the plasmid and cosmid clones used as hybridization probes. They contained the *Hin*dIII fragments R (pCM5009) and T (pCM5007) and the *Eco*RI fragments P (pRR3), V (pRR6), A (pCM3), D (pCM3), d (pGHS4), e (pGHS2), J (pCM5019) and a (pRR7) of HCMV strain Adl69

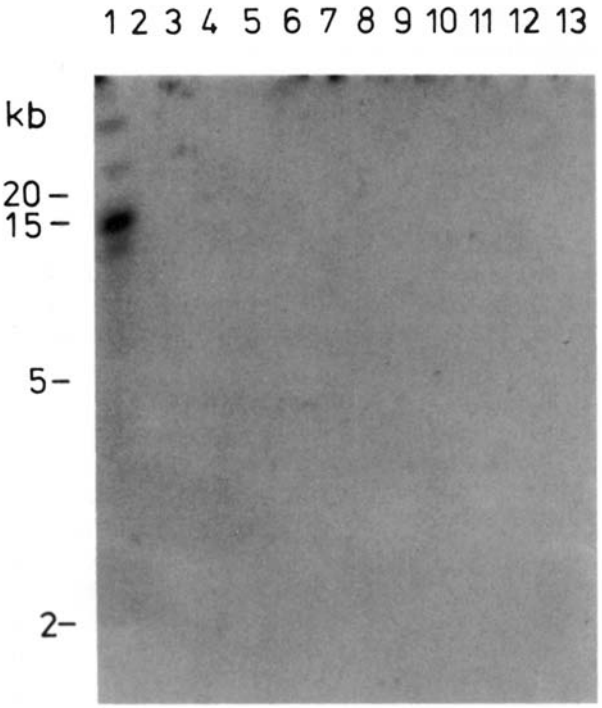

Fig. 2. Southern blot hybridization of *Eco*RI cleaved DNA from the temporal cortex of several patients with cloned radioactive ^{32}P-labelled HCMV DNA fragments. *Lanes 1–6:* DNA from schizophrenic patients; *lane 1* shows a clear hybridization signal; *lanes 7–9:* DNA from normal individuals; *lanes 10–13:* DNA from chorea Huntington patients

the brain of one control case. However, their finding might be also explained by immunosuppression, since their control case had received immunosuppressive therapy as treatment for rheumatoid arthritis.

Minor immunosuppression with increasing age or neuroleptic therapies could contribute to virus reactivation and should be considered as a possible explanation. Considering the young age of the patient and the fact that most schizophrenic patients were treated with neuroleptics, these factors were unlikely to contribute in the present case. Unfortunately, reactivation by other forms of immunosuppression or coinciding primary infection cannot be ruled out.

The suicide of the patient might indicate that he was suffering from an acute psychotic episode which could have been caused by an activation of HCMV in the temporal cortex.

Neurotropic viruses have been reported occasionally in the temporal lobe of patients with endogenous psychoses (Tayler et al. 1985; Gannicliffe et al. 1985). Adenoviruses have been found in an elderly schizophrenic (Lord et al. 1975) and DNA of herpes simplex viruses in a depressive woman (Gannicliffe et al. 1985). Furthermore, herpes simplex DNA sequences were detected by molecular hybridization in temporal lobe tissues form from five to six patients with severe epilepsy (Gannicliffe et al. 1985). This finding might be relevant for schizophrenia, since left temporal lobe dysfunction has been long suspected in schizophrenia (Flor-Henry 1969) and psychoses in temporal lobe epilepsy are sometimes clinically indistinguishable from schizophrenic psychoses (Trimble and Perez 1982).

Positive findings can easily be missed in analyses of small amounts of brain specimens because of the focal nature of CNS infections from herpes simplex and other viruses (Fraser et al. 1981; Haase et al. 1984). This might explain previous negative results of searches for viruses in schizophrenic brain tissue. In situ hybridization of larger parts of the brain may overcome these shortcomings.

In summary, the demonstration of HCMV DNA in the temporal cortex of a young schizophrenic male hints at the possibility that viral infection of the tem-

poral lobe may in some sporadic cases be a contributing factor to the development of schizophrenic disorders. There is no indication, however, that infection of the central nervous system with HCMV is an aetiological factor in the great majority of schizophrenic psychoses. Clearly more studies, preferably in situ hybridizations of whole brains, are needed to prove or disprove the cytomegalovirus hypothesis of schizophrenia.

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