

HERPES SIMPLEX VIRUS ANTIBODIES IN THE CEREBROSPINAL FLUID OF SCHIZOPHRENIC PATIENTS

L. BÁRTOVÁ, *J. RAJČÁNI, J. POGÁDY

Research Psychiatric Laboratory, Regional Psychiatry Hospital Pezinok,
and *Institute of Virology, Slovak Academy of Sciences,
817 03 Bratislava, Czechoslovakia

Received November 17, 1986

Summary. — Antibodies to herpes simplex virus type 1 (HSV-1) were tested in the cerebrospinal fluid (CSF) of 262 schizophrenic patients by virus neutralization test (VNT) and enzyme-linked immunosorbent assay (ELISA). While VNT in the presence of complement revealed antibodies to HSV-1 in 18.3% of samples, ELISA was positive in 61.2% of cases; both tests were positive in 42 samples (16%).

Key words: *herpes simplex virus; antibodies; cerebrospinal fluid; schizophrenia*

Several investigators described elevated serum antibody levels to herpes simplex virus type 1 in schizophrenic patients as compared to nonpsychotic controls (Halonen *et al.*, 1974; Gotlieb-Stematski *et al.*, 1981; Cappel and and Sprecher, 1983; Libíková, 1983). In addition, Libíková (1983) reported antibodies to HSV-1 in 42.3% of CSF samples as determined by neutralization test in the presence of complement but not in its absence. We retested a part of these CSF samples along with further ones and found a little lower positive rate (28%) of complement requiring neutralizing antibodies (CRNA) in 81 out of 288 CSF samples (Rajčáni *et al.*, 1987). In contrast, only 3 out of 231 CSF samples tested for measles antibodies showed a HI titre ≥ 8 , although 60% of the patients had HI antibodies in the serum in an average titre of 20 ± 3 . Later on, all available CSF samples were retested by ELISA and, if possible, also by VNT. The results are presented in this communication.

The CSF samples were coming from simple, paranoid, catatonic and hebephrenic schizophrenia (211 samples). The rest 51 samples were kindly submitted by Dr. O. A. Vasilyeva, All Union Mental Health Research Centre, Res. Inst. of Clinical Psychiatry, Tomsk, U.S.S.R.

VNT was made in the presence of complement (12–13 units per 0.1 ml fresh guinea pig serum; 126 units per ml) with 100–200 PFU of HSV-1 strain Kupka inoculated into Vero cells.

ELISA was performed on domestic microplates P (KOH-I-NOOR, Dalečín) coated overnight (phosphate buffer, pH 7.2) with a crude extract (10 $\mu\text{g/ml}$) of HSV-1 infected (strain KOS) human embryo cells (LEP). Control wells were coated with the extract of noninfected cells. To prepare the extract, LEP cells were harvested when showing CPE at 24–48 hr p.i. After 3 cycles of freezing and thawing, the cells were suspended in phosphate buffered saline (PBS) containing 1% BSA and thoroughly sonicated. The supernatant after low speed centrifugation had a titre of 6×10^6 PFU/ml when plated on Vero cells. After inactivation with formalin, the

* When requests for reprints should be addressed to.

Table 1. Presence of HSV-1 antibodies in the CSF of schizophrenic patients as detected by VNT and ELISA

Origin of CSF	Test	CSF dilution									Average titre log	P/T
		0	2	4	8	16	32	64	128	256		
Pezinok	VNT	179	20	9	3						1.47 ± 0.72*	32/211 ^a
Tomsk		35	8	6	2						1.5 ± 0.67*	16/ 51 ^a
Total		214 ^b	28	15	5							48/262
Pezinok	ELISA	47		30		16	45	34	28	11	5.88 ± 1.2**	134/211
Tomsk		21		3		5	5	10	7		5.64 ± 1.1**	27/ 51
Total		68		33		21	50	44	35	11		161/262

* difference insignificant

** difference insignificant

^a difference significant ($p < 0.05$)^b inhibition of CPE formation was negative after inoculation of 100 PFU of HSV-1 previously incubated with undiluted CSF and complement

P/T = positive out total

antigen was adjusted to a protein concentration 1 mg/ml and stored in 100 µl aliquots at -70°C . The CSF samples were diluted in PBS containing 1% BSA and tested at dilutions shown in Table 1. The conjugate (Swine anti-Human Ig/Px; SEVAC, Prague) was diluted 1:400. The addition of the substrate followed according to standard procedures (Hsiung, 1982). The absorbance reading was made in Dynatech minireader. The endpoints were calculated from the OD₄₉₂ ratios of the virus versus control antigen (≥ 2.1). The antigen dilution was checked at different coating concentrations ranging from 200 to 5 µg/ml against the ascitic fluid containing anti-gB (strain Kupka) monoclonal antibody in dilutions up to 65 536; a non-reactive ascitic fluid was used as negative control.

The results are shown in Table 1. VNT in the presence of complement was positive at dilutions 2, 4, and 8 in 15.1% of samples from Pezinok and 31.3% of samples from Tomsk (this difference was significant, $p \geq 0.05$). The samples which neutralized the virus in undiluted state only were considered negative. In contrast to these low CRNA titres, the ELISA test showed titres up to 256 (reactions in the range of dilutions 2–8 were regarded for negative). There was no significant difference in the positive rate between the Pezinok (63.2%) and Tomsk samples (52.9%; $p < 0.05$). Table 2 shows that both tests were positive in 16% of the samples tested, whereas ELISA was positive in additional 45.2% of cases. Both tests were negative in 36.5% of schizophrenic patients.

The interpretation of these findings must be done with caution. While the results of Gotlieb-Stematski *et al.* (1981), Cappel and Sprecher (1983) supported the possible association between HSV and psychotic depression, neither Pokorný *et al.* (1973) nor Rimón *et al.* (1978, 1983) found any association. The sensitivity of the test may be essential: RIA and ELISA are more suitable than complement fixation: neutralization in the presence of com-

Table 2. Comparison of the results of VNT and ELISA in the CSF of schizophrenic patients

Results	No. of positive CSF samples (%)	Correlation probability
VNT and ELISA positive	42 (16%)	0.874* 0.26**
VNT positive, ELISA negative	6 (2.3%)	0.123 0.059
VNT negative, ELISA positive	119 (45.2%)	0.553 0.738
VNT and ELISA negative	95 (36.5%)	0.446 0.941
VNT positive	48 (18.3%)	
ELISA positive	161 (61.2%)	
Total examined	262 (100%)	

* probability of the given ELISA result at given VNT result

** probability of the given VNT result at given ELISA result

plement or enhanced neutralization test may be positive in the CSF showing no antibody in the simple NT. The choice of serum or CSF samples may be also of importance. Rajčáni *et al.* (1987) confirmed the presence of elevated serum antibody titres to HSV-1 by retesting a great number of sera from domestic schizophrenic patients as compared to controls; this was not the case with the sera from Tomsk patients. On the other hand, as reported here, antibodies to HSV-1 were present in the CSF of both domestic and Tomsk patients. Nonetheless, such antibodies were found in senile dementia, alcohol abusers, in sclerosis multiplex and Guillan-Barré syndrome (Libíková *et al.*, 1981, 1983).

Out of the most frequent viruses affecting the CNS, measles is no candidate for an association with schizophrenia, at least not according to the results of Libíková (1983) and ours (Rajčáni *et al.*, 1987). Human cytomegalovirus (CMV) was suggested to be involved in the aetiology of schizophrenia by several investigators. Torrey *et al.* (1982, 1983) described IgM class antibody to CMV in 11% of CSF samples from schizophrenic patients; in 32% of their samples they found antibodies by NT. Albrecht *et al.* (1980) showed increased CSF to serum antibody ratio against CMV in 68% of schizophrenic patients indicating possible local antibody production. However, Rimón *et al.* (1986) found no evidence for the role of CMV in the development of mental disease. Although 17% of the patients exhibited a CSF to serum ratio > 2, no virus-specific IgM class antibody to CMV was detected.

In comparison to CMV, HSV seems to be more interesting candidate because it sustains latent in neurons. The search for CMV DNA sequences in the human brain samples (ne. hippocampi) by the hybridization technique at autopsy was negative; no complementary DNA (Aulakh *et al.*, 1981) was found either in the brains of schizophrenics or of mentally healthy persons. On the other hand, DNA sequences complementary to the HSV genome were found at autopsy in 6 out of 11 extracts from normal brains with multiple sclerosis (Fraser *et al.*, 1981) as well as in the brains of epileptic patients

(Gannacliffe *et al.*, 1985). In accordance with this, our preliminary studies revealed sequences complementary to ^{32}P -HSV DNA by spot blot hybridization in the extracts of nc. amygdalae removed at curative stereotactic surgery in 40% of the specimens examined (Kudelová *et al.*, in preparation).

Cereful collecting of the data obtained by standardized techniques might contribute to define the possible "virologic markers" associated with schizophrenia and mental deterioration.

Acknowledgements. The authors thank dr. Š. Breier for statistics calculations and Mrs. B. Pavlíková for technical assistance.

References

- Albrecht, P., Torrey, E. F., Boone, E., and Hicks, J. T. (1980): Raised cytomegalovirus antibody level in cerebrospinal fluid of schizophrenic patients. *Lancet* II, 769–772.
- Aulakh, G. S., Kleinman, J. E., Aulakh, H. S., Albrecht, P., Torrey, E. F., and Wyatt, R. J. (1981): Search for cytomegalovirus in schizophrenic brain tissue. *Proc. Soc. exp. Biol. Med.* **167**, 172–174.
- Cappel, R., and Sprecher, S. (1983): Are herpes viruses responsible for neuropsychiatric diseases? In P. V. Morozov (Ed.): Research on the Viral Hypothesis of Mental Disorders. *Adv. biol. Psychiat.* **12**, 168–173.
- Fraser, N. W., Lawrence, W. C., Wroblewska, Z., Gilden, D. H. and Koprowski, H. (1981): Herpes simplex type 1 DNA in human brain tissue. *Proc. natn. Acad. Sci. U.S.A.* **78**, 6416–6465.
- Gannacliffe, A., Saldanha, J. A., Itzhaki, R. F., and Sutton, R. M. P. (1985): Herpes simplex viral DNA in temporal lobe epilepsy. *Lancet* **1**, 214–215.
- Gotlieb-Stematski, T., Zonis, J., Arlazoroff, A., Mozes, T., Sigal, M., and Szekeley, A. (1981): Antibodies to Epstein-Barr virus, herpes simplex type 1, cytomegalovirus and measles virus in psychiatric patients. *Arch. Virol.* **67**, 333–339.
- Halonen, P. E., Rimón, R., Arohonka, K., and Jäntti, V. (1974): Antibody levels to herpes simplex type 1, measles and rubella antibodies in psychiatric patients. *Br. J. Psychiatry* **125**, 461–465.
- Huang, G. D. (1982): *Diagnostic Virology*, 3rd Edition, Yale University Press, New Haven, London.
- Libíková, H. (1983): Schizophrenia and viruses: principles of etiologic studies. In P. V. Morozov (Ed.): Research in the Viral Hypothesis of Mental Disorders. *Adv. biol. Psychiat.* **12**, 20–51.
- Libíková, H., Pogády, J., and Mucha, V. (1981): Enveloped viruses and viral immunity in schizophrenia and senile dementia, pp. 69–72. In C. Perris, G. Struve, B. Jansson (Eds.): *Biological Psychiatry*, Elsevier, North Holland, Biomed. Press.
- Libíková, H., Pogády, J., and Š. Breier (1983): Viral hypothesis of schizophrenia. Results of virological research (in Slovak). *Čs. Psychiatrie* **79**, 361–375.
- Pokorný, A., Rawls, W., Adam, E., and Mefferd, R. (1973): Depression, psychopathy and herpes virus type 1 antibodies. Lack of relationship. *Arch. gen. Psychiat.* **29**, 820–822.
- Rajčáni, J., Libíková, H., Smereková, J., Kudelová, M., Mucha, V., Pogády, J., Breier, Š., and Škodáček, J. (1987): Investigations on possible role of viruses affecting the CNS in the etiology of schizophrenia and related mental disorders. In E. Kurstak (Ed.): *Viruses, Immunity and Mental Diseases*, Plenum Press, New York (in press).
- Rimón, R., and Halonen, P. (1978): Serum and cerebrospinal fluid antibody levels to herpes simplex type 1, measles and rubella virus in patients with schizophrenia. *Ann. clin. Res.* **10**, 291–293.
- Rimón, R. (1983): Viral etiology of schizophrenia? *Ann. clin. Res.* **15**, 1–3.
- Rimón, R., Ahokas, A., and Palo, J. (1986): Serum and cerebrospinal fluid antibodies to cytomegalovirus in schizophrenia. *Acta psychiat. Scand.* **73**, 642–644.
- Torrey, E., Yolken, R., and Winfrey, C. (1982): Cytomegalovirus antibody in CSF of schizophrenic patients detected by enzyme immunoassay. *Science* **216**, 892–894.
- Torrey, F., Yolken, R. H., and Albrecht, P. (1983): Cytomegalovirus as a possible etiological agent in schizophrenia. In P. V. Morozov (Ed.): Research on the Viral Hypothesis of Mental Disorders. *Adv. biol. Psychiat.* **12**, 150–160.