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## PRELIMINARY REPORT

TEMPERATURE-SENSITIVE ALTERATION IN FUSION  
ACTIVITY OF SUBACUTE SCLEROSING PANENCEPHALITIS  
VIRUS DURING SERIAL PASSAGES IN VITRO AND  
EXPRESSION OF HEMAGGLUTININ ON THE INFECTED  
CELLS

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The Biken strain of subacute sclerosing panencephalitis (SSPE) virus, a maturation-defective variant of measles virus, was serially passed in human embryonic lung (HEL) cells at 37 C. The strain formed syncytial giant cells (GC) at both 37 C and 39 C, but the surface of infected cells did not show hemadsorption at early passages of the strain. However, GC-forming activity of the strain diminished at 39 C after 25 passages or more and hemadsorption on the infected cells became positive at both 37 C and 39 C after 40 passages or more of the strain. Hardly any infectious cell-free virus was detected in the culture fluid even after hemadsorption became positive. Possible mechanisms for the defect of SSPE virus were discussed.

Subacute sclerosing panencephalitis (SSPE) is a rare, but progressive and serious disease of the central nervous system of children and adolescents (Sever and Zeman, 1968). Although it has been revealed that persistently infecting measles virus or its variant (SSPE virus) is the cause of SSPE (Horta-Barbosa et al., 1969; Payne, Baublis and Itabashi, 1969),

the pathogenesis of the disease is unknown.

Temperature-sensitive (ts) mutants of paramyxoviruses, such as Newcastle disease virus (NDV), hemagglutinating virus of Japan (HVJ) and measles virus, have been reported to establish persistent infections in vitro easily. Most progeny virus from such persistent infections have also been reported to be ts mutants (Knight, Duff and Rapp, 1972; Nagata et al., 1972; Preble and Youngner, 1972; Haspel et al., 1973; Gould and Linton, 1975). Some ts mutants of measles virus were found to be encephalitogenic in laboratory animals

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(Haspel, Duff and Rapp, 1975; Norrby and Kristensson, 1978). Recently, Hodes (1979) reported that a strain of SSPE virus consisted of a heterogenous population containing its virus and could establish persistent infection at a restricted temperature.

During serial passage of the Biken strain of SSPE virus in vitro, I found that its fusion activity had become temperature-sensitive with expression of hemagglutinin on the infected cells. This paper reports these phenomena and discusses an aberrant property of SSPE virus.

The virus used in this study was the Biken strain of SSPE virus, isolated from brain cells of a patient with SSPE by cocultivation with human embryonic lung (HEL) cells (Ueda et al., 1975). The strain has been maintained at 37 C by cocultivation of infected cells with fresh HEL cells at 7- to 10-day intervals because of its maturation-defectiveness.

When HEL cell suspension infected with

the Biken strain was dispersed onto fresh sheets of HEL cells and cultured at 37 C, syncytia appeared after 12 h, grew in size, forming giant cells (GC), and autolysed in the following 2 to 4 days. New syncytia appeared successively in neighbouring areas. These GC and regions where GC had been formed were distinguishable as plaques by naked eye after staining with Giemsa solution.

An aliquot of 0.2 ml of HEL cell suspension infected with the Biken strain at the 17th, 31st or 42nd passage at 37 C was inoculated onto new sheets of HEL cells in 35-mm plastic petri dishes containing 2.5 ml of fresh maintenance medium: a mixture of equal volumes of Medium 199 and Eagle's minimum essential medium supplemented with 3% fetal bovine serum. The newly infected cultures were incubated at 37 C or 39 C in a 5% CO<sub>2</sub>-incubator. After incubation for 2 days, the cultures were stained with Giemsa solution containing 50% methanol.

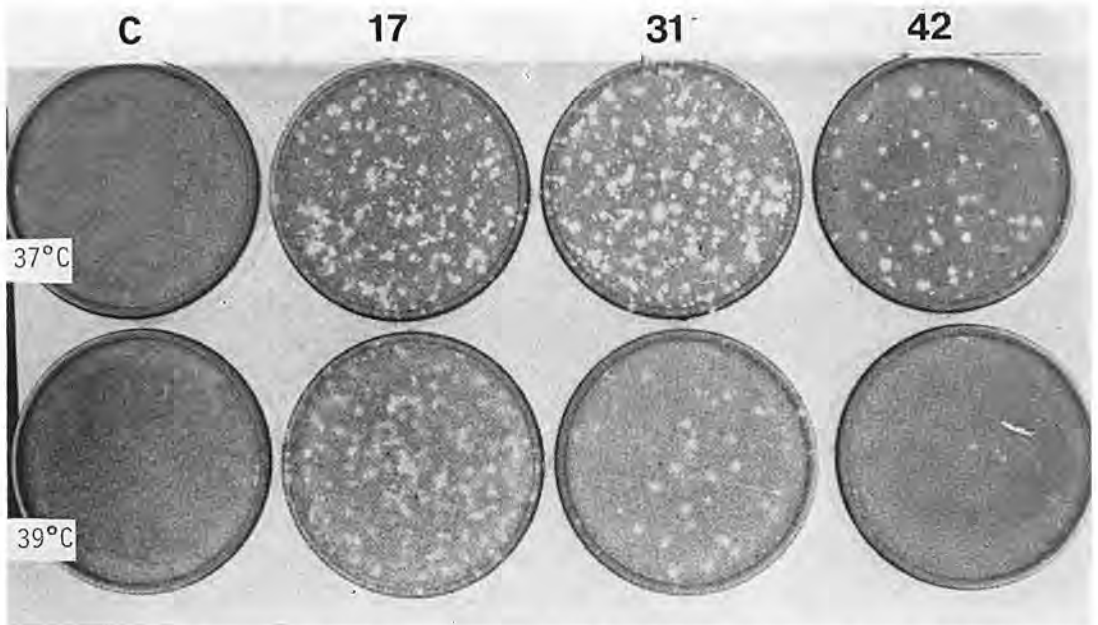


FIGURE 1. Plaque formation in HEL cell monolayers at 37 C and 39 C by the Biken strain of SSPE virus at different passage levels in HEL cells at 37 C. The infected cultures were stained with Giemsa solution 2 days after infection. C: uninfected cultures, 17, 31, 42: passage numbers in HEL cells at 37 C of the strain used for infection.

Figure 1 shows typical results of several experiments. Since it was hard to adjust the inoculum size because of the defectiveness of the strain, the number of plaques formed at 37 C was varied in samples at different passage levels. However, it is clear that the numbers of plaques formed at 39 C by the strain at the 31st and 42nd passage were much reduced. This temperature-sensitive alteration in GC-forming activity of the strain was observed in cultures infected with the strain passaged 25 times or more in HEL cells at 37 C. Although plaques were invisible, when the strain which had been passaged 40 times or more at 37 C was cultured at 39 C, syncytia containing several nuclei were observed under the microscope (Fig. 2b). When these cultures were incubated further at 37 C, GC appeared in the following 2 days.

The GC formed by the strain at early passage showed no hemadsorption (HAD) on their surface, but those formed by the strain which had been passaged 40 times or more at 37 C showed HAD. Although the GC formed at 39 C were much smaller than those formed at 37 C, HAD was seen at both temperatures (Fig. 2a, b).

As previously reported, the strain did not produce infectious cell-free virus until at least the 23rd passage in HEL cells (Ueda et al., 1975). In this study, tests were made for infectious cell-free virus in the culture fluid at passages 13, 14, 27, 28, 39, 40, 41 and 44. Infectivity was detected only in the culture fluid of cells at the 40th passage clarified by centrifugation at 3,000 rev/min for 10 min at 4 C. Its titer was very low ( $10^{0.75}$  TCID<sub>50</sub>/ml) and passage of the virus with culture fluid alone was not successful.

As reported above, fusion activity of the Biken strain became temperature-sensitive and HAD on the infected cells became positive during serial passages at 37 C. F (fusion) protein of paramyxoviruses including measles virus is involved in cell fusion (Norrby and Falksveden, 1964). Hence, the temperature-sensitive alteration in fusion activity may well

be a result of alteration in F protein. However, it is not clear whether it results from alteration in function or synthesis of the F protein, or from an aberration in insertion of the F protein into the plasma membrane. The fact that HAD became positive raises the interesting problem of whether there was an aberration in insertion of glycoproteins into the plasma membrane in infected cells. Breschkin et al. (1979) reported that all the structural proteins of measles virus were synthesized in BSC-1 cells infected with the Biken strain, but that only F protein was expressed on the cell surface. We reported previously that HAD-negative CV-1 cells infected with the Biken strain induced hemagglutination-inhibition (HI) antibody in a monkey (Ueda, Otsuka and Okuno, 1975). In addition, we detected measles HI antibody in the cerebrospinal fluid (CSF) of the patient from whom the strain was isolated (Ueda et al., 1975). This suggests that the hemagglutinin (HA protein) synthesized in cells infected with the Biken strain was not abnormal, at least immunologically. Measles HI antibody has been detected in CSF of most patients with SSPE (Sever et al., 1974) as well as in our case. Nevertheless, no HAD was detected on cells infected with many other isolates from patients with SSPE (Doi et al., 1972; Thormar et al., 1973; Makino et al., 1977). Payne et al. (1969) also reported that their isolate required several passages before HAD on the infected cells became positive. So, the alteration in HAD seen in cells infected with SSPE virus seems to be a result of an alteration in insertion of HA protein into the plasma membrane, which might be common to SSPE virus. Transmembrane insertion of the envelope glycoprotein of vesicular stomatitis virus (VSV) was recently found to require a signal or leader sequence at the amino-terminus of the nascent precursor of the glycoprotein (Irving et al., 1979), like secretory proteins (Blobel and Dobberstein, 1975; Jackson and Blobel, 1977). It is unclear at present whether glycoproteins of measles virus need such signal sequences or



a

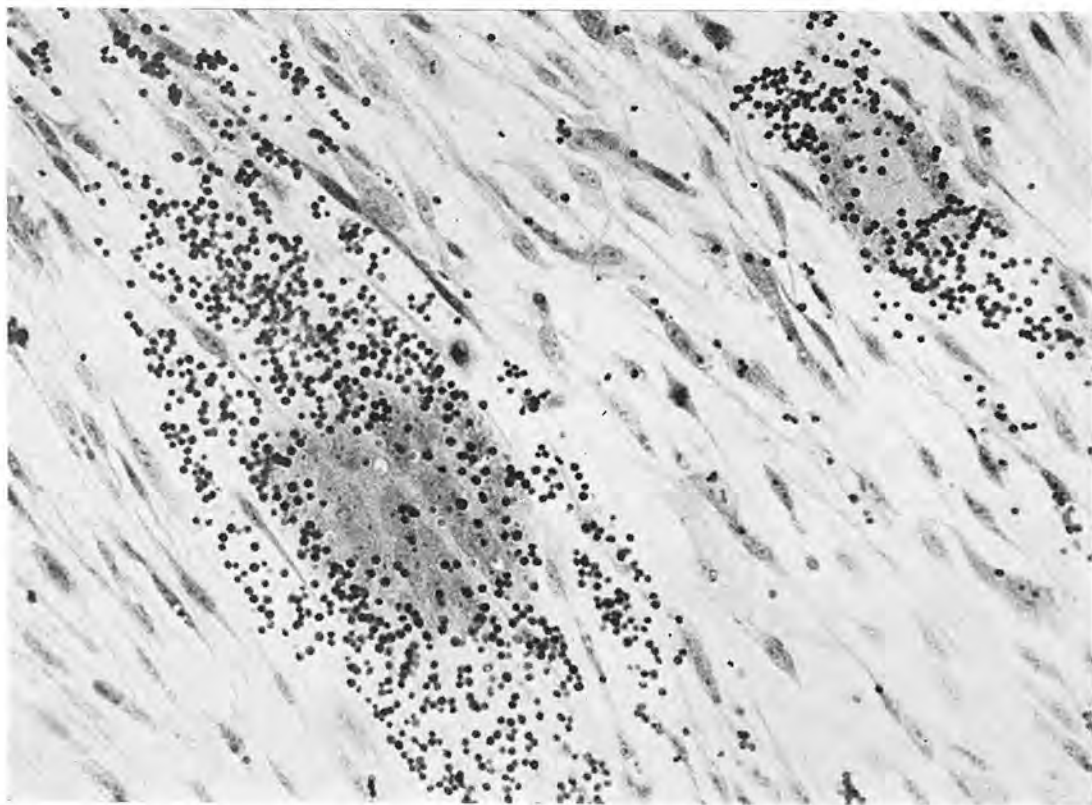
FIGURE 2. Hemadsorption (HAD) on infected HEL cells 2 days after infection with the Biken strain of SSPE virus at the 42nd passage in HEL cells at 37 C. a: HAD on a huge syncytial giant cell formed at 37 C, b: HAD on 2 small syncytia formed at 39 C. Hematoxylin and eosin stain. Photographs were taken at  $\times 50$ .

whether the signal sequences for F and HA proteins are the same. However, since such a signal sequence is considered to be universally needed for transmembrane insertion of proteins, the aberrant combination of signal sequence(s) with HA and F proteins could account for alterations in HAD and fusion, that is, expression of glycoproteins on SSPE virus-infected cells.

Several recent reports have dealt with an abnormal matrix or membrane (M) protein of SSPE virus accounting for its defect (Schluederberg et al., 1974; Wechsler and Fields, 1978; Hall, Kiessling and ter Meulen, 1978; Hall, Lamb and Choppin, 1979). The M protein is considered to mediate alignment of nucleo-

capsids under the plasma membrane where glycoproteins are expressed (Choppin and Compans, 1975). The Biken strain did not produce infectious cell-free virus even after the infected cells became able to express both F and HA proteins at 37 C. This indicates that the Biken strain has an abnormality in M protein like other strains of SSPE virus investigated.

Consequently, there seem to be multiple abnormalities in SSPE virus especially in early passages. Further studies of the molecular basis of the phenomena reported above could lead to a better understanding of SSPE virus and its genesis.



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#### REFERENCES

- Blobel, G., Dobberstein, B. 1975. Transfer of protein across membranes. I. Presence of proteolytically processed and unprocessed nascent immunoglobulin light chains on membrane-bound ribosomes of murine myeloma. *J. Cell Biol.* 67: 835-851.
- Breschkin, A. M., Morgan, E. M., McKimm, J., Rapp, F. 1979. SSPE-BIKEN: A naturally arising hemagglutination-defective mutant of measles virus. *J. Med. Virol.* 4: 67-80.
- Choppin, P. W., Compans, R. W. 1975. Reproduction of paramyxoviruses, pp. 95-178. *In* Fraenkel-Conrat, H., Wagner, R. R. [ed.] *Comprehensive Virology*. vol. 4. Plenum Press, New York.
- Doi, Y., Sanpe, T., Nakajima, M., Okawa, S., Katoh, T., Itoh, H., Sato, T., Oguchi, K., Kumanishi, T., Tsubaki, T. 1972. Properties of a cytopathic agent isolated from a patient with subacute sclerosing panencephalitis in Japan. *Jpn. J. Med. Sci. Biol.* 25: 321-333.
- Gould, E. A., Linton, P. E. 1975. The production of a temperature-sensitive persistent measles virus infection. *J. Gen. Virol.* 28: 21-28.
- Hall, W. W., Kiessling, W., ter Meulen, V. 1978. Membrane proteins of subacute sclerosing pan-

- cephalitis and measles viruses. *Nature* 272: 460-462.
- Hall, W. W., Lamb, R. A., Choppin, P. W. 1979. Measles and subacute sclerosing panencephalitis virus proteins: Lack of antibodies to the M protein in patients with subacute sclerosing panencephalitis. *Proc. Natl. Acad. Sci. USA* 76: 2047-2051.
- Haspel, M. V., Knight, P. R., Duff, R., Rapp, F. 1973. Activation of a latent measles virus infection in hamster cells. *J. Virol.* 12: 690-695.
- Haspel, M. V., Duff, R., Rapp, F. 1975. Experimental measles encephalitis: A genetic analysis. *Infect. Immun.* 12: 785-790.
- Hodes, D. S. 1979. Temperature sensitivity of subacute sclerosing panencephalitis virus and its ability to establish persistent infection. *Proc. Soc. Exp. Biol. Med.* 161: 407-411.
- Horta-Barbosa, L., Fuccillo, D. A., London, W. T., Jabbour, J. T., Zeman, W., Sever, J. L. 1969. Isolation of measles virus from brain cell cultures of two patients with subacute sclerosing panencephalitis. *Proc. Soc. Exp. Biol. Med.* 132: 272-277.
- Irving, R. A., Toneguzzo, F., Rhee, S. H., Hofmann, T., Ghosh, H. 1979. Synthesis and assembly of membrane glycoproteins: Presence of leader peptide in nonglycosylated precursor of membrane glycoprotein of vesicular stomatitis virus. *Proc. Natl. Acad. Sci. USA* 75: 570-574.
- Jackson, R., Blobel, G. 1977. Post-translational cleavage of presecretory proteins with an extract of rough microsomes from dog pancreas containing signal peptidase activity. *Proc. Natl. Acad. Sci. USA* 74: 5598-5602.
- Knight, P. R., Duff, R., Rapp, F. 1972. Latency of human measles virus in hamster cells. *J. Virol.* 10: 995-1001.
- Makino, S., Sadaki, K., Nakagawa, M., Saito, M., Shinohara, Y., Gotoh, F., Okabe, T. 1977. Isolation and biological characterization of a measles virus-like agent from the brain of an autopsied case of subacute sclerosing panencephalitis (SSPE). *Microbiol. Immunol.* 21: 193-205.
- Nagata, I., Kimura, Y., Ito, Y., Tanaka, T. 1972. Temperature-sensitive phenomenon of viral maturation observed in BHK cells persistently infected with HVJ. *Virology* 49: 453-461.
- Norrby, E. C. J., Falksveden, L. G. 1964. Some general properties of the measles virus hemolysin. *Arch. Gesamte Virusforsch.* 14: 474-486.
- Norrby, E., Kristensson, K. 1978. Subacute encephalitis and hydrocephalus in hamsters caused by measles virus from persistently infected cell cultures. *J. Med. Virol.* 2: 305-317.
- Payne, F. E., Baublis, J. V., Itabashi, H. H. 1969. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. *N. Engl. J. Med.* 281: 585-589.
- Preble, O. T., Youngner, J. S. 1972. Temperature-sensitive mutants isolated from L-cells persistently infected with Newcastle disease virus. *J. Virol.* 9: 200-206.
- Schluederberg, A., Chavanich, S., Lipman, M. B., Carter, C. 1974. Comparative molecular weight estimates of measles and subacute sclerosing panencephalitis virus structural polypeptides by simultaneous electrophoresis in acrylamide gel slabs. *Biochem. Biophys. Res. Commun.* 58: 647-651.
- Sever, J. L., Zeman, W. [ed.] 1968. Measles virus and subacute sclerosing panencephalitis. *Neurology* 18 (Part 2): 1-192.
- Sever, J. L., Krebs, H., Ley, A., Barbosa, L. H., Rubinstein, D. 1974. Diagnosis of subacute sclerosing panencephalitis. The value and availability of measles antibody determinations. *JAMA* 228: 604-606.
- Thormar, H., Jervis, G. A., Karl, S. C., Brown, H. R. 1973. Passage in ferrets of encephalitogenic cell-associated measles virus isolated from brain of a patient with subacute sclerosing panencephalitis. *J. Infect. Dis.* 127: 678-685.
- Ueda, S., Okuno, Y., Okuno, Y., Hamamoto, Y., Ohya, H. 1975. Subacute sclerosing panencephalitis (SSPE): Isolation of a defective variant of measles virus from brain obtained at autopsy. *Biken J.* 18: 113-122.
- Ueda, S., Otsuka, T., Okuno, Y. 1975. Experimental subacute sclerosing panencephalitis (SSPE) in a monkey by subcutaneous inoculation with a defective SSPE virus. *Biken J.* 18: 179-181.
- Wechsler, S. L., Fields, B. N. 1978. Differences between the intracellular polypeptides of measles and subacute sclerosing panencephalitis virus. *Nature* 272: 458-460.