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Search for Cytomegalovirus in Schizophrenic Brain Tissue (41144)

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Abstract. A human cytomegalovirus (CMV) DNA probe was developed and hybridized to DNA extracted from brain tissue of 6 patients with schizophrenia and 6 control individuals. We failed to detect any CMV-related genetic information in the DNA of these 12 individuals. These results indicate that either CMV is not involved in schizophrenia or that the genetic viral information, if present, is below the level of sensitivity of the present assay.

Speculation that viruses may play an etiological role in some cases of schizophrenia have become increasingly common in recent years (1). Earlier reports of viral particles in the spinal fluid (2–4) have been revived by the finding of viral-like activity in the spinal fluid of over one-third of schizophrenic patients (5, 6).

Recently, Albrecht *et al.* (7) reported an elevated cerebrospinal fluid to serum ratio of cytomegalovirus (CMV) antibody in 19 of 60 schizophrenics. They interpreted their data as most compatabile with an infection which occurred many years prior to the onset of the disease, and found no evidence of currently ongoing viral activity. If there had been a CMV infection in earlier years and the viral genetic information is present in latent form, however, it might be possible to detect the DNA sequences of the CMV utilizing nucleic acid hybridization techniques with the DNA derived from brain tissue.

The choice of where to look in the brain for evidence of viral disease in schizophrenic patients centers on the limbic system and basal ganglia with their immediate extensions (8, 9). The hippocampus is central to this system, with direct connections to the thalamus, hypothalamus, brainstem, frontal, and temporal cortex as well as to several other parts of the limbic system and the stria terminals.

In addition it has been reported that glutamic acid decarboxylase and choline acetyl transferase are reduced in the hippocampus of schizophrenic patients compared with controls (10). Finally, it is known that CMV has an affinity for the limbic area (11).

For these reasons, postmortem brain material from the hippocampus of six schizophrenic patients and six controls was examined for CMV-related DNA sequences using nucleic acid hybridization technique.

Materials and Methods. *Purification of DNA from human tissues.* Tissues from the hippocampus of the brain of six schizophrenic and six normal patients were obtained at autopsy and kept frozen at −80° until the isolation of DNA. Autopsies were performed within 12 hr after death. About 1 g of hippocampus tissue was processed and DNA was isolated by hydroxyapatite batch elution (12, 13) and further purified and sheared as outlined previously (13).

Purification and ^3H labeling of viral DNA. The Towne strain of CMV was grown and purified as described previously (14). The virus DNA was isolated by hydroxyapatite batch elution as outlined above (12, 13). Purified viral DNA was labeled *in vitro* using all four labeled deoxynucleoside triphosphates by the "nick-translation" method (15–17). The crosslinked and foldback segments of the probe (15–20%) were removed by hydroxyapatite chroma-

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tography (17). The specific activity of this probe was 1.5×10^7 cpm/μg.

Nucleic acid hybridization. The hybridization mixture contained 3 mg/ml of unlabeled brain DNA and 10,000 rpm of ^{3}H-DNA probe in a final volume of 75 μl of buffer. Buffer reagents and hybridization techniques used were essentially the same as described previously (13). The mixture was allowed to anneal to an Ecot value of 10,000 (18). The level of hybridization was determined by hydroxyapatite chromatoggraphy as outlined previously (13).

Results. *Characterization of the CMV probe.* The CMV [^{3}H]DNA probe hybridized 90.6% to its homologous DNA extracted from CMV-infected cells. No hybridization was observed to unrelated chicken liver DNA. A nonspecific background sticking of 11.4% of the probe to the hydroxyapatite column was observed and was subtracted from the hybridization values. This background appeared to be due to the "foldback" which was not completely removed during the first recycling.

Absence of CMV related DNA sequenced in schizophrenic brain DNA. There were no DNA sequences present complementary to human CMV in the DNA from any of the six schizophrenic or six normal patients. In all instances the hybridization level was equal to the background level which was observed with unrelated negative control chick liver DNA. The probe should have been capable of detecting one viral genome in 10 cells (14).

Discussion. The failure to detect viral DNA sequences of CMV in the hippocampus of six schizophrenic patients may be explained in one of several ways. It may indicate that there is no CMV present in schizophrenics' brains and that the finding of an elevated CSF/serum ratio of CMV antibodies is an epiphenomenon associated with altered immunological reactivity in these patients. Or it may reflect the fact that six patients may not be a sufficient number to test this hypothesis.

Alternatively, the failure to find evidence of previous CMV infection may be because the wrong part of the brain was examined. We know that viruses may be very specific in their selection of cell types (e.g., varicella-zoster) and location (e.g., poliovirus) in the brain. It is theoretically possible for a virus like CMV to affect a highly specific and circumscribed area such as the septal nuclei or the nucleus accumbuns in the limbic area and to not affect other areas. Since the limbic system is a complex circuit, infections in one specific location might affect function in all other parts of the circuit. If this is the case, an area-by-area survey of the limbic system may eventually have to be made.

Finally, it is possible that CMV is present, but our technology is not yet sensitive enough to detect it. Deterioration of brain tissue after death and limited sensitivity of the DNA probe may both contribute to negative results. In an area such as this, it is important to revive negative results and retest them from time to time as the technology improves.

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