BIKEN JOURNAL Vol. 18, 113-122, 1975

SUBACUTE SCLEROSING PANENCEPHALITIS (SSPE): ISOLATION OF A DEFECTIVE VARIANT OF MEASLES VIRUS FROM BRAIN OBTAINED AT AUTOPSY[1]

SHIGEHARU UEDA

Department of Bacteriology, Hyogo College of Medicine, Mukogawa-cho, Nishinomiya, Hyogo and Department of Virology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

YOSHINOBU OKUNO

Department of Preventive Medicine, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

YOSHIOMI OKUNO

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Yamada-kami, Suita, Osaka

YOSHIHIKO HAMAMOTO and HISASHI OHYA

Department of Pediatrics, Kitano Hospital, Nishi-ohgimachi, Kita-ku, Osaka

(Received January 6, 1975)

SUMMARY A cytopathic agent causing formation of syncytial giant cells was isolated by co-cultivation of human embryonic lung cells with brain cells obtained at autopsy from a patient with subacute sclerosing panencephalitis.

Measles specific intracellular immunofluorescence was detected in syncytial giant cells developed by the agent. Paramyxovirus-like nucleocapsids were observed by electron microscopy in nuclei of the syncytial giant cells. Measles specific immunofluorescence was also detected on the surface of unfixed syncytial giant cells. However, the syncytial giant cells did not produce either virions or hemagglutinin, and did not show hemadsorption of African green monkey red cells.

Hence, the newly isolated agent seems to be a defective variant of measles virus, and was designated as the SSPE-" BIKEN " strain.

INTRODUCTION

Subacute sclerosing panencephalitis (SSPE), including the diseases described by Dawson (1933) as " inclusion body encephalitis ", by Pette and Döring (1939) as " nodular panencephalitis " and by van Bogaert (1945) as " subacute sclerosing leukoencephalitis ", is a progressive, degenerative, neurologic disease of

1 Part of this work was presented at the 22nd Annual Meeting of the Society of Japanese Virologists (Sendai, October 10, 1974).

children and adolescents. Dawson first described inclusion bodies in the brain cells of patients with the disease, suggesting its viral etiology (Dawson, 1933).

However, it was not until 1965 that Bouteille et al. (1965) first suggested from electron microscopic studies that it was due to paramyxovirus infection. Connolly et al. (1967) reported elevated titers of measles antibodies in the sera and cerebrospinal fluid (CSF) of patients with SSPE and the presence of measles antigen which was detected in biopsied brain tissue by the immunofluorescent technique in 1967. Baublis and Payne (1968) succeeded in in vitro culture of measles antigen forming syncytia from a brain biopsy specimen of a patient with SSPE and later several groups reported the isolation of measles virus from the brain or lymph nodes of patients with SSPE (Horta-Barbosa, Fuccillo, and Sever, 1969; Katz, Oyanagi, and Koprowski, 1969; Hort-Barbosa et al., 1969; Chen et al., 1969; Payne, Baublis, and Itabashi, 1969; Kettyls et al., 1970; Degré, Vandvik, and Hovig, 1972; Sato et al., 1972; Greenham et al., 1974). Sato et al. (1972) reported the isolation of measles virus which did not produce virions from a brain biopsy specimen of a patient with SSPE in 1972. This was the first case of SSPE reported in Japan.

Different strains of measles virus isolated from the brains or lymph nodes of patients with SSPE vary in virological and biological characteristics. To clarify the pathogenesis of SSPE, comparative studies are needed on the virological and biological characteristics of different strains. Accordingly this paper reports the second case of isolation of measles virus in Japan from the brain tissue of a patient with SSPE and some virological characteristics of this strain.

MATERIALS AND METHODS

1. *Case report*

A 6 year old boy with myoclonic seizures, was admitted to Kitano Hospital on December 17, 1973. His past history was unremarkable except that he had had uncomplicated measles infection with gamma-globulin therapy at the age of 9 months. There was no family history of neurological diseases. The patient lived in an urban district of Osaka and had been in good health until a few months before admission, when he developed psychic symptoms consisted of learning disability, forgetfulness, defiant behavior, negativisms and timidity. These deteriorations of intellect and character were insidious and after a few months were followed by sudden myoclonic jerks of the extremities and head.

The myoclonic jerks became aggravated despite anticonvulsant medication and were easily evoked by excitement, but disappeared in sleep. Physical examination showed that he had a right hemiparesis and repetitive involuntary movements (dystonic or ballismic), especially on that side. He had no cranial nerve symptoms or abnormal extraocular movements, and fundscopic results were normal. Lumbal puncture yielded clear CSF under normal pressure. It contained less than 2 white cells/mm^3, 20 mg/dl of protein, of which 18.9% was gamma-globulin, and 75 mg/dl of sugar. A skull X-ray, carotid angiogram and pneumoencephalogram appeared normal. The electroencephalogram (EEG) demonstrated paroxismal, periodic 2-3 cycle/sec bursts of complexes of spikes and slow waves at a rate of 1 per 10 sec on a normal background activity. The myoclonic jerks coincided with these bursts.

The myoclonic jerks gradually increased in frequency and severity so that he became unable to sit or to walk, and ataxia, tremor, dysphagia and inappropriate verbal responses developed with time. He became expressionless, dysarthric and could only swallow with difficulty.

By February, 1974, the frequency of myoclonic jerks decreased and he became akinetic and unresponsive. Repeated examinations revealed rigido-spasticity, exaggerated tendon reflexes and positive Babinski reflexes with bilateral ankle clonus. Periodic slow wave bursts in the EEGs which were prominent in the myoclonic stage disappeared in the coma stage. Three months after the onset of the jerks, he developed noisy, irregular breathing, hyperthermia and diaphoresis, and lapsed into coma. Despite treatments with ACTH, corticosteroids, anticonvulsants and antiviral chemicals such as cyclocide, he died on March 23, 1974, about 9 months after the onset of the psychic symptoms.

Serological examinations revealed marked elevation of the measles neutralizing, hemagglutination

TABLE 1. *Measles antibody titers[a] in sera and CSF of the patient with SSPE*

	Jan. 26, '74	Jan. 30, '74		Feb. 15, '74		Mar. 22, '74
	Serum	Serum	CSF	Serum	CSF	Serum
NT[b]	13.0	13.5	8.5	12.0	7.5	12.5
HI	12	11	7	—	—	—
CF	8	9	4	—	—	—

[a] 2^n. [b] Neutralization test.

inhibition and complement fixing antibody titers in both the serum and CSF (Table 1). Skin tests for PPD, candida and killed measles vaccine were negative. Clinical and laboratory data supported the diagnosis of SSPE.

2. *Serological examination*

Measles neutralizing antibody titers in the serum and CSF of the patient were measured by the microneutralization test (Ueda, 1971). The hemagglutination inhibition (HI) test and complement fixation (CF) test were carried out by the methods of Toyoshima et al. (1965). For the HI test on the CSF, kaolin treatment was omitted, following the suggestion of Sever et al. (1974).

3. *Co-cultivation of the brain cells with human embryonic lung (HEL) cells*

A small piece (about 0.2 cm^3) of brain tissue was obtained from the right parietal cortex of the patient at autopsy and kept for about 10 hr in chilled growth medium consisting of a mixture of equal volumes of Medium 199 (M 119) and Eagle's minimal essential medium (MEM) supplemented with 20% fetal bovine serum (FBS), 100 μg/ml of streptomycin and 100 units/ml of penicillin.

Then, the piece of brain tissue was incubated in 30 ml of 0.25% trypsin solution for 30 min at 37 C with gentle shaking at intervals. Isolated brain cells were sedimented by low-speed centrifugation and washed once with MEM. The washed brain cells were suspended in 15 ml of growth medium at a final concentration of 4×10^3 cells/ml, and mixed with 1.5×10^6 HEL cells. The mixture was seeded into 3 plastic dishes (6 cm in diam), and the dishes were incubated at 37 C in an atmosphere of 5% CO_2 in air. After incubation for 3 days the co-cultured cells had formed a complete monolayer. The growth medium was changed to maintenance medium consisting of a mixture of equal volumes of M 199 and MEM supplemented with 2% FBS, and this maintenance medium was renewed twice a week.

After 14 days the co-cultivated cells were treated with trypsin and seeded at the same original cell density. At the time of this second subculture, coverslip cultures in Leighton tubes were made for immunofluorescent and cytological examinations.

4. *Immunofluorescent staining*

1) Staining of acetone-fixed cells

Coverslip cultures of the co-cultivated cells or HEL cells infected with the CP agent were fixed in cold acetone for 10 min. Immunofluorescence was examined by the indirect method.

2) Staining of unfixed cells

The method of Häyry and Defendi (1970) was adopted to detect possible antigen on the surface of HEL cells infected with the CP agent.

3) Antisera

The antisera employed for both types of immunofluorescent staining were acute and convalescent sera from patients with typical and atypical measles, and sera from children before and after measles immunization. Sera and CSF obtained at different stages from the patient were also used.

Fluorescein-labelled rabbit antiserum against human gamma-globulin (Eiken Chemical Co., Ltd., Tokyo) was obtained commercially.

5. *Hematoxylin and eosin (HE) staining*

Coverslip cultures of the co-cultured cells or HEL cells infected with the CP agent were fixed with Bouin's solution for 15 min and stained with HE for cytological examination.

6. *Electron microscopy*

The infected monolayer cultures grown in plastic dishes (6 cm in diam) were fixed *in situ* with phosphate-buffered 2% glutaraldehyde for 30 min, and then scraped off the surface of the dish with a

rubber policeman and cetrifuged into a pellet. They were then post-fixed in 1% osmium tetroxide in phosphate buffer for 1 hr and embedded in Spurr's low viscosity epoxy resin (Spurr, 1969) after dehydration in a graded alcohol series and treatment with glycidyl n-butyl ether.

Ultrathin sections were cut with a LKB-4801A microtome, stained with uranyl acetate and lead citrate, and examined under a Hitachi HU-11Ds electron microscope.

7. *Passage and storage of the CP agent*

Trypsinized HEL cells infected with the CP agent were suspended in fresh maintenance medium at 5- to 10-fold the original cell density. The suspension of infected cells was seeded onto confluent monolayers of HEL cells in either glass bottles or plastic dishes, and cultured at 37 C.

As stock of the CP agent, trypsinized HEL cells infected with the CP agent were suspended in growth medium containing 10% dimethylsulfoxide (DMSO) at the one-tenth the original cell density and stored at −80 C.

8. *Hemadsorption and hemagglutination*

Volumes of 0.3 ml of 0.5% suspension of African green monkey red cells were added to coverslip cultures in Leighton tubes at each passage of the CP agent. After 2 hr of incubation at 37 C, the cultures were washed 4–6 times with 3 ml of phosphate-buffered saline and immediately examined microscopically or stained with HE.

Cultures of the CP agent in bottles were disrupted by freezing-thawing 3 times in a dry ice-acetone bath or by ultrasonication. The supernatant fluid after centrifugation at 3,000 rev/min for 15 min at 4 C was tested for hemagglutinating activity.

9. *Plaque reduction test by measles antibody*

Volumes of 0.5 ml of HEL cells infected with the CP agent suspended in the maintenance medium were mixed with an equal volume of hyperimmune rabbit serum against the Tanabe strain of measles virus which had been diluted to a measles neutralizing antibody titer of 1:10 or as a control with an equal volume of normal rabbit serum. After 1 hr of incubation at 37 C, 0.2 ml volumes of the mixtures were inoculated onto confluenct monolayers of HEL cells in 4 plastic dishes (6 cm in diam). The dishes were incubated for 1 hr at 37 C in an atmosphere of 5% CO_2 in air to allow infected cells to come in contact with HEL cell monolayers. The dishes were then overlayed with 6 ml of medium consisting of M 199 (90%), FBS (10%), $NaHCO_3$ (2.25 mg/ml) in 1% Special Agar Noble (Difco Lab., Detroit), and incubated at 37 C in an atmosphere of 5% CO_2 in air. The second overlay was done 4 days after the first one with the same medium containing neutral red (0.12 mg/ml). Plaques were counted 3 days after the second overlay.

10. *Detection of infectious cell-free virus*

The HEL cell cultures infected with the CP agent were disrupted at the 4th, 17th, 18th and 23rd passages by freezing-thawing 3 times in a dry ice-acetone bath or by ultrasonication. Then they were centrifuged at 3,000 rev/min for 15 min at 4 C and 0.5 ml of the supernatant fluid was inoculated into HEL cell coverslip cultures in Leighton tubes. After 1 hr of incubation at 37 C, 1 ml of fresh maintenance medium was added. The tubes were kept at 37 C for 3 weeks and the medium was renewed at intervals. During this period, the coverslip cultures were examined by both immunofluorescent microscopy and HE staining.

RESULTS

1. *Development of syncytial giant cells*

Syncytial giant cells first appeared after 2 sequential subcultures of co-cultivation of the brain cells with HEL cells, that is after 3 weeks of co-cultivation.

Then, the agent inducing syncytial giant cells was successfully passaged by "cell-to-cell" infection. Syncytial giant cells usually appeared within 12 hr after seeding infected cells onto fresh HEL cell cultures. They grew to 1–2 mm in diameter and usually began to autolyze from the center and then became detached from the culture vessels. The site of giant cell detachment became covered by ingrowth of surrounding cells, and another giant cell appeared again in the "healed" monolayer.

On HE staining, syncytial giant cells were seen to contain diffuse eosinophilic matrices in the cytoplasm and patchy eosinophilic inclusion bodies were occasionally detected in the nuclei (Fig. 1).

2. *Immunofluorescent staining*

When fixed with acetone, syncytial giant cells only showed intracellular fluorescence with sera containing measles antibody, such as convalescent sera of patients with typical or atypical measles, sera after measles immunization, and the serum and CSF of the patient. Other sera containing no measles antibody failed to give a specific fluorescence (Table 2). The fluorescence was observed as fine grains diffusely distributed in the cytoplasm of syncytialgiant cells (Fig. 2). Patchy fluorescence was occasionally observed in some nuclei of syncytial giant cells. The fluorescence in microvilli and on cell membranes was weaker than that observed in cells infected with wild or attenuated strains of measles virus.

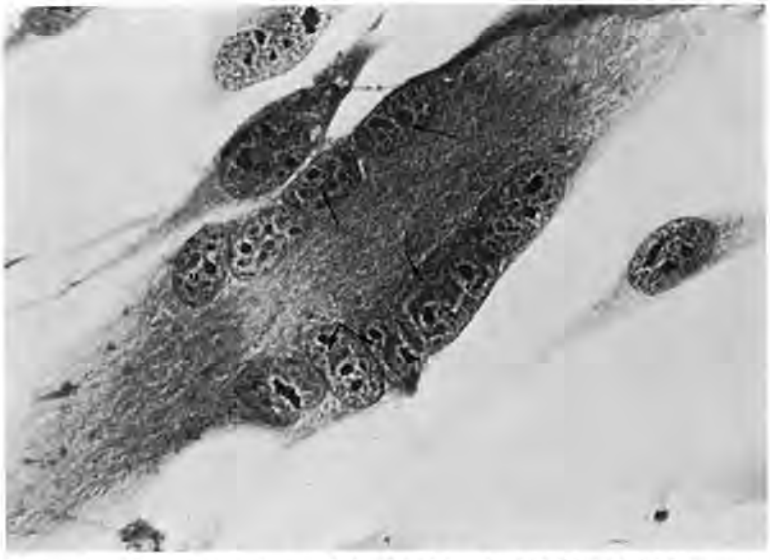


FIGURE 1. *A syncytial giant cell developed by the SSPE-"BIKEN" strain in the HEL cell culture. Intranuclear eosinophilic inclusion bodies are indicated by arrows. Magnification: ×800.*

On staining of unfixed cells, ring-shaped and speckled fluorescence was observed around and on the cell surface of syn-

TABLE 2. *Summary of immunofluorescent study with several measles sera on HEL cells infected with the SSPE-"BIKEN" strain and measles virus*

Sera	Dilution	SSPE virus		Measles virus[a]		Uninfected	
		Acetone-fixed	Unfixed	Acetone-fixed	Unfixed	Acetone-fixed	Unfixed
Measles							
acute	1 : 4	−	−	−	−	−	−
convalescent	1 : 64	+	+	+	+	−	−
Atypical measles							
acute	1 : 4	−	−	−	−	−	−
convalescent	1 : 64	+	+	+	+	−	−
Measles immunization							
pre	1 : 4	−	−	−	ND[b]	−	ND
post	1 : 4	+	+	+	ND	−	ND
SSPE							
serum	1 : 64	+	+	+	+	−	−
CSF	1 : 8	+	+	+	+	−	−

[a] Nagahata strain. [b] Not done.

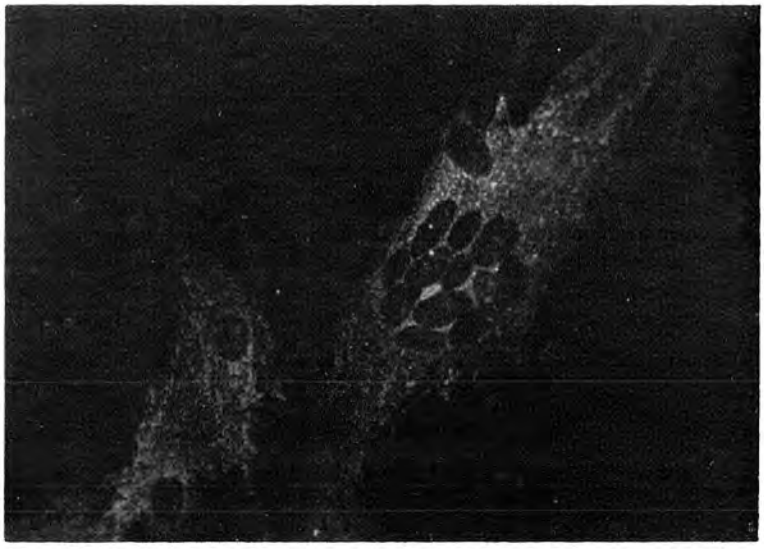
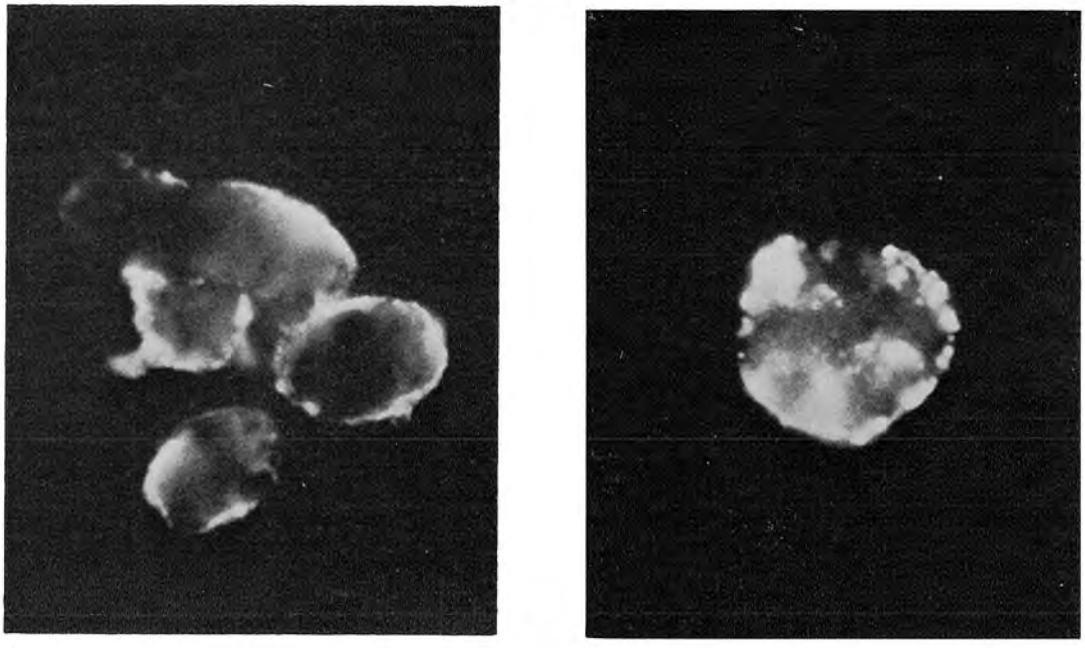


FIGURE 2. *Two syncytial giant cells developed by the SSPE-" BIKEN " strain in a HEL cell culture show intracytoplasmic immunofluorescence. Stained with convalescent serum from a patient with typical measles. Magnification: × 400.*

cytial giant cells when staining was done with sera containing measles antibody or CSF of the patient (Fig. 3a, b).

3. *Electron microscopy*

The nuclei of syncytial giant cells showed sparse matrices. The cytoplasm had lost most of the normal structures, and the endoplasmic reticulum in particular had no microsomes. Aggregated strands resembling paramyxovirus nucleocapsids were found in the nuclei of syncytial giant cells. The strands lacked a " fuzzy " coat and had an outer diam-



a) b)

FIGURE 3. (*a*) *Ring-form,* (*b*) *speckled immunofluorescence on the surface of syncytial giant cells. Stained with convalescent serum from a patient with typical measles. Magnification: × 1,000.*

eter of about 120 A (Fig. 4).

Neither budding of virions nor cell-free virions were observed.

4. *Hemadsorption, hemagglutinin and infectious cell-free rirus*

Since the establishment of the brain cells co-cultivated with HEL cells 6 months ago, the syncytial giant cell forming agent has now been passed 23 times through HEL cells.

Usually, HEL cells infected with wild or attenuated strains of measles virus show strong adsorption of African green monkey red cells and produce hemagglutinin of high titer. However, syncytial giant cells developed by the CP agent failed to adsorp red cells or produce hemagglutinin.

No infectious cell-free virus was detected in the fluid of freeze-thawed or ultrasonicated cultures of the CP agent.

5. *Plaque reduction test by measles antibody*

The plaques produced by HEL cells infected with the CP agent decreased in number and size when the infected cells were treated with measles antibody (Table 3).

TABLE 3. *Plaque reduction of HEL cells infected with the SSPE-"BIKEN" strain by measles antibody*

Antibody	No. of plaques	Mean	Reduction %
+	29, 27, 27, 26	27.3	52.0
—	66, 55, 45, 44	52.5	

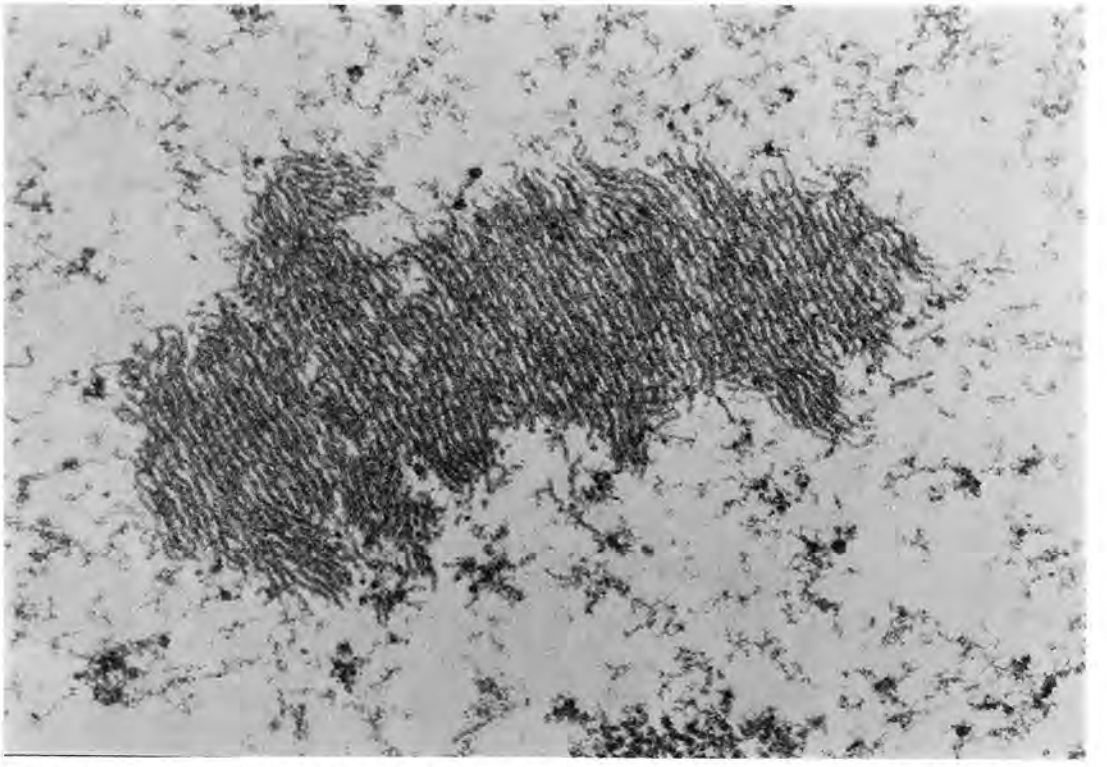


FIGURE 4. *An aggregate of nucleocapsids in the nucleus of a syncytial giant cell developed by the SSPE-"BIKEN" strain in a HEL cell culture. Magnification: ×60,000.*

DISCUSSION

The CP agent which was newly isolated in co-cultures of HEL cells with brain cells of a patient with SSPE forms syncytial giant cells. Measles specific immunofluorescence was detected mainly in the cytoplasm and occasionally in the nuclei of syncytial giant cells. This fluorescence was diffusely distributed in the cytoplasm and appeared as speckls in the nuclei. But the CP agent produces neither virions nor hemagglutinin, so it seems to be a defective variant of measles virus and was designated as the SSPE-" BIKEN " strain.

Various distributions of immunofluorescence have been reported by different groups on different strains (Freeman et al., 1967; Baublis and Payne, 1968; Horta-Barbosa et al., 1969, 1971; Oyanagi et al., 1971; Degré et al., 1972; ter Meulen et al., 1972; Hamilton et al., 1973), and the distribution seems to depend on either the stage after infection or whether the strain produces virions or not.

The syncytial giant cells developed by the SSPE-" BIKEN " strain had surface antigen(s) of measles virus, as shown by indirect immunofluorescent staining and the plaque reduction test. Doi et al., (1972) reported similar results of a plaque reduction test with the Niigata-1 strain of SSPE virus which did not produce virions. Moreover, in studies on the same strain Dubois-Dalcq et al. (1974) clearly showed the surface antigen by electron microscopy using immunoperoxidase. In the present work the surface antigen was stained with the serum or CSF from the patient as well as with convalescent sera from patient with measles. This suggests the existence of antibody against surface antigen in the serum and CSF of the patient. It is still uncertain whether the surface antigen detected by immunofluorescent staining and that shown by the plaque reduction test were actually the same. However, the existence of antibody reacting with the surface antigen in the CSF of the patient might have affected the long-term or subacute clinical course of the disease. Moreover the negative skin test of the patient to a killed measles virus vaccine seems to indicate a defect in cellular immunity, resulting in failure to eliminate latently infected cells in the brain with measles antigen(s) on the surface, as postulated by Burnet (1968).

The defectiveness of SSPE virus and/or abnormality of cellular and humoral immunoresponse of the patient might cause the onset of SSPE, though studies are required on which is the cause and which the effect.

The SSPE-" BIKEN " strain failed to produce virions and hemagglutinin even after the 23rd passage through HEL cells. Nevertheless, high titers of HI antibody were detected in the serum and CSF of the patient. This discrepancy between the lack of production of hammagglutinin by the SSPE-" BIKEN " strain and the existence of high titers of HI antibody in the patient, and the relationship between hemagglutinin and the surface antigen of cells infected with the SSPE-" BIKEN " strain are under study.

ACKNOWLEDGMENTS

We thank Prof. K. Fukai of the Department of Preventive Medicine and Dr. S. Nii of the Department of Pathology, this Institute for valuable advice on election microscopy.

REFERENCES

Baublis, J. V., and F. E. Payne. 1968. Measles antigen and syncytium formation in brain cell cultures from subacute sclerosing panencephalitis (SSPE). Proc. Soc. Exp. Biol. Med. 129: 593–597.

Bouteille, M., C. Fontaine, Cl. Vedrenne and J. Delarue. 1965. Sur un cas d'encéphalite subaiguë à inclusions. Etude anatomo-clinique et ultrastructurale. Rev. Neurol. 113: 454–458.

Burnet, F. M. 1968. Measles as an index of im-

munological function. Lancet 2: 610–613.

Chen, T. T., I. Watanabe, W. Zeman, and J. Mealey, Jr. 1969. Subacute sclerosing panencephalitis: Propagation of measles virus from brain biopsy in tissue culture. Science 163: 1193–1194.

Connolley, J. H., I. V. Allen, L. J. Hurwitz, and J. H. D. Millar. 1967. Measles-virus antibody and antigen in subacute sclerosing panencephalitis. Lancet 1: 542–544.

Dawson, J. R. 1933. Cellular inclusions in cerebral lesions of lethargic encephalitis. Am. J. Pathol. 9: 7–16.

Degré, M., B. Vandvik, and T. Hovig. 1972. Subacute sclerosing panencephalitis: Isolation and ultrastructural characterization of a measles-like virus from brain obtained at autopsy. Acta Pathol. Microbiol. Scand. [B] 80: 713–723.

Doi, Y., T. Sampe, M. Nakajima, S. Okawa, T. Kato, H. Ito, T. Sato, K. Oguchi, T. Kumanishi, and T. Tsubaki. 1972. Properties of a cytopathic agent isolated from a patient with subacute sclerosing panencephalitis in Japan. Jap. J. Med. Sci. Biol. 25: 321–333.

Dubois-Dalcq, M., L. H. Barbosa, R. Hamilton, and J. L. Sever. 1974. Comparison between productive and latent subacute sclerosing panencephalitis viral infection in vitro. An electron microscopic and immunoperoxidase study. Lab. Invest. 30: 241–250.

Freeman, J. M., R. L. Magoffin, E. H. Lennette, and R. M. Herndon. 1967. Additional evidence of the relation between subacute inclusion-body encephalitis and measles virus. Lancet 2: 129–131.

Greenham, L. W., D. B. Peacock, T. J. Hill, B. Brownnell, and W. H. Schutt. 1974. The isolation of S.S.P.E. measles virus in newborn mice. Arch. Gesamte Virusforsch. 44: 109–120.

Häyry, P., and V. Defendi. 1970. Surface antigen(s) of SV40-transformed tumor cells. Virology 41: 22–29.

Hamilton, R., L. Barbosa, and M. Dubois. 1973. Subacute sclerosing panencephalitis measles virus: Study of biological markers. J. Virol. 12: 632–642.

Horta-Barbosa, L., D. A. Fuccillo, and J. L. Sever. 1969. Subacute sclerosing panencephalitis: Isolation of measles virus from a brain biopsy. Nature 221: 974.

Horta-Barbosa, L., R. Hamilton, B. W. Witting, D. A. Fuccillo, J. L. Sever, and M. L. Vernon. 1971. Subacute sclerosing panencephalitis: Isolation of suppressed measles virus from lymph node biopsies. Science 173: 840–841.

Horta-Barbosa, L., D. A. Fuccillo, W. J. London, J. T. Jabbour, W. Zeman, and J. L. Sever. 1969. Isolation of measles virus from brain cell cultures of two patients with subacute sclerosing panencephalitis. Proc. Soc. Exp. Biol. Med. 132: 272–277.

Katz, M., S. Oyanagi, and H. Koprowski. 1969. Subacute sclerosing Panencephalitis: Structures resembling myxovirus nucleocapsids in cells cultured from brain. Nature 222: 888–890.

Kettyls, G. D., H. G. Dunn, N. Dombsky, and I. M. Turnbull. 1970. Subacute sclerosing panencephalitis: Isolation of a measles-like virus in tissue culture of brain biopsy. Canad. Med. Ass. J. 103: 1183–1184.

Oyanagi, S., V. ter Meulen, M. Katz, and H. Koprowski. 1971. Comparison of subacute sclerosing panencephalitis and measles viruses: an electron microscope study. J. Virol. 7: 176–187.

Payne, F. E., J. V. Baublis, and H. H. Itabashi. 1969. Isolation of measles virus from cell cultures of brain from a patient with subacute sclerosing panencephalitis. N. Engl. J. Med. 281: 585–589.

Pette, H., und G. Döring. 1939. Über eine einheimische Panencephalomyelitis vom Charakter der Encephalitis japonica. Dtsch. Z. Nervenheilk. 149: 7–44.

Sato, T., K. Oguchi, T. Tsubaki, T. Kumanishi, Y. Doi, T. Sanpe, and H. Ito. 1972. Isolation of measles agent from a patient with subacute sclerosing panencephalitis and inoculation in animals. Shinkei-gaku Topics 2: 71–77. [In Japanese]

Sever, J. L., H. Krebs, A. Ley, L. H. Barbosa, and D. Rubinstein. 1974. Diagnosis of subacute sclerosing panencephalitis. The value and availability of measles antibody determinations. JAMA 228: 604–606.

Spurr, A. R. 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. 26: 31–43.

ter Meulen, V., M. Katz, Y-M. Käckell, G. Barbanti-Brodano, H. Koprowski, and E. Lennette. 1972. Subacute sclerosing panencephalitis: In-vitro characterization of viruses isolated from brain cells in culture. J. Infect. Dis. 126: 11–17.

Toyoshima, K., T. Kitawaki, K. Otsu, M. Mutai, S. Omura, and N. Kunita. 1965. Studies on the

serological standardization of measles virus. Biken J. 8: 87–94.
Ueda, S. 1971. Comparison of measles antibody titers measured by the micro- and macro-methods. Biken J. 14: 155–160.
van Bogaert, L. 1945. Une leuco-encéphalite sclérosante subaiguë. J. Neurol. Neurosurg. Psychiat. 8: 101–120.