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Cytomegalovirus and Schizophrenia
A Test of a Viral Hypothesis

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Summary: Cerebrospinal fluid (CSF) from 20 chronically hospitalised male schizophrenics and from 12 patients admitted with acute schizophrenia were examined for antibodies against cytomegalovirus. A sensitive and specific enzyme-immunoassay was used to detect IgG or IgM classes of antibodies in the CSF of the schizophrenic patients and of ten orthopaedic patients, who served as controls. No significant amounts of IgM antibody were found in the CSF of either group. A significant titre of IgG was found in only one of the 32 schizophrenics, an acute patient, but in four of the orthopaedic patients. The results do not support an association of cytomegalovirus infection with schizophrenia; if such an association occurs, it must be unusual.

In 1973 Torrey summarised the evidence which could support the hypothesis that a virus, possibly a slow and latent one, plays a role in the aetiology of schizophrenia (Torrey, 1973). A study of 47 acute patients with psychotic, mostly schizophrenic, illnesses admitted to a district general hospital unit, in most cases with a history of previous schizophrenic episodes, was then published (Tyrell *et al*, 1979; Crow *et al*, 1979). Eighteen of the 47 had a virus-like agent (VLA) in their cerebrospinal fluid (CSF) which had a cytopathic effect on cultured cells, and this sub-group had a significantly higher mean IgA level. As they also detected VLA (virus-like agents) in eight with multiple sclerosis or other neurological illnesses, it was postulated that the VLA may be widely distributed in the community, but only becomes pathogenic in a genetically predisposed subpopulation.

Subsequently, Albrecht *et al* (1980) reported a series of 60 chronic schizophrenics from one hospital, 68% of whom had an increased CSF-to-serum ratio for antibodies to cytomegalovirus (CMV), including one patient who had been drug-free for a month or more. In that report, an abnormal titre was taken as more than two standard deviations above the mean of 26 controls, drawn from employees and drug addiction rehabilitation patients from the same institution.

Clearly, these reports could only be regarded as suggestive. Tyrell *et al* reported that VLA was not specific for schizophrenia, and there was an absence of normal controls. Torrey's finding of CMV antibodies in a chronically hospitalised sample of patients but not employees of the same hospital could have been due to an epidemic in the hospital some years before the control group were exposed to the same environment.

Method

Selection of subjects

The CSFs of two samples of patients and of one control sample were examined. These included 20 chronically hospitalised male schizophrenics from St Fintan's Hospital, Ireland, with a mean age of 46.9 years and average stay in hospital of 13.8 years, and 12 patients (males and females) tested following an acute admission to Charing Cross Hospital, London, as well as ten orthopaedic patients with negative findings at the time of CSF examination of myelography.

The symptomatic and diagnostic profile, based on CATEGO criteria and derived from a Present State Examination (PSE) interview, on the Syndrome Check List (SCL) (Wing *et al*, 1974) is presented in Table I. It shows that almost all patients from both groups would satisfy criteria for chronic or acute schizophrenia according to ICD-9. Ten of the chronic patients had been off medication for at least three weeks, and ten patients were on continuous medication; the medication status of the acute sample was mixed.

Technique

Two ml of CSF was collected by lumbar puncture from each patient in the chronic sample, immediately stored at −197° centigrade in liquid nitrogen, and subsequently transported by air to the Charing Cross Laboratory. CSF from the acute sample was refrigerated up to one hour, and then stored at −70°C. PSE and SCL ratings were processed with the CATEGO program to arrive at a diagnostic syndrome and the most approximate ICD-9 diagnosis.

The CSFs were diluted 1:5 and examined for CMV specific IgG and IgM by a commercially available enzyme immuno-assay (EIA) technique 'Enzygnost' (Behring), in which the antibody sought in the specimen is bound to antigen on the test plate. Antigen and negative control antigen are obtained from human diploid fibroblasts, respectively infected and not infected with CMV. The antigen-antibody complex reacts with the enzyme conju

TABLE I

Cytomegalovirus antibody in the CSF of schizophrenics and controls

	ICD-9	*CATEGO Diagnostic categories*	*n*	*CMV-IgG Titres*			*CMV-IGM Titres*
				*<5**	*10*	*≥40*	*<5**
Chronically hospitalised schizophrenics	295.3	SC	12	12			12
	295.6	RS	1	1			1
	295.2	O	5	5			5
	296.0	M	2	2			2
Acute schizophrenics	295.3	S	8	7	1		8
	295.2	O	1	2			1
	297.9	P	3	3			3
Orthopaedic controls	–	–	10	6	3	1	10

*Titres <5 show no evidence of antibody.

N.B. The ICD numbers refer to diagnostic labels and the CATEGO categories refer to Diagnostic Symptom groups as follows: 295 is Schizophrenic Psychosis—.3 paranoid type,—.6 residual,–.2 catatonic type; 296.0 is Mania; 297.9 is Paranoid states; S is Schizophrenia, O is other psychosis including catatonica; RS is residual schizophrenia, P is paranoid psychosis other than First-Rank (S), M is Mania and mixed psychoses.

gates, which are made by coupling highly avid rabbit antisera with alkaline phosphatase. The IgG/Alkaline Phosphatase reagent is γ-chain specific and the IgM/ Alkaline Phosphatase reagent is μ-chain specific. Unbound reactants are removed by washing processes. On the addition of substrate solution, a yellowish-green colour develops.

Results

The results are presented in Table I. No significant amounts of IgM antibody were found in the CSF of either the schizophrenic or orthopaedic controls. A significant IgG antibody titre over 5 was found in only one of the 32 schizophrenic patients, an acute patient, but in four of the orthopaedic patients, one of whom had a titre of over 40 (Table II). Thus, no association between CMV virus and schizophrenia has been found in these samples.

TABLE II

CSF cytomegalovirus antibodies in psychiatric patients and controls

	CMV-IgM ELISA	*CMV-IgG ELISA*		
	<5	*<5*	*10*	*>40*
Schizophrenics	20	20	–	–
Chronic (Ireland)	20	20	–	–
Acute (London)	12	11	1	–
Orthopaedic controls (London)	10	6	3	1*

*This table shows that only one patient, a non-schizophrenic control, is likely to have had a cytomegalovirus infection in the past.

Discussion

A consideration of antibody changes in schizophrenia should take into account the evidence describing immunoglobulin levels in schizophrenia, because antibodies are immunoglobulins. Several investigators have reported abnormal immune function in schizophrenic patients (Friedman *et al*, 1967; Heath & Krupp, 1967). Pulkinnen (1977) determined serum IgA, IgG and IgM immunoglobulin concentrations in 76 schizophrenics on admission and attempted to correlate his results with the psychopathology, background, and prognosis of the patients. The highest IgM concentrations were found in withdrawn schizophrenics, but any connection between prognosis and immunoglobulin concentration was at least partly explicable on the grounds of age at the onset of the disease. Sugarman *et al* (1982) conducted a study of serum antibody levels in alcoholic, depressive, and schizophrenic patients. They compared IgG, IgA, IgM, IgE and IgD levels in the three groups of patients with those in healthy controls, finding no significant difference in total immunoglobulin levels between patients and controls. Torrey *et al* (1978) found an increase in CSF IgG concentration in 35% of chronic schizophrenics, although Albrecht *et al* (1980) failed to reproduce this result. Delisi *et al* (1981) quantified IgG, IgA and IgM in CSF and plasma from schizophrenic patients and controls, using an immuno-fluorescent technique. A generalised reduction in immunoglobulin levels was observed in the schizophrenic patients compared with controls—an opposite result to Torrey's. Whilst in general these studies raise the possibility of abnormal immune functioning in schizophrenia, the results are inconsistent with each other and no conclusions can be drawn. The effect of immunoglobulin levels of endemic infectious agents, together with those of dietary deficiency and lowered levels of activity seen in

institutionalised patients, needs to be examined critically, since many studies in this field have used chronic institutionalised patients. Many of the findings of the previous studies may be related to institutional risks of group infection rather than to schizophrenia.

Certain clinical and epidemiological features of schizophrenia suggest that it may be due to a virus (Torrey, 1973) and evidence of slow or latent virus infection in schizophrenic patients has been sought. Torrey *et al* (1978) examined the sera and CSF of 66 patients with functional psychoses for immunoglobulins and antibodies against measles, herpes simplex, rubella, and CMV. The patient group was comprised of 14 manic depressives, nine with schizo-affective disorders, 13 first-admission schizophrenics, and 30 schizophrenics with multiple admissions; 80 patients with various neurological diseases served as controls. In this study, the CSFs of six out of 17 multiple-admission schizophrenic patients had definite elevations of IgG or measles antibody and differed significantly from controls. Over one-third of the CSFs in all groups were positive for herpes simplex antibodies, but there were no significant differences between the groups. Eight per cent of patients had detectable rubella antibody in CSF, but the authors claim that in none of them was the titre, determined by haemagglutination-inhibition tests on CSF, sufficiently high to be of significance. No CMV antibodies were detected in any of the CSFs of the schizophrenic patients; CMV antibody was detected in only one CSF—that from a patient suffering from multiple sclerosis.

Albrecht *et al* (1980) examined the serum and CSF in 60 schizophrenic patients and 26 controls for antibodies against cytomegalovirus, vaccinia virus, herpes simplex, and influenza A virus. A CSF/serum antibody ratio more than two standard deviations above the mean of the controls was taken as an indication of local antibody production in the central nervous system. In that study, 68% of the patients had an increased CSF/serum ratio for CMV antibody, 14% for vaccinia antibody, 4% for HSV antibody, and 15% for influenza A virus antibody, suggesting a disproportionate association of CMV virus with schizophrenia. All virus antibodies in that particular study were measured by a virus-plaque neutralisation test. However, the sensitivity of virus neutralisation tests for antibodies varies greatly, depending upon the strain of virus and the type of tissue culture cells used; it is not possible, or even desirable to equate the results obtained by this technique with those obtained using other test systems. Since these studies were done, more sensitive and specific techniques for the detection of viral antigens and antibodies have been developed, including radio-immune assay (RIA) and enzyme-immune-assay (EIA/ELISA). These tests are considerably more sensitive and specific for detecting antibody than second-generation tests such as complement fixation, immunofluorescence, and haemagglutination inhibition (Booth, 1983). Torrey *et al* (1982) used EIA techniqes to detect the presence of antibody to CMV in the CSF of 178 patients with schizophrenia, 17 patients with bipolar disorders, and 11 other psychiatric patients; controls were found from 79 neurological patients and 41 normal control subjects. Testing all CSFs at a dilution of 1:10, the CSFs of 20 of the schizophrenic patients and three of the patients with bipolar disorders were found to have significant increases in IgM antibody to CMV.

In the study reported here, a newly developed enzyme-linked immunoabsorbent assay (ELISA) test was used to investigate the presence or absence of IgG and IgM class antibodies against CMV in the CSF of acute and chronic schizophrenics. ELISA techniques have been critically evaluated and reported to be highly sensitive—comparable with tests for Coxsackie A and B viruses (Hermann *et al*, 1979; Katze & Crowell, 1980). Using this technique, a significant IgG antibody was found in only one schizophrenic patient; IgM class antibodies were not found in any of the CSF specimens examined. This result is in accordance with that obtained by Gotlieb-Stematsky *et al* (1981) who using an indirect immunofluorescent technique, failed to detect antibodies against CMV in any of the CSFs of 18 schizophrenic patients examined. Similarly, Aulakh *et al* (1981) failed to implicate CMV in schizophrenia; they developed a human CMV DNA probe which was hybridised to DNA extracted from brain tissue obtained at autopsy from six patients with schizophrenia and six control individuals. The sensitivity of their technique was such that the method should have been capable of detecting one viral genome in ten cells, but, nevertheless, they failed to detect any CMV-related genetic material in any of the 12 individuals examined. They concluded that either CMV is not involved in schizophrenia or that the viral genetic information, if present, is below the sensitivity of the assay, and hypothesise that the finding of elevated CSF/serum ratios of CMV antibodies is an epiphenomenon, associated with altered immunological reactivity in these patients. Certainly, our results suggest that abnormal titres of antibodies against CMV, or IgM antibodies against CMV, detectable by ELISA, are not found commonly in

the CSF of acute or chronic schizophrenic patients and are unlikely to be of aetiological significance. These results give no support to an association of CMV infection with schizophrenia; if such an association occurs, it must be unusual. The failure to replicate earlier reports associating CMV and schizophrenia could be due to the more specific techniques used in this study, but a more likely explanation is that previous authors selected patients from an institution which had an endemic infection in the past, unrelated to the aetiology of schizophrenia.

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