LOCALIZATION OF ARBOVIRAL ANTIGEN IN BRAIN TISSUES FROM PATIENTS WITH ENCEPHALITIS

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INTRODUCTION

There is a degree of histologic uniformity in the lesions of the brain caused by viruses (Joseph, 1967). In only a very few viral infections e.g. rabies and cytomegalovirus infections, are pathognomonic inclusions detected. Hence, the identification of the virus causing the encephalitis is not possible on the basis of the lesions seen in haematoxylin and eosin (H and E) stained tissue sections. Moreover, in very tiny biopsies there may not be any lesions pertaining to viral encephalitis *per se*, detectable by light microscopy in routinely stained tissues. Thus, the observation of viral antigenic material *in situ* by immunohistochemical staining procedures provides a valuable tool for the study of the aetiology of encephalitides caused by viruses.

This report describes the application of two immunoenzymatic techniques for the detection of arboviral antigen in brain tissues from patients with cases of suspected viral encephalitis or encephalopathy.

MATERIALS AND METHODS

Reagents: Mouse IgG was purified from normal mouse serum by using protein A Sepharose CL4B (Pharmacia). Antiserum to purified mouse IgG was raised in rabbits. Rabbit IgG obtained from hyperimmune serum to JE virus, was purified on DE-52 (Whatman) ion-exchange column equilibrated with 0.0175 M phosphate buffer (pH 6.9). Rabbit anti mouse IgG was conjugated to horseradish peroxidase (type VI, Sigma) or biotinylated. Avidin peroxidase (Sigma) was used.

Tissue: Brain tissues were obtained from 38 patients with a clinical diagnosis of encephalitis or encephalopathy from various parts of India. They were obtained after death, either at postmortem or with a Vim Silverman Needle (Seth *et al.*, 1974).

These tissues were processed for histology by standard techniques after formalin fixation. Some of the tissues after processing had been stored in paraffin blocks for ten years.

The controls used were brain tissues obtained at autopsy from persons who had died without neurological illness as well as brain tissues obtained from normal mice and from mice experimentally infected with JE virus.

The sections were dewaxed in xylene and brought to water through alcohol. They were covered with 10% H_2O_2 in methanol. After washing in water the tissues were trypsinized for 2-4 hours in 0.1% trypsin and 0.1% $CaCl_2$ in distilled water at 37°C. Washing was

then carried out in distilled water. The sections were treated with 0.1 M periodic acid and with 0.02% sodium borohydride. Sections were thoroughly washed in distilled water, then in phosphate buffer saline (pH 7.2). They were treated with normal serum (rabbit) at a dilution of 1:4 (prepared in 1% bovine serum albumin in distilled water) for 30 minutes, and then washed with PBS. Sections were incubated with mouse antiserum to JE virus at 37°C for 30 minutes, followed by three washes of PBS for 15 minutes each. Sections were treated with conjugate (rabbit antimouse IgG) tagged with horseradish peroxidase (Wilson and Nakane, 1978) at a dilution of 1:100 in rabbit serum in phosphate saline (RSPS), and then with Diamino benzidine tetrahydrochloride (DABT, 50 mg in 100 ml Tris and 100 ml of 30% H_2O_2 in methanol) for 15 mins. After three washes of PBS they were counterstained with methyl green for 20 minutes. They were then dehydrated, cleared in xylol and mounted in DPX.

After treating the sections with mouse antiserum to JE virus, the sections were treated with biotinylated rabbit anti-mouse IgG for 30 minutes at room temperature. Sections were washed in PBS and then were covered with Avidin HRP (Kendall *et al.*, 1983) for 15 minutes, followed with DABT. They were counterstained with methyl green, dehydrated, cleared in xylol and mounted in DPX.

The JE virus infected mouse brain positive control was treated as above whilst the negative controls were treated with normal serum and further processed as mentioned above.

RESULTS

Twenty three tissues out of 38 (60.5%) tested for Group B arboviral antigen were identified by both the indirect immunoperoxidase and the biotin avidin peroxidase methods. However, the biotin avidin peroxidase method gave a more intense staining reaction.

The positive results for immunoperoxidase staining was recognized as a yellowish brown granular reaction product in antigen containing cells whereas the background appeared as a pale brown colour (Fig. 1). The antigen was detected mainly in the neurons and occasionally in glial cells. Nonspecific staining was virtually absent, as no positive reaction was observed in sections from negative controls whereas the positive controls showed the presence of antigen. The intensity of the staining reaction was graded from + to +++.

There were 15 (39.5%) specimens negative for arboviruses by both these methods. No antigen was detected in control tissues obtained at postmortem from persons who had no neurological illness or from JE virus infected mouse brain treated with normal serum.

On histologic examination of the brain tissues stained by H and E, there was no

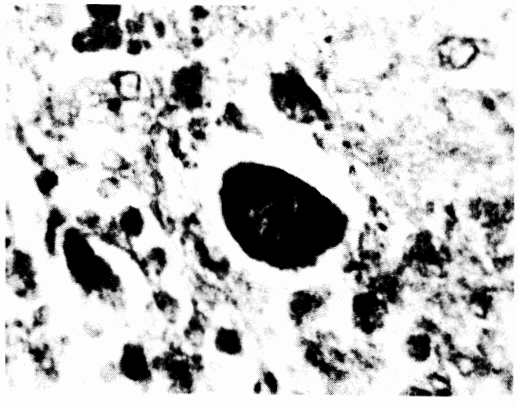

Fig. 1–Cerebellum stained by the immunoperoxidase method. Note a dark granular staining of the antigen in a Purkinjee cell and some other neurons.

pathology seen in 34 (89.5%) brain tissues, which had been obtained with a Vim Silverman Needle (VS). A diagnosis of encephalitis could be arrived at in the remaining four (10.5%) brain tissues; three obtained at autopsy and one with a VS needle. In one of these tissues Negri bodies were detected indicating a rabies viral encephalitis. This was proved to be rabies on mouse inoculation. No arboviral antigen was demonstrated in the brain tissue of this patient who died of rabies virus encephalitis.

DISCUSSION

The use of immunolabelling techniques have been increasingly employed in recent years. Immunoperoxidase techniques are used to detect a variety of cell products as well as viral antigen and other specific proteins (Heyderman and Neville 1977; Delellis *et al.*, 1979; Brown *et al.*, 1985). The immunoperoxidase technique has an advantage over the immunofluorescent technique as it does not need the use of a special microscope. Furthermore, once the endogenous peroxidase has been destroyed by alcohol and H_2O_2, nonspecific reactions do not pose a problem.

The results indicate that the immunolabelling reaction was stronger in the biotin avidin peroxidase method than in the indirect immunoperoxidase method. This improved sensitivity is due to the potential for amplification by biotin avidin peroxidase complex (Guesdon *et al.*, 1979). It is observed that JE virus infected mouse brain treated with normal serum, and the tissue from the patient with rabies virus encephalitis were negative. Both these indicate the specificity of the reaction.

It is of particular interest that the tiny brain tissues in which no abnormal histology could be detected by light microscopy could be diagnostic of an arboviral infection as revealed by immunolabelling techniques. The use of monoclonal antibodies would increase the specificity, especially as there is cross reaction between the closely related JE and West Nile arboviruses.

Another advantage of immunostaining is that positive results could be obtained on tissues which were stored in paraffin blocks indicating that the antigen can be detected even after many years.

In epidemic situations where there are fatalities, the collection of brain specimens could serve to pinpoint the aetiological agent involved, by the use of this method.

SUMMARY

Brain tissues from 38 patients with a clinical suspicion of encephalitis or encephalopathy were examined by two immunoenzymatic techniques for the detection of arboviral antigen. Group B arboviral antigen was identified in 23 of these tissues. This simple method could be used for the diagnosis of the causal agent of encephalitis.

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