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Intracranial synthesis of specific IgG antibody in cerebrospinal fluid of neurocysticercosis patients

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Abstract: To determine the source of *Cysticercus*-specific IgG antibody in cerebrospinal fluid(CSF), paired samples of serum and CSF were collected from confirmed neurocysticercosis, other neurologic diseases and normal control. The antibody levels in serum and CSF were measured by enzyme-linked immunosorbent assay (ELISA). With the measurement of total protein, albumin and IgG concentration in serum and CSF, the contribution of IgG in CSF were calculated in transudation, exudation and intracranial synthesis using the formula of Tourtellotte and Ma (1978). Mean concentrations of total protein, albumin, IgG and proportional IgG levels in CSF by transudation, exudation and intracranial synthesis were elevated in neurocysticercosis. But only the intracranial synthesis of IgG showed a statistically significant correlation with the specific IgG antibody levels in CSF. In CSF from lateral ventricle in the 4th ventricular neurocysticercosis, the protein concentrations were normal and the specific antibody levels were negative. However, in consecutively secured lumbar CSF from the same patients, the former were increased and the latter were positive. These results indicated that, in neurocysticercosis, the specific IgG antibody in CSF was a local product of intracranial synthesis.

Key words: *Cysticercus*, neurocysticercosis, IgG antibody, CSF, ELISA, intracranial synthesis of IgG

INTRODUCTION

A demonstration of specific antibody in neurocysticercosis is of value in supporting the diagnosis not only in patients with definite findings of brain computerized tomography(CT) but also in patients with ambiguous ones (Chang *et al.*, 1988). Serologic tests such as complement fixation (CF) (Nieto, 1956), indirect hemagglutination (Biagi *et al.*, 1961), ELISA (Diwan *et al.*, 1982; Espinoza *et al.*, 1982; Mohammad *et al.*, 1984; Cho *et al.*, 1986) and radioimmunoassay (RIA) (Miller *et al.*, 1984) have been applied in detecting specific antibody in CSF as well as in serum. Of these tests, ELISA is now widely used because of its rather high sensitivity and versatility.

In the diagnosis of neurocysticercosis, serologic test employing CSF seems quite reasonable and more pertinent than use of serum. As Flisser and Larralde (1986) mentioned, however, there had been a number of uncertainty that should be clarified prior to the use of CSF. First, it was not clear whether sensitivity of the diagnosis was really increased by use of CSF. Neurocysticercosis patients with definite findings of brain CT may show positive antibody levels only in serum but not in CSF or *vice versa*. In this respect, Cho *et al.* (1986) reported that 71 confirmed neurocysticercosis patients in Korea were divided into 4 combinations of specific antibody responses when paired samples of serum and CSF were tested by ELISA; 50

patients (70.4%) of specific IgG antibody positive in serum (serum (+)) and specific IgG antibody positive in CSF (CSF (+)), 9 cases (12.6%) of specific IgG antibody negative in serum (serum (−)) and CSF (+), 5 cases (7.0%) of serum (+) and specific IgG antibody negative in CSF (CSF (−)), and 7 cases (9.9%) of serum (−) and CSF (−). Clearly tests using both samples add sensitivity of about 10%.

Secondly, it was also not clear whether specific antibody in serum of pure muscular cysticercosis could perfuse into CSF (Flisser and Larralde, 1986). In this connection, Cho *et al.* (1986) also reported two patients of cysticercosis with lesions in muscles confirmed by X-ray but with normal brain CT, in whom the antibody was positive only in serum while negative in CSF. In tests of either ELISA or RIA, serum was diluted to 1 : 100 or more than CSF (Miller *et al.*, 1984; Mohammad *et al.*, 1984; Cho *et al.*, 1986). This implied that even a slight contamination of CSF with positive serum could reverse the negative results of CSF. Data from the above two patients, therefore, suggested that antibody in serum could not be transferred to CSF in such patients.

Although the above examples are correct, it is still necessary to establish that specific antibody in CSF of neurocysticercosis patients was produced *de novo* in central nervous system (CNS). In diagnosis of meningitides, antibody tests in CSF is now regarded less meaningful than antigen detection because antibody is permeated through damaged blood-brain barrier (BBB) in CNS lesion (Leibowitz and Kennedy, 1972; Griffin, 1981). Related with this topic, Spina-Franca *et al.* (1976) reported that, in neurocysticercosis, the elevated IgG concentrations in CSF were related with titers of CF tests in CSF. Recently, Miller *et al.* (1985) described that 4 of 6 patients of neurocysticercosis patients produced IgG *de novo* in CNS. From these reports it seems likely that specific IgG antibody in CSF were synthesized inside of BBB. However, direct evidence that CNS synthesized IgG is the specific IgG antibody is still in paucity except for the oligoclonal antibody differences between serum and CSF (Miller *et al.*, 1985).

Furthermore, the quantitative relations of exudation and intracranial synthesis of specific antibody seems important in understanding the source of the antibody in CSF as well as in percussing the pathophysiologic aspect of BBB damage in neurocysticercosis. This study was undertaken to provide evidence that specific IgG antibody in CSF of neurocysticercosis patients was actually synthesized in CNS.

MATERIALS AND METHODS

1. Subjected patients

(1) Neurocysticercosis

A total of 82 confirmed neurocysticercosis patients were included in this study. The diagnosis was made in individual patients on the bases of (1) brain CT findings in all (a single or multiple low densities with or without calcifications), (2) brain surgery in 21 cases and (3) skin nodule biopsy in 11 cases. All cases manifested clinically neurologic symptoms such as seizures, headache and/or neurologic deficits. Serologic tests for *Cysticercus*-specific IgG antibody in serum and CSF were conducted in all patients. And the results were a strong back-up for the final diagnosis in 75 out of 82 subjected patients (Table 1).

(2) Patients with other neurologic diseases and normal control

A total of 45 patients with neurologic or neurotic symptoms were included as the controls (Tables 1 and 2). Five of them were with tuberculous meningitis confirmed by smear and acid-fast staining of CSF. Six were diagnosed as tuberculoma or other infectious granuloma of CNS by surgery or by medical observation. Eight patients were viral meningitis or encephalitis. Six were patients of benign/malignant tumors of CNS, *i.e.*. arachnoid cyst, ependymoma, astrocytoma, angiolipoma diagnosed by surgery and a metastatic bronchogenic carcinoma

diagnosed by brain CT and biopsy of the main lesion. Six patients were medically diagnosed as CNS vascular diseases by angiography, brain CT and bloody CSF, and these included subarachnoid hemorrhage, brain contusion/hematoma and aneurysm. Nine patients were miscellaneous diseases such as demyelinating diseases (2 patients), porphyria (1), normal pressure hydrocephalus of unknown etiology (1), Meniere's disease (1), Behcet disease (1), Shy-Drager syndrome (1), Parkinson's disease (1) and diphenylhydantoin toxicity (1). The remaining 5 patients were diagnosed belatedly as neurotic diseases and a cervical spondylosis. They were regarded as normal control in this study.

2. Serum and CSF

From each patient, paired samples of serum and CSF were simultaneously secured. CSF from all but 2 patients of neurocysticercosis were collected by lumbar puncture. Ventricular CSF were secured from lateral ventricle in 3 patients of neurocysticercosis in addition to lumbar CSF. Two patients were examined only with ventricular CSF. The CNS lesions, in the 4th ventricle of those 5 cases, were confirmed by ventriculography.

CSF were collected before brain surgery except in 2 neurocysticercosis patients because serum/CSF were screened for specific antibody. CSF from 2 patients of neurocysticercosis were, however, collected after a week of the surgery. In some neurocysticercosis patients, samples were collected after praziquantel treatment.

3. Specific IgG antibody test by ELISA

The specific IgG antibody levels in serum and CSF were examined by ELISA as described by Cho *et al.* (1986). Briefly, wells in polystyrene micro-titer plate (Costar, USA) were coated with 200μl of antigen (cystic fluid of *Taenia solium* metacestodes, Choi *et al.*, 1986; Larralde *et al.*, 1986) in protein concentration of 2.5μl/ml (diluted in carbonate buffer, pH 9.6) overnight at 4°C. After washing, serum/CSF were incubated for 2 hours at 36°C. Serum was diluted in 1:100 with phosphate buffered saline (PBS) (pH 7.4)/0.5% Tween 20, while CSF was undiluted. Peroxidase-conjugated anti-human IgG goat IgG (heavy- and light-chain specific, Cappel, USA) was used in dilution of 1:5,000 in PBS/Tween 20. Substrate composed of 99ml of distilled water, 1ml of 1% *o*-phenylene diamine and 50μl of 6% H_2O_2. Stopping the reaction with 20μl of 8% H_2SO_4, color was read for absorbance(abs.) with Gilford spectrophotometer. Cut-off value of positive reaction in both serum and CSF was abs. 0.18 as described by Cho *et al.*(1986). To minimize variation between tests, a positive reference serum of which average abs. was 1.0 was run together; the read abs. for each sample was divided by abs. from the positive reference serum.

4. Measurement of total protein, albumin and IgG in serum and CSF

Serum snd CSF were stored at −40°C after ELISA. All the samples were thawed simultaneously and concentrations of total protein, albumin and IgG were measured.

Total protein concentration in serum was measured by Biuret method. Albumin in serum and total protein in CSF were measured by Bradford method (1976). Albumin concentration in CSF was calculated from total protein in CSF after electrophoresis and densitometry of each CSF. IgG concentration in serum was measured by turbidoimmunoassay supplied by Boerhinger-Manheim (Eckmann *et al.*, 1970) while IgG in CSF was by radial immunodiffusion (Fahey and McKelvey, 1965) using Nissiu N-immunoring test kit (Japan).

5. Calculation of IgG in CSF contributed by transudation, exudation and intracranial synthesis

By applying the formula of Tourtellotte and Ma (1978), the amount of non-specific IgG in CSF was calculated for respective contribution by transudation, exudation and intracranial synthesis. The formula was:

$$IgG_{syn} = \left[IgG_{csf} - \left(\frac{IgG_s}{369}\right) - \left(Alb_{csf} - \frac{Alb_s}{230}\right) \times \left(\frac{IgG_s}{Alb_s}\right) \times 0.45\right] \times 5$$

In actual calculation, exudation, $(Alb_{csf} - Alb_s/230) \times IgG_s/Alb_s \times 0.45$ was turned out as minus value in 18 of 127 tested cases. The minus values were regarded as 0 in calculating intracranial synthesis of IgG. The above formula computed intracranial synthesis of IgG *in a day*. In this study, we did not multiply with factor 5 to compare the relative contributions in mg/dl unit.

6. Relationship between specific IgG antibody levels in CSF and *de novo* intracranial synthesis of IgG

The relation between specific IgG antibody levels in CSF of neurocysticercosis patients (abs.) and IgG in CSF contributed by transudation, exudation and intracranial synthesis (mg/dl) were statistically analysed by multiple linear regression and simple linear regressions.

RESULTS

1. Specific IgG antibody test in serum and CSF

As shown in Table 1, neurocysticercosis patients were not necessarily positive for specific IgG antibody. The sensitivity of the antibody test using serum was 76.3%(61/80) and that using CSF was 78.8%(63/80). When the results of paired samples were combined, the sensitivity was 91.3%(73/80). Out of 45 patients of other neurologic diseases and normal control, 5(11.1 %) showed false positive antibody test. Out of 5 false positive cases, 4 were positive only in serum and 1 only in CSF.

Mean and standard deviation(SD) of abs. were shown in Table 2 by disease category. In neurocysticercosis patients, abs. in serum ranged 0.03～1.77 while those in CSF did 0.00～2.73. Abs. in false positive cases of other diseases were near cut-off value.

2. Protein concentrations in serum and CSF

Serum concentrations of total protein and albumin in 82 neurocysticercosis patients were not different from those of 45 other neurologic diseases and normal control (Table 2). But serum concentrations of IgG were higher in 21 of 82 neurocysticercosis patients (above 1.9g/dl). In other neurologic diseases and normal control, IgG in serum were within normal limits.

Total protein concentrations in CSF of 80 neurocysticercosis patients ranged 15～350mg/dl. Only 11 patients showed the values within

Table 1. Results of *Cysticercus*-specific IgG antibody tests in serum and CSF by ELISA in subjected patients

Disease category	No. of cases	No. of patients with#			
		serum (+)		serum (−)	
		CSF(+)	CSF(−)	CSF(+)	CSF(−)
Neurocysticercosis	80*	51	10	12	7
Tuberculous meningitis	5	0	0	1	4
Viral meningitis/encephalitis	8	0	0	0	8
Infectious granuloma	6	0	1	0	5
Intracranial tumor	6	0	0	0	6
Vascular diseases	6	0	0	0	6
Miscellaneous	9	0	2	0	7
Normal control	5	0	1	0	4

* Two cases of neurocysticercosis were excluded because only ventricular CSF were examined.
** Cut-off value of positive and negative tests was abs. 0.18 in both serum and CSF.
\# serum(+): specific IgG antibody positive in serum
serum(−): specific IgG antibody negative in serum
CSF (+): specific IgG antibody positive in CSF
CSF (−): specific IgG antibody negative in CSF

Table 2. Mean±SD of protein measurements, calculated contribution of IgG in CSF and abs. of specific IgG antibody in serum and CSF of the subjected patients

Disease category	No. of cases	Contents in serum* of			Contents in CSF** of			Contribution of IgG in CSF by			Abs. of antibody in	
		total protein	albumin	IgG	total protein	albumin	IgG	transudation	exudation++	intracr-an. synthesis++	serum	CSF
Neurocysticercosis	82/80+	7.0 ±0.8	4.4 ±0.5	1.66 ±0.38	67.4 ±48.1	42.3 ±34.3	14.0 ±16.2	4.52 ±1.05	2.94 ±3.14	6.88 ±12.74	0.46 ±0.39	0.68 ±0.63
Tuberculous meningitis	5	7.1 ±0.8	4.1 ±0.7	1.28 ±0.36	122.0 ±31.0	80.0 ±26.7	30.4 ±23.4	3.48 ±0.97	8.38 ±4.36	18.53 ±20.78	0.07 ±0.03	0.09 ±0.11
Viral meningitis/encephalitis	8	7.1 ±0.9	3.9 ±0.3	1.37 ±0.28	61.0 ±35.1	44.7 ±29.5	7.6 ±6.8	3.72 ±0.76	4.17 ±4.33	1.30 ±2.30	0.07 ±0.05	0.02 ±0.02
Infectious granuloma	6	8.0 ±0.6	4.7 ±0.1	1.36 ±0.31	44.2 ±13.0	28.0 ±11.3	3.3 ±2.2	3.68 ±0.83	1.18 ±1.39	0.17 ±0.37	0.06 ±0.07	0.03 ±0.04
Intracranial tumor	6	7.1 ±0.5	4.1 ±0.5	1.21 ±0.23	36.6 ±15.2	21.4 ±12.1	1.9 ±1.6	3.27 ±0.63	0.80 ±1.11	0	0.06 ±0.05	0.01 ±0.01
Vascular diseases	6	7.8 ±0.5	4.3 ±0.4	1.32 ±0.31	65.3 ±6.7	47.9 ±11.0	5.8 ±2.4	3.59 ±0.84	3.89 ±1.81	0	0.06 ±0.04	0.03 ±0.02
Miscellaneous	9	6.9 ±0.7	3.9 ±0.4	1.39 ±0.35	46.2 ±14.2	33.4 ±13.8	2.8 ±1.1	3.77 ±0.94	2.24 ±0.18	0	0.08 ±0.09	0.03 ±0.04
Normal control	5	7.5 ±0.9	4.4 ±0.3	1.13 ±0.12	32.2 ±5.7	23.1 ±5.6	1.7 ±0.7	3.01 ±0.27	0.46 ±0.41	0	0.09 ±0.08	0.02 ±0.02

* g/dl ** mg/dl + 82 cases were examined by serum while 80 cases by lumbar CSF.
++ Minus values of exudation and intracranial synthesis were regarded as zero(0).

normal ranges (up to 40mg/dl). All of 5 tuberculous meningitis, 4 of 6 infectious granuloma, 5 of 8 viral meningitis/encephalitis, 2 of 6 intracranial tumors, all of 6 CNS vascular diseases, 4 of 9 miscellaneous diseases and none of normal control showed elevated concentration of total protein in CSF.

Albumin in CSF was elevated in neurocysticercosis, tuberculous meningitis, viral meningitis/encephalitis and vascular diseases. The highest mean was observed in tuberculous meningitis (Table 2).

IgG concentrations in CSF were elevated over normal limit (6.0mg/dl) in 51 of 80 neurocysticercosis, all of tuberculous meningitis, 3 of 8 viral meningitis/encephalitis, and 1 of 6 infectious granuloma patients. Mean IgG concentration in CSF was above normal in patients of neurocysticercosis, tuberculous meningitis and viral meningitis. But they were in normal values in all patients of vascular diseases, intracranial tumors, miscellaneous diseases and normal control (Table 2).

3. Relative contribution of IgG in CSF by transudation, exudation and intracranial synthesis

(1) Contribution by transudation

Because it was calculated by simple division of serum concentration of IgG by 369, mean value of IgG contributed by transudation was higher in neurocysticercosis than in other diseases.

(2) Contribution by exudation

Mean contribution by exudation was high in tuberculous meningitis (8.38mg/dl), viral meningitis (4.17mg/dl), vascular diseases (3.89mg/dl) and neurocysticercosis (2.94mg/dl) respectively.

(3) Contribution by intracranial synthesis

It was calculated by subtracting IgG concentrations contributed by transudation and exudation from total IgG in CSF. In patients with intracranial tumor, vascular diseases, miscellaneous diseases, and normal control, intracranial synthesis of IgG were not observed. In patients of tuberculous meningitis (5/5), viral meningitis (3/8), infectious granuloma (2/6) and

Table 3. Relationship between total protein concentration and specific IgG antibody levels in CSF

Total protein in CSF (mg/dl)	No. of patients of neurocysticercosis				total	other neurologic diseases
	serum (+)		serum (−)			
	CSF (+)	CSF (−)	CSF (+)	CSF (−)		
~ 20	1	0	0	0	1	2
21~ 40	5	4	0	2	11	17
41~ 60	21	6	5	4	36	12
61~ 80	12	0	3	1	16	7
81~100	7	0	2	0	9	2
101~120	1	0	2	0	3	2
121~	4*	0	0	0	4	3**
Total	51	10	12	7	80	45

* Two neurocysticercosis patients who were examined after neurosurgery were included here
** One tuberculous meningitis patient with false positive specific IgG antibody was included

Table 4. Relationship between albumin concentration and specific IgG antibody levels in CSF

Albumin in CSF (mg/dl)	No. of patients of neurocysticercosis				total	other neurologic diseases
	serum (+)		serum (−)			
	CSF (+)	CSF (−)	CSF (+)	CSF (−)		
~ 10	4	0	0	0	4	1
11~ 30	14	8	3	5	30	19
31~ 50	18	2	4	2	26	15
51~ 70	11	0	3	0	14	4
71~ 90	0	0	2	0	2	4**
91~110	1	0	0	0	1	1
111~	3*	0	0	0	3	1
Total	51	10	12	7	80	45

*, ** same as in Table 3

neurocysticercosis (46/80, 57.5%), IgG was produced in CNS.

4. Relation between protein contents and specific IgG antibody in CSF

As shown in Tables 3, 4 and 5, 80 neurocysticercosis patients were divided into 4 groups according to the positivity for specific IgG antibody tests in serum and CSF. And distribution of cases was observed at different levels of total protein, albumin and IgG in CSF.

In neurocysticercosis, total protein concentration in CSF was higher in CSF(+) cases regardless of either serum(+) or serum(−). Mean and SD of total protein was 74.2±56.7 mg/dl in serum(+)/CSF(+) cases, 42.6±10.4 mg/dl in serum(+)/CSF(−) cases, 72.7±22.8 mg/dl in serum(−)/CSF(+) cases and 49.7±12.3mg/dl in serum(−)/CSF(−) cases (Table 3). Higher concentrations of total protein over 60mg/dl were found in CSF(+) cases.

As shown in Table 4, albumin concentrations in CSF were high in neurocysticercosis patients with CSF(+). Mean and SD were 46.1±40.5 mg/dl in serum(+)/CSF(+) cases, 24.4±10.7 mg/dl in serum(+)/CSF(−) cases, 47.8±20.7 mg/dl in serum(−)/CSF(+) cases and 29.6±8.4mg/dl in serum(−)/CSF(−) cases.

More marked differences in IgG concentrati-

Table 5. Relationship between IgG concentration and specific IgG antibody levels in CSF

IgG in CSF (mg/dl)	No. of patients of neurocysticercosis				total	other neurologic diseases
	serum (+)		serum (−)			
	CSF (+)	CSF (−)	CSF (+)	CSF (−)		
～ 2.0	3	3	0	2	8	14
2.1～ 4.0	3	3	0	0	6	19
4.1～ 6.0	6	4	2	3	15	1
6.1～ 8.0	8	0	5	1	14	2
8.1～10.0	5	0	0	0	5	4
10.1～12.0	2	0	3	0	5	0
12.1～14.0	2	0	0	0	2	1
14.1～	22*	0	2	1	25	4**
Total	51	10	12	7	80	45

*, ** same as in Table 3

ons in CSF were revealed in neurocysticercosis by positivity of specific IgG antibody in CSF. In 51 serum(+)/CSF(+) cases, mean and SD of IgG in CSF were 18.0±18.3mg/dl; in 10 serum(+)/CSF(−) cases, they were 3.4±2.4 mg/dl; in 12 serum(−)/CSF(+) cases, 8.7± 4.5mg/dl and in 7 serum(−)/CSF(−) cases, 6.7±6.1mg/dl.

5. Findings with ventricular CSF

In this study, ventricular CSF were obtained from 5 neurocysticercosis patients with the 4th ventricular lesions. Of these, 3 patients were examined additionally their lumbar CSF while 2 were not. Protein profiles in ventricular CSF were compared with paired lumbar CSF. Specific IgG antibody levels were also compared.

Table 6. Comparison of protein profiles and specific IgG antibody levels in 5 ventricular CSF and 3 paired lumbar CSF in nuerocysticercosis patients with the 4th ventricle lesions

Protein/antibody	Mean ± SD in ventricular CSF	Mean ± SD in lumbar CSF
Total protein (mg/dl)	18.8±5.1	77.7±6.4
Albumin (mg/dl)	10.9±2.3	53.5±6.5
IgG (mg/dl)	0	12.0±4.3
Calculated contribution of IgG by (mg/dl)		
transudation	4.22±0.79	4.35±0.60
exudation	0*	5.03±0.64
intracranial synthesis	0	3.25±5.59
Specific IgG antibody (abs.)	0.10±0.04	1.40±0.55

* −1.20±0.17 in actual calculation

As shown in Table 6, protein concentrations in ventricular CSF were within normal ranges while those in lumbar CSF were elevated. Similarly, specific IgG antibody levels (abs.) were in negative ranges in 5 ventricular CSF without exception. But in 3 lumbar CSF high abs. were observed.

IgG concentrations contributed by exudation and intracranial synthesis were not calculated in ventricular CSF whereas they were 5.03± 0.64 and 3.25±5.39mg/dl in lumbar CSF respectively.

6. Relationship between specific IgG antibody and sources of IgG in CSF

In order to characterize IgG in CSF from different sources, abs. of specific IgG antibody in CSF and IgG concentrations from 3 sources were analysed by multiple linear regression. When abs. of the antibody in CSF is "Y", IgG in CSF from transudation (in mg/dl) is "A", that from exudation is "B" and that from intracranial synthesis is "C", the relations between them were:

$$Y = -0.072A - 0.0068B + 0.019C + 0.8915$$

Multiple correlation coefficient (R) of the

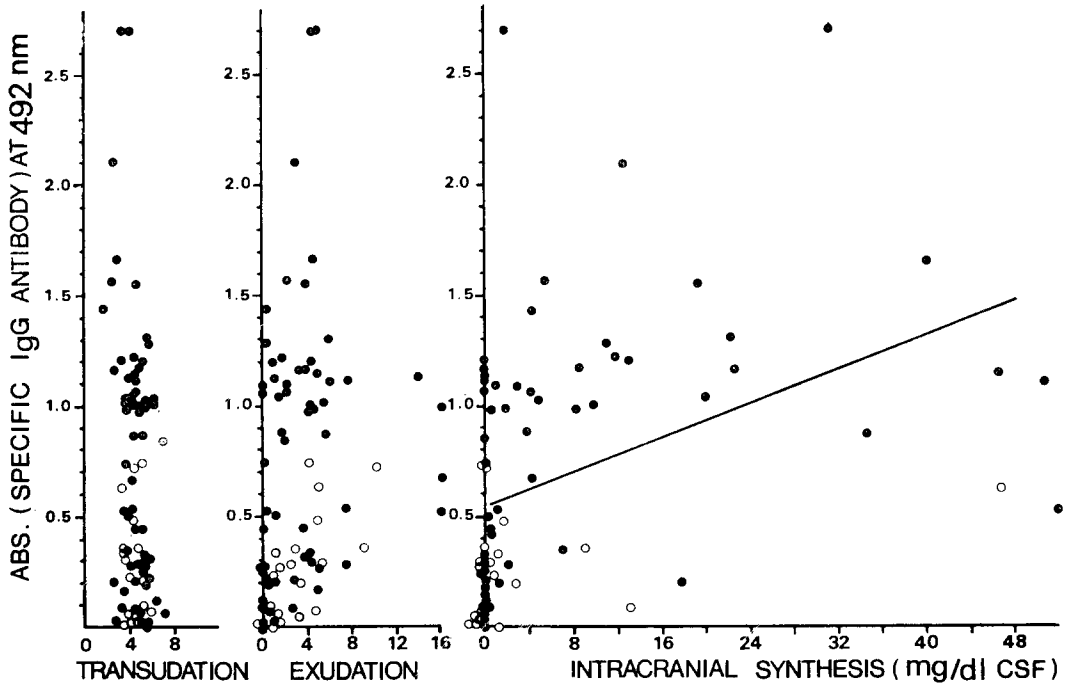

Fig. 1. Relationship between abs. of specific IgG anticody in CSF and claculated contribution of CSF IgG by transudation, exudation and intracranial synthesis. Closed circle (•): a neurocysticercosis patients of serum (+), open circle (○): a patient of serum (−).

equation was 0.4114. And the relation between them had statistical significance ($F=5.61$, $P<0.01$). Among them, regression coefficient between abs. and transudation was −0.072 ($P=0.274$) and that between abs. and exudation was −0.0068 ($P=0.677$). Unlike two sources, IgG contributed by intracranial synthesis showed statistically significant regression coefficient of 0.019 in neurocysticercosis patients ($P<0.01$).

Again correlations between abs. of specific IgG antibody and IgG concentration in CSF from 3 sources were separately considered (Fig. 1). Correlation coefficient (r) between abs. and IgG from transudation was −0.213 ($P>0.1$) and that between abs. and exudation was 0.149 ($P>0.1$). And that between abs. and intracranial synthesis was 0.393 ($P<0.01$). The regression equation between abs. of specific IgG antibody in CSF (Y) and intracranial synthesis of IgG (X) was:

$$Y=0.0194X+0.5438 \quad (F=14.225,\ P<0.01)$$

7. Associated exudation of IgG in neurocysticercosis

From the above statistical analyses, it became evident that there is a definite correlation between IgG synthesis in CNS and specific IgG antibody levels in CSF in neurocysticercosis patients. Therefore, it strongly indicates that specific IgG antibody in CSF was produced locally in CNS rather than transferred from serum. It did not necessarily mean that exudation of non-specific IgG into CSF did not occur in CNS lesions of neurocysticercosis. To confirm the frequency of association of exudation with *de novo* synthesis. the number of patients with significant exudation was analysed as in Table 7.

The criteria of significant exudation were: (1) ratio of CSF albumin$\times 10^3$/serum albumin was 9.0 or above (Link and Tibbling, 1977) and/or (2) calculated contribution of IgG in

Table 7. Association of exudation with intracranial synthesis of IgG in CSF in neurocysticercosis patients

Category	No. of cases	No. of cases whose origin of IgG in CSF is			
		intracranial synthesis	exudation*	synthesis & exudat.*	neither synth. nor exudation
Serum(+)/CSF(+)	51	14	6	22	9
Serum(+)/CSF(−)	10	1	1	0	8
Serum(−)/CSF(+)	12	3	3	4	2
Serum(−)/CSF(−)	7	2	2	0	3
Total	80	20	12	26	22

* Calculated exudation of IgG in CSF, 3.0mg/dl and/or CSF albumin $\times 10^3$/serum albumin, 9.0 or above

CSF was 3.0mg/dl or more. Out of 80 neurocysticercosis patients, 38(47.5%) showed significant exudation. As much as 54.9%(28/51) of serum(+)/CSF(+) patients showed the associated exudation. In CSF(+) cases, 55.6%(35/63) were associated with exudation while 17.6% (3/17) showed significant exudation in CSF (−) cases.

DISCUSSION

This study showed that IgG in CSF was increased in most of neurocysticercosis patients as reported by Spina-Franca *et al.*(1976). Mean IgG concentration of our patients was 14.0mg/dl. But the value was extremely variable by individual patient. And it appeared to be a difficult procedure to determine the normal value of IgG in CSF. Nerenberg *et al.*(1978) reported that IgG in CSF was 4.6 ± 1.9mg/dl in normal persons when measured by radioimmunoassay. In this study, IgG in CSF was 1.7 ± 0.7mg/dl in 5 normal control. If 6.0mg/dl is set arbitrarily as upper normal limit of IgG in CSF, 51 of 80 neurocysticercosis patients are regarded to have elevated concentration of IgG in CSF. Out of them only 2 cases (3.9%) were specific IgG antibody negative in CSF. Contrarily, out of 29 patients whose IgG in CSF were under 6.0mg/dl, 14(48.3%) were the antibody positive in CSF.

Our study indicated that out of 80 patients, 34 showed no evidences of intracranial synthesis of IgG while 46 did. In 34 patients without the evidence of synthesis, 20 (58.8%) were specific IgG antibody positive in CSF. And of 46 patients with the evidence, 3 (6.5%) were the antibody negative in CSF. The above complex relations between specific IgG antibody in CSF and IgG concentration/CNS IgG synthesis in CSF, suggested that the increased IgG (mostly produced in CNS) were represented by positive results of specific IgG antibody in CSF. But the reverse was not true. Even in cases with normal IgG concentration in CSF, thus without the evidence of CNS synthesis of IgG, specific IgG antibody was frequently positive in CSF. As revealed in the regression equation between abs. in CSF and CNS synthesis of IgG ($Y = 0.0194X + 0.5438$), abs. of specific IgG antibody of about 0.54 seems to be a limiting value that reflect the CNS synthesis of specific IgG antibody.

The quantity of specific IgG antibody can not be measured in their weight or concentration. Only relative activity can be measured. Therefore, it is impossible at this moment how the positive results of specific IgG antibody in CSF can be related with normal IgG concentrations in CSF or without evidence of CNS synthesis of IgG.

Many pathogenetic factors made the clinical pictures of neurocysticercosis as well as results of laboratory examinations extremely variable (Stapien, 1962; Loo and Braude, 1982; Mc Cormick *et al.*, 1982; Nash and Neva, 1984; Earnest *et al.*, 1987). Especially the stage of infection significantly affected the protein contents and antibody levels in CSF in neurocysticercosis (Sotelo *et al.*, 1985). In patients

with inactive calcified lesions, inflammatory CSF findings disappeared, and antibody levels turned to negative while pathologic diagnosis is still neurocysticercosis.

This study established that specific IgG antibody levels in CSF were related to the amount of CNS synthesis of IgG rather than to IgG transferred from serum. However, correlation coefficient between the antibody and synthesis (0.393) was relatively low though highly significant. This may be due to different stages of infection of the subjected patients and to variable numbers of infected parasite. It is expected that the correlation coefficient would be higher if cases of a certain pathologic stage of the infection are only considered.

As shown in Table 7, exudation of non-specific IgG into CSF through damaged BBB were evident in neurocysticercosis. Significant exudation was recognized in 38 of 80 patients. Interestingly, most of patients with exudation of IgG (26/38) showed concomitant CNS synthesis of IgG. In 63 cases of specific IgG antibody positive in CSF, 35(55.6%) were associated with exudation. Of them, 26(74.2%) showed concomitant CNS synthesis of IgG. However, in 17 specific IgG antibody negative cases in CSF, only 4(23.5%) showed exudation of IgG while none of them were associated with CNS synthesis of IgG. These results suggest strongly that the exudation of protein through damaged BBB occurs mostly at local granulomatous lesions especially when plasma cell activities are in their peak rather than old fibrotic lesions.

Another point to be made here is the relations of protein contents and IgG antibody levels in ventricular CSF. As Nieto(1959) mentioned, the antibody test using ventricular CSF was always negative when the lesions were located at the 4th ventricle. As he explained, it was because of low protein concentration. Also in this study, total protein and albumin concentrations were low and IgG was not detected by radial immunodiffusion in ventricular CSF. Contrarily, in the paired lumbar CSF, the proteins including IgG were increased; and specific IgG antibody were positive. These results in paired ventricular/lumbar CSF can easily be explained when sink tank role of CSF is considered. But it is unacceptable unless the lesion at the 4th ventricle is playing a perfect role of check valve. Protein moves in water by diffusion. Therefore, the antibody in the 4th ventricle can move to any direction even to lateral ventricle. Because the protein concentrations in ventricular CSF were low and the antibody was negative, the above explanation of check valve role of the lesion would simulate the real situation there. Anyway, the difference in the antibody levels between ventricular and lumbar CSF also supports the conclusion on the intracranial production of specific IgG antibody at the lesion(Sotelo *et al.*, 1985).

The present study should be supplemented by further studies to confirm the local *de novo* production of specific antibody in neurocysticercosis. Oligoclonal antibody differences were proved in paired samples of serum and CSF by isoelectric focusing (Miller *et al.*, 1985). SDS-PAGE/western blot may be another tool demonstrating the specific antibody differences. Immunocytochemical evidence of specific IgG antibody at the lesion can also prove the local production in CNS.

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=국문초록=

뇌유구낭미충증 환자의 뇌척수액내 특이 IgG 항체의 기원

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뇌 유구낭미충증의 진단에는 혈청뿐만 아니라 뇌척수액내 특이 IgG항체가도 측정하여 혈청학적 진단의 민감도를 약 10% 높일 수 있다. 이 경우 뇌척수액내 특이 IgG항체가 그 환자의 혈청에서 혈관-뇌 장벽을 넘어 이전된 것인지 또는 두개강내 병변에 형성된 육아종에서 생성한 것인지를 결정하는 일은 뇌척수액내 특이 IgG항체가 측정의 의미를 부여하는 데에 중요하다. 이 연구는 그 기원을 찾기 위하여 실시하였다.

확진된 뇌 유구낭미충증 환자 82례와 기타 신경계질환 환자 및 건강대조인 45례에서 모은 혈청과 뇌척수액에서 각각 총단백질, 알부민 및 IgG 농도를 측정하였다. 그리고 Tourtellotte and Ma (1978)의 공식에 의하여 뇌척수액내 IgG 농도를 여출(濾出, transudation; 혈관-뇌장벽이 정상인 상태에서 생리적으로 뇌척수액으로 이전된 것), 삼출(滲出, exudation; 혈관-뇌장벽이 파괴된 상태에서 병적으로 이전된 것) 및 두개강내 생산 등 세가지 기원으로 분리하였다. 그리고 혈청 및 뇌척수액내 특이 IgG항체가를 효소 면역측정법으로 측정하였다. 뇌척수액내 특이 IgG항체가와 각기원별 IgG량과의 관계를 통계학적으로 검정하였다. 그 결과를 요약하면 다음과 같다.

1. 뇌 유구낭미충증 환자 뇌척수액에는 총단백질, 알부민 및 IgG평균치가 상승되어 있었다. 각 단백질 농도는 혈청내 특이 IgG항체가와는 관계없이 뇌척수액내 IgG항체가 양성자에서 높았다.

2. 뇌 유구낭미충증 환자의 뇌척수액내 IgG량(14.0mg/dl)은 여출(4.52mg/dl), 삼출(2.94mg/dl) 및 두개강내 생산(6.88mg/dl)으로 구성되었다. 뇌척수액내 특이 IgG항체가는 여출 및 삼출에 의한 IgG양과는 통계학적 유의성이 없는 관계이었으나 두개강내 생산량과는 유의한 관계가 있었다.

3. 제 4뇌실에 병변이 확인된 뇌유구낭미충증 환자의 측뇌실에서 얻은 뇌척수액에는 총단백질, 알부민 및 IgG량이 정상범위이었고 특이 IgG항체는 음성이었다. 같은 환자의 요추천자로 얻은 뇌척수액에서는 각 단백질이 상승되어 있었고 특이 IgG항체가는 양성이었다.

4. 뇌 유구낭미충증 환자의 뇌척수액내 IgG량중 삼출에 의한 양이 증가된 예에서는 대부분 두개강내 IgG 생산이 동반되고 있었다. 따라서 삼출에 의한 뇌척수액내 IgG 출현은 뇌유구낭미충증의 진행중 어느 시기에 특히 심한 것으로 추정된다.

이상의 결과에서 유구낭미충증 환자의 뇌척수액내 특이 IgG항체는 두개강내 병변에서 생산된 것임을 알 수 있었다. 따라서 뇌척수액을 이용한 특이 IgG항체 측정은 진단에 도움이 된다고 생각된다.