Acta Medica Okayama

Volume 24, *Issue* 6 1970 *Article* 3

DECEMBER 1970

Electron microscopic demonstration of a new virus isolated from a patient with SMON

Zensuke Ota*

*Okayama University,

Electron microscopic demonstration of a new virus isolated from a patient with SMON*

Zensuke Ota

Abstract

The first successful electron-microscopic observation of a virus isolated from a patient with SMON was performed. The morphological and developmental characteristics of this virus suggests that this type of virus has not been isolated from humans. Hence, it is considered that the virus observed is of a new type and presumably the causative agent of SMON. The author wishes to express his profound thanks to Prof. TADASHI OFUJI for painstaking proof reading of the manuscript and also acknowledgement is due to Mr. NOBUO HAYASHI, Mr. NOBORU SAIHARA, Mr. TAKASHI NAKAMURA and Miss TOSHIYO OMIZU for their technical assistance of electron microscopy.

---

*PMID: 4258715 [PubMed - indexed for MEDLINE]

Acta Med. Okayama **24**, 573—577 (1970)

ELECTRON MICROSCOPIC DEMONSTRATION OF A NEW VIRUS ISOLATED FROM A PATIENT WITH SMON

Zensuke OTA

Department of Internal Medicine, Okayama University Medical School, Okayama, Japan (Director: Prof. T. Ofuji)

Received for publication, October 1, 1970

Hitherto unknown encephalomyeloneuropathy that characteristically follows acute or chronic abdominal distress has been observed throughout the country since about 1955 and is tentatively designated as subacute myelo-optico-neuropathy, or briefly as SMON (1).

According to our epidemiological surveys on the prevalence of this condition in Yubara district of Okayama Prefecture (2), it is most likely that this condition is infectious and the causative agent is a virus.

Several known viruses isolated from patients' materials have been claimed to be the causative agent (3, 4). However, none of them gave positive results in confirmation experiments (2). Recently, INOUE (5) observed cytopathogenic effect of millipore-filtered extract of patient's feces on culture cells and considered this as the successful isolation of the causative virus of SMON.

It is the purpose of this communication to present a successful electron microscopic observation of a virus isolated by INOUE.

MATERIALS AND METHODS

The virus was Sato strain isolated by INOUE (5) which had been maintained by passages in BAT 6 cells. The BAT 6 cell line has been established from a subcutaneous tumor of a hamster induced by bovine adenovirus type 3 and maintained by INOUE (5). The virus and BAT cells were kindly supplied from Dr. Y. INOUE, Virus Research Institute, Kyoto University, for this study. The tissue culture method was the same as INOUE's description (5). Some of the culture media for virus propagation contained a small amount of tetracycline. For electron microscopic observation, cells showing cytopathogenie effect 4 to 6 days after the virus infection were collected, fixed with glutaraldehyde and osmium tetroxide and embedded in Epon. The sections were observed by the electron microscope after uranyl acetate and lead stainings.

(This study was presented at The General Congress of SMON Research Committee held in Tokyo on June 30, 1970)

RESULTS

Fig. 1 shows the earliest stage of virus formation. A dark arch-shaped structure is observed directly under a protrusion of the cell membrane.

Fig. 2 illustrates the following stage of virus formation. A virus particle is seen budding from a microvillus. The particle is composed of an outer membrane 140 mμ in diameter, *i. e.* envelope, an inner membrane 90 mμ in diameter, *i. e.* nucleocapsid and an electron-lucent core.

In Fig. 3 there is a virus particle with sparse spikes 10 mμ long on the envelope in a cytoplasmic vacuole. The particle has a small surface protrusion, pedicel, which is presumably connected with the membrane of the cytoplasmic vacuole.

Fig. 4 displays a virus particle of doughnut shape in a cytoplasmic vacuole. The diameter of the envelope is 100 mμ and that of the nucleocapsid 70 mμ.

All of the virus particles seen in Figs. 1, 2, 3 and 4 are immature. Although some of them are not connected with the cell membrane in the sections, they appear to be in budding process from the cell membrane.

A mature virus particle is illustrated in Fig. 5. The particle consists of an envelope 140 mμ in diameter, relatively electron-lucent viroplasma 27 mμ wide and a round, electron-dense nucleoid 85 mμ in diameter. A marginal layer that is 10 mμ in width and less electron-dense than the central portion is descernible within the nucleoid. This layer seems to correspond to the nucleocapsid structure of the immature virus particle. The envelope of the particle is still in contact with the protruded vacuolar membrane (arrow). This means that the particle is in budding process.

The following is the summary of the results obtained. The virus particle is produced by budding process from the membranes of the cell surface, microvilli and vacuoles. The particle in budding process, *i. e.* immature particle, is composed of an envelope derived from the membraneous structures of the cells, an electron-dense nucleocapsid and an electron-lucent central core. The nucleocapsid shows an arch figure in the earliest stage of budding process and gradually becomes circular or ring-shaped. At the end stage of budding, the nucleocapsid forms a nucleoid with an increase in electron density at the electron-lucent central core and then the immature particle becomes mature. After maturation, the particle is released from the pedicle to be free. The mature virus particles exist in the outside of the cells or in the inside of the cytoplasmic vacuoles. The diameter of the virus particles, mature or immature, varies from 100

mμ to 140 mμ and that of the nucleocapsid from 70 mμ to 90 mμ.

In addition to the above observation, numerous mycoplasma ranging from 150 mμ to 800 mμ in diameter were observed together with the virus particles in the specimens prepared from tetracycline-free cultures as in Fig. 6.

The virus particles above mentioned were seen either in tetracycline-containing or tetracycline-free cultures. However, in both specimens virus particles, particularly mature ones, were relatively rare.

The virus particles and mycoplasma were not observed by the electron microscope in the control cells of the BAT 6 cell line.

DISCUSSION

The virus particle observed in the present study closely resembles mouse leukemia virus in the morphological aspect. However, the followings are the characteristics of the present virus different from leukemia virus. The mature virus particle in this study has a denser nucleoid, the formation of which is completed before its release from the pedicle. According to INOUE's study (5), this virus shows a clear cytopathogenic effect and is analysed to be DNA in nucleic acid type. The formation process of this virus virion is much more similar to Japanese encephalitis virus, since the nucleoid of both viruses is completed before their release from cells (6).

Although broad studies on this virus have not yet been accumulated, it is considered that this virus can be a causative agent of SMON. As for mycoplasma, it is presumed that this might be a passenger microorganism, since in many known morbid conditions, mycoplasma has been isolated merely as a passenger (7).

SUMMARY

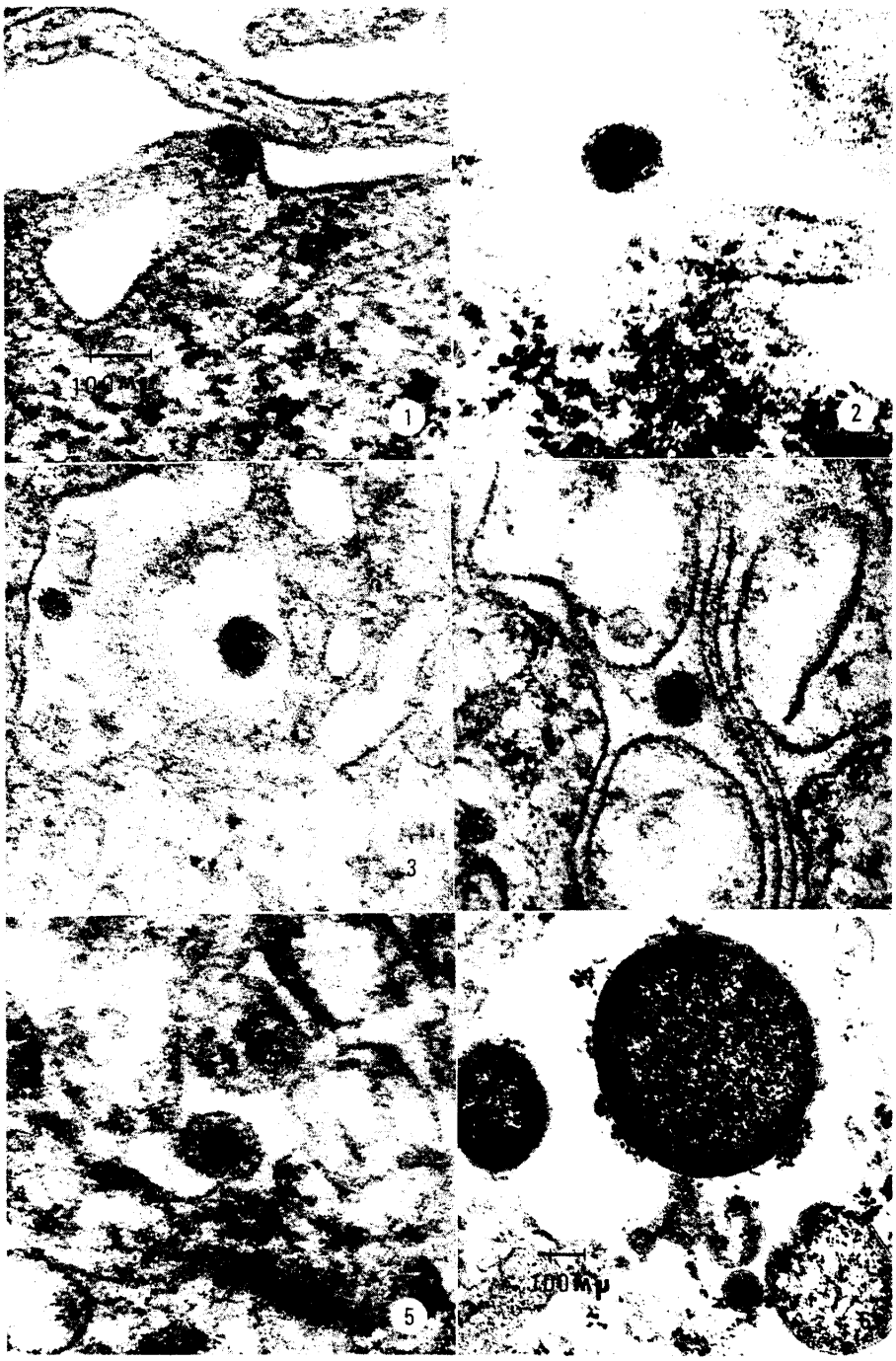
The first successful electron-microscopic observation of a virus isolated from a patient with SMON was performed. The morphological and developmental characteristics of this virus suggests that this type of virus has not been isolated from humans. Hence, it is considered that the virus observed is of a new type and presumably the causative agent of SMON.

ACKNOWLEGEMENT

The author wishes to express his profound thanks to Prof. TADASHI OFUJI for painstaking proof reading of the manuscript and also acknowledgement is due to Mr. NOBUO HAYASHI, Mr. NOBORU SAIHARA, Mr. TAKASHI NAKAMURA and Miss TOSHIYO OMIZU for their technical assistance of electron microscopy.

REFERENCES

1. TSUBAKI, T., TOYOKURA, Y. and TSUKAGOSHI, H.: Symposium on non-specific encephalomyelitis (1) subacute myelo-optico-neuropathy following abdominal symptoms—a clinical and pathological study. *J. J. S. I. M.* **53**, 779, 1964
2. OTA, Z.: An epidemiological and clinical survey on the prevalence of SMON in Yubara district of Okayama. *Jap. Med. J.* **2407**, 15, 1970
3. SHINGU, M. *et al.*: Isolation of ECHO type 21 virus from patients with "infectious column myelitis", a myelitis following abdominal signs. *J. J. A. Inf. D.* **39**, 139, 1965
4. MIYAHARA M. *et al.*: A case of so-called "subacute-myelo-optico-neuropathy" (SMON) from which Coxcackie type A 4 was isolated. *J. J. S. I. M.* **57**, 1113, 1968
5. INOUE, Y. K., NISHIBE, Y. and NAKAMURA, Y.: A new virus frequently isolated from feces of patients with SMON. *Igaku No Ayumi* **72**, 321, 1970
6. OTA, Z.: Electron microscopic study of the development of Japanese encephalitis virus in porcine kidney stable (PS) cells. *Virology* **25**, 372, 1965
7. DMOCHOWSKI, L. *et al.*: Viruses and mycoplasma (PPLO) in human leukemia. *Cancer* **18**, 1345, 1965



Figs 1 to 5 : × 80, 000 Fig 6 : × 40, 000