Acta virol. **36**: 585–588, 1992

SSPE IN SLOVAKIA: IMMUNOCYTOCHEMICAL STUDY

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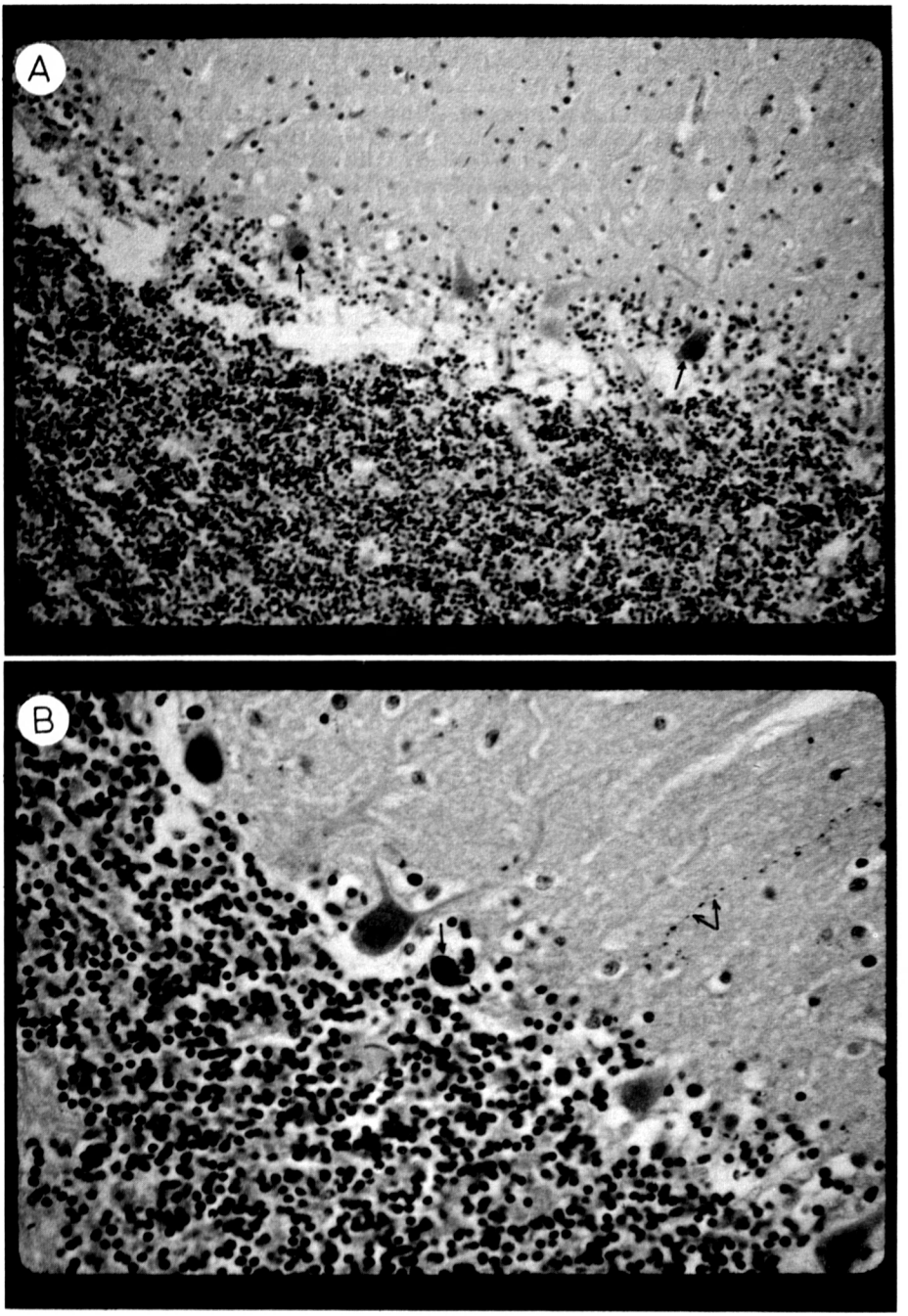
Received February 19, 1992

Summary. – In a retrospective study of two patients from Slovakia with clinical, virological and histopathological diagnosis of subacute sclerosing panencephalitis (SSPE), measles virus antigen was detected by immunocytochemical labelling studies. The formalin fixed, paraffin-embedded thin brain sections labelled with anti-measles antibodies and avidin-biotin complex peroxidase were counterstained with haematoxylin. Only a single area of brain was examined in each patient: cerebellum and parietal lobe. Viral antigen positive reaction was identified within Purkinje cells and extending along dendritic processes in cerebellum, and also in oligodendrocytes of subparietal white matter.

Key words: measles virus; subacute sclerosing panencephalitis; immunocytochemistry

SSPE is a rare, slowly progressing, fatal disease of the central nervous system (CNS) predominantly affecting children and adolescents. Measles virus (MV) persisting in the CNS is involved in etiopathogenesis of SSPE (terMeulen *et al.*, 1983). MV structural proteins were demonstrated in the brain tissue of SSPE patients by immunofluorescence and immunohistochemistry (Connoly *et al.*, 1967; Lenette and Magoffin, 1968; Jenis *et al.*, 1973; Budka *et al.*, 1982; Haase *et al.*, 1985). We report here the detection of MV antigen by immunoperoxidase technique in thin sections of formalin fixed, paraffin-embedded brain tissue of two SSPE patients from Slovakia.

The typical clinical course with progressive mental deterioration and characteristic neurological signs and EEG pattern were observed in both SSPE children, who died 8 years ago. One of them (case 1) was 7 years old male with clinical manifestation of SSPE at the age of 7 years and initially had measles at 8 months. The other patient (case 2) was 13 years-old male who suffered from SSPE at the age of 12 years. He had not history of measles but had contact with his ill relative one year ago without any clinical symptom of measles. Both patients were vaccinated and revaccinated against measles. Antibody titer to MV estimated by haemagglutination-inhibition test was 1:256 (case 1) and 1:128 (case 2) in sera and 1:16 (case 1) and 1:8 (case 2) in cerebrospinal fluid.


A
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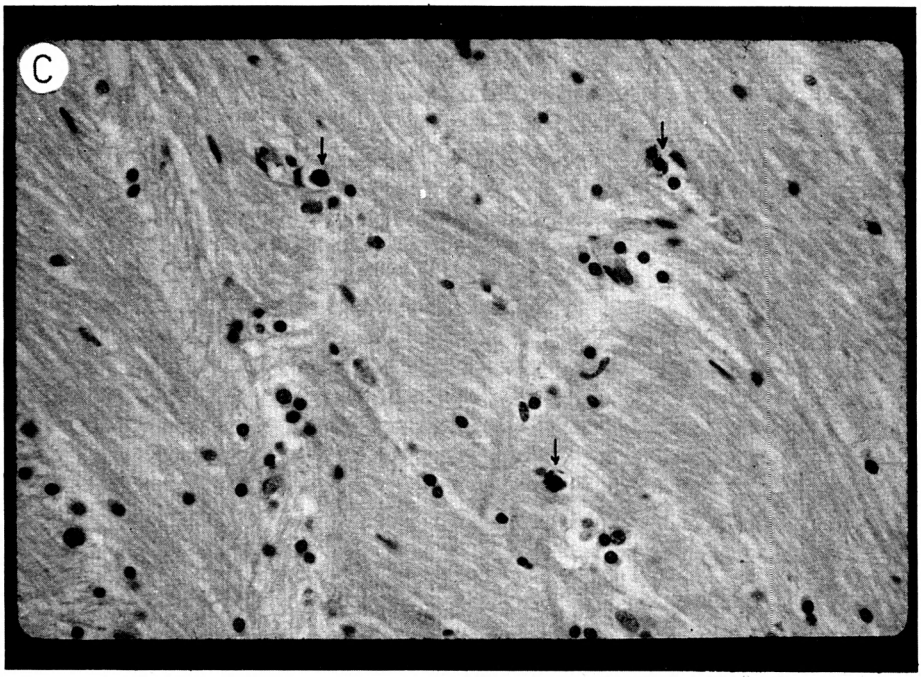

Fig. 1
MV antigen in brain tissue
Representative labelled cells are marked by arrows.
A. Cerebellar tissue from case 1. Intranuclear strong specific MV antigen positive staining in Purkinje cells. Magn. 12 x 20.
B. Cerebellar tissue from the same case. Strong specific MV antigen positive staining in nucleus of Purkinje cells. The dots represent dendritic accumulation of virus specific antigen. Magn. 12 x 40.
C. Subparietal white matter from case 2. Sporadic marked nuclear and cytoplasmic antigen positive staining in oligodendrocytes. Magn. 12 x 40.

Thin sections from paraffin-embedded tissue were deparafinized and labelled immunocytochemically for MV antigen. Endogenous peroxidase activity was blocked by incubation in 0.3 % H_2O_2 in methanol for 30 min. After washing in PBS the sections were incubated with rabbit anti-measles serum (1:1000 dilution) for 30 min at room temperature. After washing in PBS, tissue sections were exposed to biotinylated anti-rabbit IgG antibodies, followed by incubation with peroxidase-conjugated avidin-biotin complex (Vectastain ABC Kit^R, Vector Labs., U. S. A.) for 30 min at room temperature. Diaminobenzidine tetrachloride (Sigma, U. S. A.) was used to develop the color. Slides was counterstained with Gill's haematoxylin. The specificity of the immunoreaction with antibodies against MV antigen was tested using preimmune serum lacking MV antibodies, which resulted in negative staining.

Only a single area of brain was examined in each patient: cerebellum (case 1) and parietal lobe (case 2). In both cases MV antigen was found within the brain tissue. In cerebellum only a few antigen-containing cells were presented. Intensive intranuclear MV antigen positive reaction was detected in Purkinje

cells (Fig. 1A), and also extending along dendritic process (Fig. 1B). In white matter of parietal lobe moderate number of antigen positive cells were observed. All positive cells were oligodendrocytes. In most of these cells marked intranuclear MV antigen accumulation was identified (Fig. 1C).

Previous immunofluorescence labelling studies have shown that most area of the brain may be involved, but cerebellum is usually spared and spinal cord is inconsistently involved (Ohya *et al.*, 1974; Esiri *et al.*, 1981). Different investigators found MV antigen almost selectively in cytoplasm, nuclei, and processes of pyramidal cells in cerebral cortex, and oligodendrocytes of white matter (Lenette, 1968; Esiri *et al.*, 1981; Budka *et al.*, 1982; Norby *et al.*, 1985). Our detection of MV antigen positive reaction in oligodendrocyte of subparietal white matter from the SSPE brain tissue was very similar to those reported by other authors. However, accumulation of MV antigen in Purkinje cells and dendritic processes in cerebellum from SSPE brain, as confirmed here by immunocytochemical method, was not previously noticed. Immunocytochemical identification of MV antigen in brains from two patients provides besides diagnostic verification also some new data on intracerebral spread and distribution of MV in SSPE.

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