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Immunohistochemical Demonstration of Viral Antigens in Japanese Encephalitis*

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Summary. Japanese encephalitis virus antigens were immunohistochemically demonstrated in formalin-fixed paraffin sections from an autopsied brain. Glial nodules were always associated with antigen-positive cell debris. Glia shrubs in the cerebellar cortex appeared to be formed along the apical dendrite of Purkinje cells. Most, but not all, of the neurons involved in neuronophagia were viral antigen positive. Antigenic masses were occasionally encountered in the center of so-called acellular plaques. Neurons with strong viral antigens were sporadically found in normal-appearing regions in the thalamus and cerebral cortex. Viral antigens were demonstrable only in neurons and not in glial or vascular endothelial cells.

Key words: Japanese encephalitis – Viral antigen – ABC method – Neuronophagia – Glial nodule

Introduction

Demonstration of viral antigens in the affected tissues has been shown to be a most useful procedure in the diagnosis of acute viral encephalitis, such as herpes simplex encephalitis (Nahmias et al. 1982). Although the histopathology of Japanese encephalitis has been well documented (Kaneko and Aoki 1928; Zimmerman 1946; Greenfield 1963), little is known about the distribution of viral antigens in the brain (Johnson et al. 1985). Demonstration of viral antigens is particularly important in cases where death ensues before the development of serological and histopathological manifestations of the infection.

In this communication, viral antigens were clearly demonstrated by the avidin-biotin-peroxidase complex (ABC) method (Hsu et al. 1981) in paraffin sections from a formalin-fixed brain of an autopsy case of Japanese encephalitis, and the localization of the antigens will be discussed in conjunction with histopathological features of the infection.

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Case Report

A 39-year-old man with a history of convulsive disorder associated with cysticercosis for 4 to 5 years and an acute history of fever and disturbed consciousness of 3 days duration was referred to the hospital. On admission, the patient was comatose and had a fever of 40° C. His blood pressure was 120/90 and his pulse rate was 120/min. His respiration rate was 34/min and physical examination disclosed moist rales in the lungs. He had a WBC count of 8,800 with a differential of 79% poly and 21% lymph. The pupils were small and equal. A stiff neck was evident and the tendon reflex was hypoactive in both the upper and lower extremities. Despite supportive therapies, the patient remained in status epilepticus and died on the day of admission. An autopsy was performed 18 h after death. The brain was fixed by immersion in 10% formalin. Macroscopically, the brain was slightly edematous and vascular markings on the cut surfaces of the cerebrum were slightly increased. In addition, numerous tiny cystic lesions of cysticercosis were disseminated in the cerebral cortex and white matter.

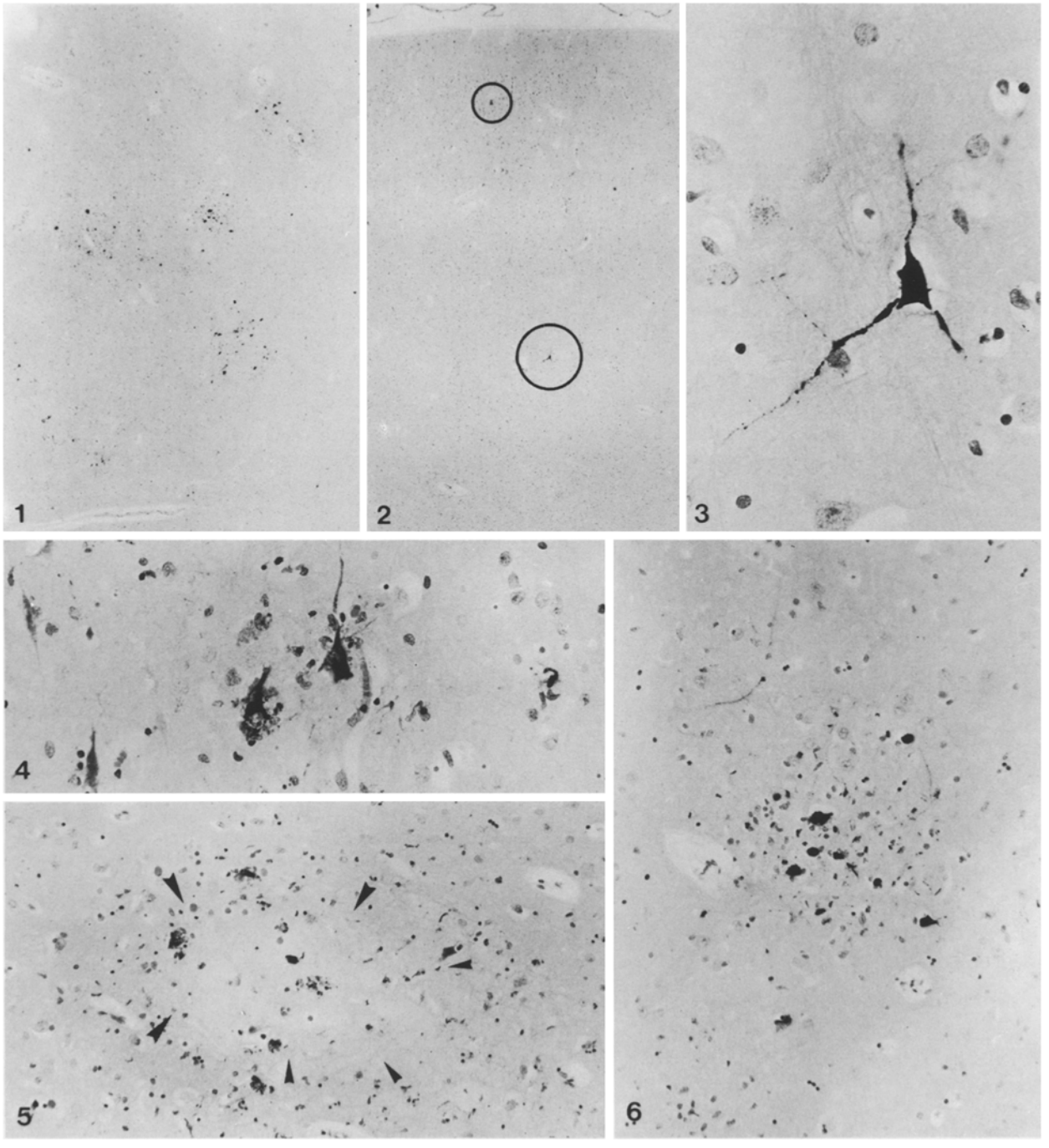
Histological and Immunohistochemical Procedures

Paraffin blocks from portions of the frontal cortex, thalamus, hippocampus, and cerebellar cortex were available for the study. Sections were stained with HE and by the method of Klüver and Barrera.

Viral antigens were stained by the ABC method using polyclonal rabbit antibodies raised against the Nakayama strain of Japanese encephalitis virus (a kind gift from Dr. A. Oya, National Institute of Health, Tokyo, Japan) as a primary antibody at a dilution of 1:2,000.

Results

The cardinal histopathological features of acute viral infection in the present case were: (1) the presence of



Figs. 1–6. Demonstration of Japanese encephalitis virus antigens by the ABC method. Counterstained with Kern-Echte-Rot

Fig. 1. Groups of antigen-positive neurons in the hippocampus. ×30

Fig. 2. Antigen-positive neurons (*encircled*) in grossly normal frontal cortex. ×30

Fig. 3. A higher power view of a neuron heavily loaded with viral antigens (the neuron in the *larger circle* in Fig. 2). ×450

Fig. 4. Two neurons with viral antigens and the one in the *left lower corner* are involved in neuronophagia. ×300

Fig. 5. Presence of antigenic masses in the center and at the edge of acellular plaque (indicated by *arrowheads*). ×150

Fig. 6. Presence of degenerated neurons with viral antigens within a glial nodule. ×150

glial nodules randomly distributed in the hippocampus and of glia shrubs in the molecular layer of the cerebellar cortex, (2) evidence of neuronophagia most frequently seen in the hippocampus, (3) the presence of small edematous necrotic foci – acellular plaques – in the hippocampus and cerebral cortices. In the frontal cortex, deeply eosinophilic pyknotic neurons were sporadically found independent of rather diffuse parenchymal mononuclear cell infiltration or proliferation of rod cells. Perivascular and subarachnoidal cell infiltrations were inconspicuous and were only rarely seen in the subarachnoid space of the cerebral convexity and around venules in the hippocampus.

By immunohistochemistry, groups of viral antigen-positive neurons were demonstrated in all of the specimens examined and they were often accompanied by focal accumulations of monocytes and lymphocytes (Fig. 1). In the frontal cortex, antigen-positive neurons were sporadically found in the absence of inflammatory cell infiltration (Figs. 2, 3). Most of neurons involved in neuronophagia were heavily loaded with viral antigens (Fig. 4). Viral antigen-positive degenerating neurons and cell debris were commonly seen in the center and vicinity of acellular plaques (Fig. 5). Viral antigen-positive cells and cell debris were detectable in the middle of a majority of glial nodules (Fig. 6) and tiny antigens masses were often seen in the cytoplasm of inflammatory cells. The glia shrubs in the cerebellar cortex apparently formed around the viral antigen-positive Purkinje cells and their dendritic processes (not shown). The viral antigens were solely localized to neurons and they were not detected in glia or endothelial cells. The result of antigen staining was completely negative in control cases.

Discussion

Sporadic occurrence of antigen-positive neurons in the absence of any inflammatory cell reaction is noteworthy. In some neurons, both the perikaryon and dendritic processes were fully loaded with viral antigens (Fig. 3). This suggests that the virus could complete its replication cycle and the shedding of progeny virus might take place before the arrival of hematogenous inflammatory cells (Kitamura 1975). This also indicates that the extent of infection could be much wider than that which could be estimated from the extent of inflammatory cell reactions. Development of minute necrotic foci, often called acellular plaque, is one of the histopathological characteristics of Japanese encephalitis (Kaneko and Aoki 1928). Although this could be the result of either focal circulatory disturbances or direct insults by virus replication (Takeya 1962), the demonstration of viral antigens in the lesion in the present study supports the view that this is due to the direct consequence of virus infection (Zimmerman 1946). The mechanism of the development of such lesions, however, remains to be elucidated.

References

Greenfield JG (1963) Japanese B encephalitis. In: Blackwood W, McMenemey WH, Meyer A, Norman RM, Russell DS (eds) Greenfield's neuropathology, 2nd end. Arnold, London, pp 201–203

Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:577–580

Johnson RT, Burke DS, Elwell M, Leake CJ, Nisalak A, Hohe CH, Lorsomrudee W (1985) Japanese encephalitis: immunocytochemical studies of viral antigen and inflammatory cells in fatal cases. Ann Neurol 18:567–573

Kaneko R, Aoki Y (1928) Über die Encephalitis epidemica in Japan. Ergebn Inn Med Kinderheilkd 34:342–456

Kitamura T (1975) Hematogenous cells in experimental Japanese encephalitis. Acta Neuropathol (Berl) 32:341–346

Nahmias AJ, Whitley RJ, Visintine AN, Takei Y, Alford CA Jr, The Collaborative Antiviral Study Group (1982) Herpes simplex virus encephalitis: laboratory evaluations and their diagnostic significance. J Infect Dis 145:829–836

Takeya S (1962) Histopathologie der japanischen Sommerencephalitis. Adv Neurol Sci 6:75–87 (in Japanese with German abstract)

Zimmerman HM (1964) The pathology of Japanese B encephalitis. Am J Pathol 22:965–991

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