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PRELIMINARY REPORT

EXPERIMENTAL SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE) IN A MONKEY BY SUBCUTANEOUS INOCULATION WITH A DEFECTIVE SSPE VIRUS[1]

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It has become apparent that subacute sclerosing panencephalitis (SSPE) is related to latent infection of the central nervous system (CNS) by a defective or suppressed form of measles virus. However, it is unknown how this measles virus persists in the CNS.

Adels et al. (1968) first attempted to transmit SSPE to experimental animals, and since then there have been a number of studies on experimental SSPE by groups of virologists, pathologists and neurologists. In these studies the technique of intracerebral inoculation has usually been used and animals have developed acute encephalitis after a short incubation period. Wear and Rapp (1971) induced chronic infection with measles virus in the brain of suckling hamsters from immune mothers. Recently, Raine et al. (1974) reported the development of a laboratory model for SSPE in weanling hamsters which survived acute encephalitis. However, it is difficult to clarify how measles virus invades the CNS or what are the necessary conditions of the host from experiments on intracerebral inoculation of animals.

Horta-Barbosa et al. (1971) isolated measles virus from the lymph nodes of patients with SSPE, suggesting that in human SSPE suppressed measles virus infection outside the CNS preceded latent infection of the CNS. Accordingly we developed a laboratory model of SSPE by subcutaneous inoculation of a monkey with a defective SSPE virus.

CV-1 cells grown in 3 Roux bottles were infected with the SSPE-" BIKEN " strain of a defective measles virus, which was isolated from the brain of a patient with SSPE and which does not produce either hemagglutinin or virions (Ueda et al., 1975). After incubation for 2 days, when the monolayer of CV-1 cells was composed of syncytial giant cells, cells were collected by trypsinization, washed once with Eagle's minimal essential medium (MEM)

1 Summary of this work was read at the Symposium on slow virus infections, March 11–12, 1975, Tokyo.

and suspended in 4 ml of Medium 199. This suspension contained 6×10^7 cells, of which 30–50% were infected. A single African green monkey which had been kept for about 2 years in an isolated cage in the animal colony of the Research Institute for Microbial Diseases, Osaka University and which had no detectable measles neutralizing or hemagglutination inhibition (HI) antibodies was inoculated subcutaneously into the right thigh with the cell suspension.

The monkey was healthy and did not show any neurologic symptoms or other clinical signs until 8 weeks after inoculation. Then its movements became slow. In the following 2 weeks, it became unable to stand or walk and could hardly pick up apples. Its lower extremities showed flexion contracture with plastic rigidity. On the day of sacrifice, 70 days after inoculation, the monkey had lost its voice and showed reduced body weight. It drivelled and showed tremor in both hands. Eye movements were not affected but paralysis of the right upper eyelid was observed. The measles HI antibody titer in the serum was elevated to 1 : 1024 2 weeks after inoculation and was subsequently maintained at a high level (Fig. 1).

A block of about 1 cm^3 of brain tissue was obtained from the right parietal cortex and cut into pieces. These pieces of brain tissue were washed once with Eagle's MEM and suspended in 60 ml of growth medium, consisting of a mixture of equal volumes of Medium 199 and Eagle's MEM supplemented with 20% fetal bovine serum, 100 μg/ml of streptomycin and 100 units/ml of penicillin. The suspension was distributed into 10 plastic dishes (6 cm in diameter) and incubated at 37 C in an atmosphere of 5% CO_2 in air. The brain cells growing out from the explants were collected by trypsinization and seeded again at the same original cell density into plastic dishes 13 days after explantation. Since then, coverslip cultures of the cells have been made at each subculture for cytological and immunofluorescent examinations.

Cells with a glial appearance grew rapidly and did not form syncytia. However, measles antigen was detected in single cells of the 3rd sequential subculture, 40 days after explantation, by indirect fluorescent staining using convalescent sera from patients with measles (Fig. 2). On staining with hematoxylin and eosin, these cells were seen to contain cytoplasmic or intranuclear inclusion bodies. No cell-free virions have yet been detected in the culture of brain cells.

Pathological and other examinations are still in progress, but the results obtained up to the present suggest that this monkey provides a laboratory model of SSPE more closely related

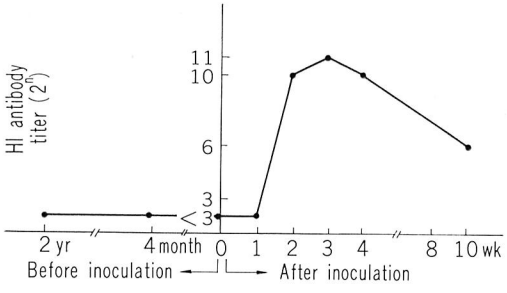

FIGURE 1. *Measles HI antibody response of the monkey before and after inoculation with CV-1 cells infected with the SSPE-"BIKEN" strain.*

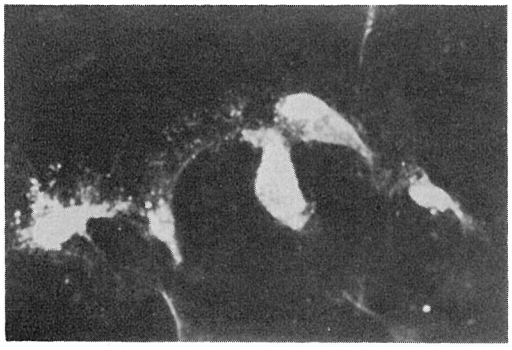

FIGURE 2. *Measles specific immunofluorescence in single cells growing out from the monkey brain explant. Stained by the indirect method using convalescent serum from a patient with measles. Magnification: ×400.*

to human cases than models obtained by intracerebral inoculation of infectious materials. With this type of model it will be possible to examine virological, pathological and neurological changes, not only in the CNS, but also in other parts of the body from the early to the late stage of SSPE.

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