

PRELIMINAR CHARACTERIZATION OF A NEW VIRUS ISOLATED FROM THE SPINAL FLUID OF PATIENTS WITH MENINGITIS IN SÃO PAULO, BRAZIL (1)

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SUMMARY

A total of 138 patients with the age of 4 months to 57 years were attended in different hospitals of São Paulo State with aseptic meningitis. A probable new agent was isolated from the cerebrospinal fluid of 35 of 53 specimens examined. Replication of the agent with similar characteristics was detected by CPE produced in the MDCK cell line. Virus-like particles measuring about 40 nm in diameter were observed by negative staining electron microscopy. No hemagglutinating activity was detected at pH 7.2 by using either human, guinea pig, chicken and at pH ranged 6.0 — 7.2 with goose red blood cells. The agent was not pathogenic to newborn or adult mice. Virus infectivity as measured by CPE was sensitive to chloroform and not inhibited by BuDR, suggesting that agent is an enveloped virus with RNA genome.

KEY WORDS: Aseptic meningitis; Aetiologic agent; Characterization of meningitis associated virus.

INTRODUCTION

The aetiology of viral infections of the central nervous system has rarely been studied. The echoviruses, coxsackie viruses and mumps virus are most likely to be associated with epidemics of virus meningitis or encephalitis^{4, 6, 7, 8}, although other viruses might be implied with sporadic cases as adenovirus, herpes virus and influenza virus^{1, 14}. In Finland a predominance of herpes simplex virus as an etiological agent of sporadic acute encephalitis was detected⁹. KENNEDY et al⁷ found evidence of a role of enterovirus, measles, herpes simplex, respiratory syncytial virus and adenovirus in childhood with encephalopathies. Flaviviruses have been incriminated as the cause of epidemic human encephalitis in several parts of the world^{2, 3, 5}. Among different arboviruses isolated in Brazil, Rocio virus is considered one of the most important aetiologic agent of human encephalitis¹¹.

From December 1982 to May 1983, an increase in the proportion of cases of aseptic meningitis was found out in 4 of the 12 administrative regions of São Paulo State. During this period were notified 138 cases of this illness which affected both male and female patients with the age which varied from 4 months to 57 years. The clinical symptoms of the ill patients were, headache

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(100%), fever (90%), vomits (90%), stiff neck (90%), and the aspect of CSF samples were limpid in 55.5% and slightly turbid in 45.5% of cases. The cerebrospinal fluid presented moderate hypercitosis with preponderance of lymphocytes. The general conditions of these patients were good without a single case of death or sequelae, without encephalitis, paralysis or parotiditis and developed to cure within 3 to 4 days. The outbreak was not associated to any intermediate vector and the attempt to correlate the illness with bacteria by routine tests were all negative.

The present study analyses the isolation of probable new virus from the cerebrospinal fluid (CSF) of patients with acute meningitis and presents some basic characteristics of the recently isolated virus for taxonomic identification.

MATERIAL AND METHODS

Specimens — The laboratory received CSF of 53 and paired sera of 17 patients with aseptic meningitis of the following cities: Rio Claro, Campinas, Jundiaí, Ribeirão Preto, Barretos, Bauru and São Paulo (fig. 1). The age of ill patients ranged from 4 months to 57 years (Table 1). Sera and samples of CSF were transported in liquid nitrogen to the laboratory.

Virus isolation — The CSF were inoculated into the continuous cell lines cultures of Hep2

TABLE 1
MAV isolation from CSF of patients distributed according to the age groups

Age of patients (Years)	No. of CSF Studied	positives	
		No.	%
0-4	6	5	83.3
5-9	14	9	64.3
10-19	4	3	75.0
20-39	14	10	71.4
40-57	4	4	100
Unknown	11	4	36.4
TOTAL	53	35	66.0

(human carcinoma of larynx), BHK-21 (baby hamster kidney), Vero (green monkey kidney) and MDCK (dog kidney) and in the newborn and adult mice by intracerebral route, for virus isolation attempts. The inoculated tubes were incubated at 35°C in a stationary position for 14 days, changing the maintenance medium every 3 days. The tubes were examined regularly for cytopathic effect. The maintenance medium consisted of Eagle MEM plus 2% inactivated chicken serum for Hep2 and BHK-21, while for Vero and MDCK cell cultures only Eagle MEM without serum was used. Undiluted CSF was inoculated in volumes of 0.05 — 0.1 ml per tube. When the quantity of specimen was insufficient it was diluted at about 10 times. All the specimens received 3 blind passages which were held for 14 days each before being considered negative.

Electron microscopy examination — The culture medium of the infected cell culture which showed CPE was withdrawn and the cells were scraped with a Pasteur pipette into a drop of PBS and after freezing and thawing 3 times was submitted to centrifugation. The supernatant was negatively stained with 10% sodium silicotungstate and examined in a 2 Philips EM-400-T microscope operated at 80 kw.

Hemagglutinating activity — The hemagglutinating activity was tested with human, guinea pig and chicken erythrocytes (RBC) at pH 7.2 and goose RBC at the pH ranging from 6.0 to 7.2, by using standard methodology¹⁰.

Serological tests — Paired samples of sera of 17 patients were submitted to hemagglutination inhibition test for influenza, parainfluenza,



Fig. 1 — Map of São Paulo State with localization of Cities where MAV occurred.