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NOTES

Persistence of Inoue-Melnick Virus and Antibody in Cerebrospinal Fluid

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Serial cerebrospinal fluid samples were obtained from 14 multiple sclerosis patients over a period of several months and were tested under code for Inoue-Melnick virus. In five of the positive patients, virus was present in 28 of 32 specimens collected over a period of 2 to 5 months. Four patients, from whom a total of 34 specimens were taken, yielded only a single isolate each. Five patients contributed 28 specimens, all of which were negative. In six patients (three virus positive and three virus negative), neutralizing antibody was detected in serum and in cerebrospinal fluid.

In 1982 we reported on the isolation of an agent from the cerebrospinal fluid (CSF) of four patients with chronic central nervous system (CNS) diseases (three with multiple sclerosis [MS] and one with amyotrophic lateral sclerosis), but none from 27 CSF specimens from patients with acute illness (5). More recently, we studied CSF specimens from 25 patients with a variety of chronic diseases of the CNS and obtained virus from 10 patients, with no difference in the frequency of isolations from those with MS or other CNS disorders (6). The isolated agents could be separated into three antigenic types (2), one of which seems to be the same as the subacute myelooptico neuropathy virus previously isolated in Japan (3). Until its clinical significance is more fully understood, the nondescript term Inoue-Melnick virus (IMV) is being used (2, 7).

This report is concerned with the isolation of IMV from consecutive CSF samples obtained from 10 MS patients treated with interferon injected intrathecally over a span of several months (4). The CSF samples were collected in 1982 from eight female and two male patients in Buffalo, N.Y., stored frozen at below −40°C, and sent in four shipments on dry ice to Houston, Tex., where they were held at −70°C until tested. Serum samples were also obtained from each patient. Among the patients, aged 19 to 41 years old, the duration of illness varied from 5 to 21 years. In addition, serial samples were tested from four MS patients in Houston, none of whom received interferon.

The identification of IMV by the characteristic changes which it produces in MRC-5 cells and which can be neutralized by specific IMV antiserum was conducted as reported previously (2, 7). The test tubes containing the CSF (diluted 1:10), the serum (diluted as indicated), the cell culture passage fluid, or the neutralized mixture were coded, and the code was placed in a sealed envelope before the cultures were inoculated. The specific result of any prior test was not known to the investigators at the time that subsequent tests were conducted on any specimen. Tests were also done under code in which CSF and serum samples in twofold dilutions were tested for antibodies against 100 50% tissue culture infectious doses of the previously isolated Baylor 9 strain, a virus of broad antigenicity crossing with both type 1 and type 2 IMV (2). The details from a typical protocol for virus isolation are shown in Table 1 and for neutralization in Table 3.

TABLE 1. Results of simultaneous virus isolation tests on a negative and a positive patient

Patient no.	Dates of CSF samples (1982)	Code no.	Reading on day[a]:			
			5		6	
			A	B	A	B
1	7 June	9	−	−	−	−
	9 June	6	−	−	−	−
	15 June	38	−	−	−	−
	22 June	40	−	−	±	−
	28 June[b]	11	−	−	−	−
3	12 June	39	±	+	+	+
	14 June	3	±	−	+	+
	16 June	8	±	±	+	+
	29 June	7	±	±	+	+
	1 July	37	+	±	+	+
	7 July[c]	15	±	±	+	±

[a] Each specimen was diluted 1:10 and coded, and then 0.1 ml was inoculated into three culture tubes. Two experienced observers (A and B) separately read the coded cultures on day 5 and recorded their results on separate pages. Readings were made again on day 6 with neither observer having available the previous day's readings. Again, they were read and recorded separately. Subsequently, the four pages of recordings were decoded by a third person as shown above, and the results were listed according to the dates of the specimens. −, No cytopathic changes; +, definite cytopathic changes; ±, mild, unclear changes in cells.

[b] A later CSF sample of 17 July was also negative in a subsequent test.

[c] Four later samples (12 August, 16 September, 22 October, 19 November) were obtained, and three of these were positive.

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TABLE 2. Virus isolations from serial CSF samples of MS patients

Patient no.	Age (yr)	Sex	Duration of MS (yr)	Period of sample collection (1982–1983)	Isolations from serial specimen no.[a]: 1	2	3	4	5	6	7	8	9	10
1	29	F	5	7 June–17 July	−	−	−	−	− :	−				
3	40	F	10	12 June–19 November	+	+	+	+	+	+ :	+	±	+	+
4	19	F	5	22 June–1 December	−	−	−	−	−	− :	+			
5	33	F	13	1 June–29 June	−	+	+	+	−	+	+	+		
6	41	F	8	9 June–19 November	−	−	−	−	− :	−	−	−	−	
7	41	M	21	22 June–15 October	−	−	−	−	− :	−	+	−		
8	36	M	16	14 June–12 November	+	±	+	+	+	+ :	+	+	+	
9	32	F	7	1 June–21 September	−	−	−	−	−	−	− :	−	−	
10	28	F	8	30 June–4 January	−	−	+ :	−	−	−	−	−	−	
11	35	F	12	30 June–4 January	−	+	− :	−	−	−	−	−	−	−

[a] The specimens to the left of the colons arrived in the first two shipments. All the specimens from each patient in these shipments were tested together under code in 1982. The specimens to the right of the colons arrived in the third and fourth shipments and were tested in 1983.

In Table 1 the detailed results for two patients, one negative and one positive for virus in CSF, are presented. Of five samples obtained from patient 1 during June, all were negative, as was a later sample taken in mid-July. Of six samples collected from 12 June through 7 July from patient 3, all were positive. These six samples (three sent in the first shipment and three in the second) were positive in the same coded test in which the five samples of patient 1 were negative. In regard to the consistency of the findings, four later samples were obtained from this patient; one specimen, taken in August, was sent in the third shipment, and three samples, taken in September, October, and November, arrived in the fourth shipment. Virus continued to be detected in three of these four specimens.

Of the 10 patients from Buffalo studied in detail, 7 yielded virus (Table 2). In three patients, virus was present in almost all the specimens collected over a period of 5 months. Three patients were completely negative, and four patients yielded only a single positive specimen.

There was consistency in the findings relative to individual patients, as is evident in the finding with specimens of patients 3 and 8: they were consistently positive in the first two shipments and continued to be positive in the third and fourth shipments, tested separately long after the samples in the first two shipments. No virus could be detected in three patients, and only a single positive sample was detected in the remaining four patients among the 34 specimens tested.

Samples of CSF and serum taken on the same day were studied for neutralizing antibody by conventional procedures that have been developed for other viruses (8). Serum and CSF specimens were first heated at 56°C for 30 min, a procedure which inactivates virus, should it have been present in the specimen. Again, the tests were coded.

The detailed procedure for carrying out the test and the protocol of a test in which specimens taken on the same day from a virus-positive and a virus-negative patient are shown in Table 3. Antibody was found in both serum and CSF. For the virus-positive patient, the titer was 1:20 in both serum and CSF, and for the virus-negative patient, it was 1:40 in the serum and 1:10 in the CSF. Four other patients, two positive and two negative for virus, were studied in a similar fashion, and all the heated specimens neutralized the virus, with low serum/CSF titer ratios.

We confirmed the isolation of IMV from the CSF in patients with chronic CNS disease and took advantage of the opportunity to study consecutive samples taken in Buffalo

TABLE 3. Virus neutralization tests on specimens from a virus-positive patient and a virus-negative patient[a]

Patient no.	Dates of CSF and serum specimens (1982)	Dilution	Code no.	Reading on day: 5 A	5 B	6 A	6 B
8	Serum (6 July)	1:10	16	−	−	−	−
		1:20	9	−	−	−	−
		1:40	5	±	±	+	+
		1:80	8	±	−	+	+
	CSF (6 July)	1:10	12	−	−	−	−
		1:20	7	−	−	−	−
		1:40	2	±	±	+	+
		1:80	3	±	±	+	+
9	Serum (3 June)	1:10	11	−	−	−	−
		1:20	13	−	−	−	−
		1:40	10	−	−	−	−
		1:80	6	±	+	+	+
	CSF (3 June)	1:10	1	−	−	−	−
		1:20	4	−	−	+	+
		1:40	14	±	±	+	+
		1:80	15	±	+	+	+

[a] Specimens were heat inactivated at 56°C for 30 min and diluted 1:10, 1:20, 1:40, and 1:80, and 0.5-ml portions were mixed with 0.5 ml of IMV (Baylor 9 strain; tissue culture passage 8, diluted to $10^{-3.0}$). This stock in previous titrations contained $10^{5.5}$ 50% tissue culture infectious doses per 0.1 ml. Tubes were coded as indicated above and incubated for 1 h at 37°C, and then 0.2 ml of each mixture was inoculated into three MRC-5 cultures. The cultures were slowly rolled at 37°C, with coded readings made on days 5 and 6 by two observers (A and B) as indicated in footnote *a* of Table 1. The four pages of readings were decoded by a third person. In addition, negative controls were included as uninfected MRC-5 cultures and positive controls as cultures inoculated with control virus stock at 10^{-3}, 10^{-4}, and 10^{-5}, as is customary in virus neutralization tests.

TABLE 4. Consecutive CSF samples were usually consistently positive or negative

Patient category	No. of patients	No. of specimens tested	No. of virus-positive specimens
Multiple positive	5	32	28
Single positive	4	34	4
Negative	5	28	0

over a period of up to 5 months. Three virus-yielding patients provided a total of 27 specimens, and 23 of these were positive. Of the remaining seven patients, three were totally negative (24 specimens tested) and four contributed only a single positive specimen (7 to 10 specimens per patient). In Houston, two MS patients who yielded IMV were also examined for persistent virus in the CSF, and both were found to be positive over a period of 6 weeks. Two other Houston patients were both negative when samples were taken over a long period. The results of both studies are summarized in Table 4.

Neutralizing antibody was studied in six patients. Three patients were selected because virus had been isolated and three because it had not been isolated. Antibody was found in both serum (ranging from 1:10 to 1:80) and CSF (ranging from 1:10 to 1:20) in six patients. The low serum/CSF titer ratios suggest that antibody was produced in the CNS. Since antibody could be detected in specimens that had yielded the virus when tested unheated, immune complexes might be present in the CSF, a suggestion that has been made by others (1, 9, 10).

In a recently reported study of coded CSF samples from 24 patients with a variety of chronic CNS disorders (6), we also found a strong association of virus isolation with the presence of antibody in the CSF. In the latter study, of nine patients yielding virus in the unheated CSF, seven were also antibody positive when the CSF was diluted 25-fold and heated as described above. Of 15 virus-negative patients, only one was positive for antibody at this dilution. We must emphasize that no regular pattern was evident in regard to association of virus isolation with any specific CNS disease or syndrome in the earlier study (6) or with any stage of illness in the MS patients in the study reported here. However, the occurrence of a persistent virus in the CSF seems worthy of further attention.

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