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ISOLATION OF TETTNANG CORONAVIRUS FROM MAN?

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Summary. — A virus, identified as Tettnang virus, was isolated from the cerebrospinal fluid (CSF) of an 18 months old child with pharyngitis accompanied by an encephalitic reaction. The isolation of virus was followed by seroconversion. The aetiological role of the virus in the given disease is discussed.

Key words: *Coronavirus*; *Tettnang virus*; *human respiratory infection*

Tettnang virus was first isolated in the Federal Republic of Germany in 1970 (Rehse-Küpper *et al.*, 1973) and included into the International Catalogue of Arboviruses (Berge, 1975) as a possible arbovirus. In Czechoslovakia it was first recorded in 1976—1977 (Danielová *et al.*, 1978; Kožuch *et al.*, 1978). Interest in the virus increased after it had been suggested that it could be the aetiological agent of Garin-Bujadoux-Bannwarth disease manifested as polyneuritis or meningopolyneuritis (Ackermann and Hörstrup, 1980; Rehse-Küpper *et al.*, 1980). In a serological survey carried out in this connection on convalescents after various neuropathies we found antibody to the virus in about 2.5% of the persons examined (Málková *et al.*, 1980). Recently, the virus was reported to be closely related to mouse hepatitis virus (MHV), a member of the genus *Coronavirus* (Bárdoš *et al.*, 1980).

Virus isolation. CSF from an 18 months old girl who in the autumn of 1978 had recovered from rhinopharingitis accompanied by a cerebellar syndrome was used. The isolation experiment was done by the method of passages in suckling (up to 24 hr old) SPF mice (Černý Vůl farm, VELAZ) by combined inoculation of 0.01 ml intracerebrally, 0.1 ml intraperitoneally and 0.1 ml subcutaneously.

Virus identification. Indirect immunofluorescence (IF) and the complement fixation (CF) reaction were used. In the IF assay, the isolated virus was used as antigen in the form of smears or impression smears from the brains of sick mice. The smears were treated with hyperimmune mouse sera against tick-borne encephalitis, Tribeč, Uukuniemi, Eyach and Tettnang viruses and stained with swine anti-mouse conjugate (SwAM/FITC, SEVAC, Prague) absorbed with mouse liver powder and immediately before used also with a 20% mouse brain suspension. The time of treatment of the preparations with sera and conjugate was 30 min each. The preparations were counterstained with Evans blue or thiazine red. The following controls were included: *a*) impression smears from uninfected mouse brains treated as described above; *b*) impression smears stained with the conjugate alone; and *c*) the corresponding hyperimmune

sera with homologous antigens. The CF reaction was carried out by a micromethod (Lennette, 1974) with antigen prepared according to Clarke and Casals (1958).

Antibody detection. Acute and convalescent sera were examined by IF, and in CF and neutralization tests. The IF assay was done on impression smears from infected mouse brains which revealed specific fluorescence in more than 50% of the tissue; the preparations were stained with swine anti-human conjugate (SwAHu/FITC, SEVAC, Prague). The further procedure was as given above (Holubová, 1980). CF antibody was assayed as described above. The virus neutralizing capacity of the sera was assayed in suckling mice by intracerebral inoculation of mixtures of a constant amount of antigen with serum dilutions. The mixtures were incubated for 2 hr at 25° C.

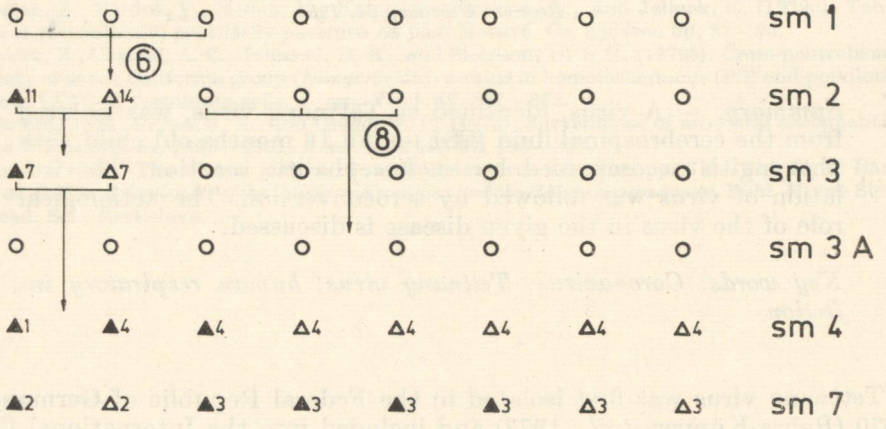

Fig. 1.
Isolation of Tettnang virus from the patient's CSF
▲ and △: mice dead or sick, respectively, on the indicated day p.i.
Figures in circles: mice killed and used for passaging on the indicated day (6 or 8) p.i.
sm 1, sm2, etc.: suckling mouse passage 1, 2, etc.

Virus isolation. The CSF used for virus isolation was taken 13 days after the first symptoms (3 days after the appearance of neurological signs). The course of the isolation experiment is illustrated in Fig. 1. The first infections occurred in single mice in the 2nd passage, the incubation period varying from 11 to 14 days. In subsequent passages the incubation period became shorter and starting with the 7th passage was stabilized at 2—3 days. At the same time all inoculated mice became infected and died. The infection of the animals was manifested by a short prodromal stage and involvement of the central nervous system (CNS). Because the original material had deteriorated, reisolation was not attempted.

Virus identification. Smears and impression smears from infected suckling mouse brains in the 7th and 9th passage were examined with the hyperimmune sera listed above. Positive reactions were only obtained with anti-Tettnang 63 hyperimmune serum, both in IF and CF tests. A comparison of our isolate with human coronavirus OC 43 (courtesy Dr. M. Brůčková, Institute of Hygiene and Epidemiology, Prague) by IF and CF tests showed both viruses being closely antigenically related.

Table 1. Antibody response of the patient

	Serum sample	Antigen			
		Isolated virus		Tettnang 63	
		IF	CF	IF	CF
1	2 Nov. 1978	0	0	0	0
2	16 Nov. 1978	4	0	4	0
3	29 March 1979	16	4	16	4
4	10 May 1979	8	±	8	±
5	17 Sept. 1979	0	ND	0	ND
Hyperimmune serum anti-Tettnang 63		128	64	128	128

IF and CF: antibody titres determined by indirect immunofluorescence and the CF test, respectively. ND = not done.

Antibody detection. The results are summarized in Table 1. IF revealed an antibody increase from negativity in the acute to a titre of 16 in the serum sample taken 5 months after onset of the disease. Then followed a decrease up to a complete disappearance of antibody 11 months after onset of disease. CF antibody was actually found only in serum taken 5 months after onset of disease, namely in a titre of 4, which indicates a higher sensitivity of the IF assay. The neutralization test in suckling mice gave negative results.

Tettnang virus has so far been isolated from ticks (Rehse-Küpper *et al.*, 1973; Danielová *et al.*, 1978; Kožuch *et al.*, 1978) and mice (Bárdoš *et al.*, 1980). The present isolation from human CSF might indicate a relationship of the virus also to man, as was already previously suggested by the finding of antibody in sera of selected groups of patients (Málková *et al.*, to be published). Moreover, the virus might appear as the probable aetiological agent of the disease in question.

Taking into account that Tettnang virus is antigenically closely related to both animal MHV and the human coronavirus OC 43, there arises the question as to which virus type is involved. We assume rather an animal type, because the virus was isolated in mice. Human types, although they could be adapted to mice (McIntosh *et al.*, 1967), require for their isolation elaborate cultivation media, mostly of human origin. As concerns the disease itself, characterized by a pharyngitis, it fals in the clinical picture caused by coronaviruses in humans (Monto, 1976). In our opinion, the CNS involvement accompanying the present case of disease, could have been due to a direct action of the virus, in view of its isolation from the CSF. But so far we cannot say as to whether this is a typical feature of the disease.

In connection with the possibility of spontaneous coronavirus infections of mice there also arose the question of an eventual coincidence of Tettnang virus isolation from mice and the formation in the patient of antibody to human coronavirus antigenically related to Tettnang virus. We consider this possibility as little probable, but a definite answer will be possible after further isolations of Tettnang virus from humans.

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