BIKEN JOURNAL
Preliminary Report

Detection of Measles Viral Antigen in the Lymph Nodes of Cynomolgus Monkeys Infected with Measles

On infection of human beings and monkeys with measles the appearance of giant cells in the respiratory organs and of so-called Warthin-Finkeldey cells in the lymphatic tissues have been characteristically observed.

In our preceding experiments not only were such pathological changes recognized extensively in many tissues and organs of cynomolgus monkeys infected with epidemiological (or wild) virus, but the existence of intranuclear and cytoplasmic inclusion bodies in giant cells in the lymph nodes was recognized. (Nii and Kamahora, 1963b; Nii *et al.* 1963c). Hitherto, these inclusions have only been observed cytologically in reticular giant cells. As inclusion bodies in cells are a definite sign of infection, their detection in this case indicates the growth of measles virus in the lymphatic tissues and thus confirmes the important role played by mesenchymal cells during measles infection which has been stressed by many authors.

In addition to the above cytopathological findings, a further proof of the growth of the virus would be to measure the distribution of antigen in the tissues of infected monkeys using the fluorescent antibody technique.

This report describes attempts to demonstrate the existence of measles viral antigen in affected lymph nodes and to confirm the significance of the lymphatic tissues during measles infection.

The experimental procedures used for infection of cynomolgus monkeys with epidemiological virus were described previously (Nii and Kamahora, 1963b; Nii *et al.* 1963c). The lymph nodes of the infected animals (Cases No. 28 and 23) were examined by the fluorescent antibody technique as well as by the routine histological method.

At autopsy the lymph nodes were each cut in half and one half was immediately stored at $-20°C$ until just before examination of fluorescence, while the other half was immersed in usual histological fixatives. Histological findings on these tissues were reported previously. Frozen sections of the affected lymph nodes were made at about $-17°C$ and slices of approximately 4μ thickness were prepared. Then the slices were immediately fixed by immersion in acetone at $-20°C$, or were air dried before fixation.

The serum of patient who had suffered from severe measles complicated by encephalitis was obtained about one month after the onset of his illness. To prepare fluorescein conjugated antibody the same method as that reported by Nii *et al.* (1963a) was used and the globulin was conjugated with fluorescein isothiocyanate (Baltimore Biological Laboratories) essentially by the method of

Marshall *et al.* (1958). Before staining preparations of infected tissues, the specificity of these procedures was ascertained using FL cells infected with Toyoshima strain virus. The appearance of fluorescent materials in the infected FL cells was in good accordance with the report by Enders et al. (1956). These materials were finely distributed in the cytoplasm at an early stage of infection (Figs. 1 and 2) and later in gross brilliant masses in various areas of the cytoplasm. In the final stage, whole syncytial cell masses were filled with fluorescence but the existence of antigen in the nuclei was uncertain.

All lymph nodes tested, such as the axillar, inguinal and mesenterial lymph nodes which were obtained from two monkeys, had measles viral antigen in the tissues, (Figs. 3 and 4). As controls, the lymph nodes of a monkey which had been inoculated with the Toyoshima strain of measles about half a year previously and was already immune to the agent, were used. No viral antigen could be detected in these tissues.

The distribution of fluorescent materials in the lymph node was in good accordance with that of the pathological lesions of the lymph nodes and these materials were found most frequently in marginal sinuses and adjacent portions of the cortical cords as well as in the germinal centers. They were observed in the medullary cords rather less frequently. Sometimes many fluorescent cells were found to be agglutinated and these cell masses may correspond to measles giant cells or Warthin-Finkeldey cells. It was distinctly seen that many single cells, resembling reticular cells, had antigens regardless of the concomitant existence of Warthin-Finkeldey cells in the same field. In histological preparations stained with haematoxylin and eosin, the Warthin-Finkeldey cells were one of the most obvious pathological changes. Therefore, it must be noted that the fluorescent antibody technique demonstrated many more affected cells in the lymph node than the usual histological technique. However, it is possible that the cells containing fluorescent materials were not all affected by virus and some might be reticulum cells phagocytosing viral antigens derived from disrupted infected cells.

In addition to the so-called Warthin-Finkeldey cells seen in the lymphatic tissues, the detection of viral antigen as well as inclusion bodies in the affected lymph nodes provide positive proof that the growth of measles virus undoutedly occurs in the lymphatic tissues of host animals.

REFERENCES

Enders, J. F. (1956). Observations on certain viruses causing exanthematous diseases in man. *Amer. J. Med. Sci.* **231,** 622-637.

Marshall, J. D., Eveland, W. C. and Smith, C. W. (1958). Superiority of fluorescein isothiocyanate (Riggs) for fluorescent-antibody technique with a modification of its application. *Proc. Soc. Exptl. Biol. Med.* **98,** 898-900.

Nii, S. and Kamahora, J. (1963a). Location of herpetic viral antigen in interphase cells. *Biken J.* **6,** 145-154.

Nii, S. and Kamahora, J. (1963b). Detection of giant cells bearing inclusion bodies in lymph nodes

of monkeys inoculated with materials obtained from human cases of measles. *Biken J.* **6,** 229-231.

Nii, S., Kamahora, J., Mori, Y., Takahashi, M., Nishimura, S. and Okuno, Y. (1963c). Experimental pathology of measles in monkeys. *Biken J.* **6,** 271-297.

SHIRO NII* and JUNTARO KAMAHORA**

* *Department of Pathology,*
** *Department of Tumor Viruses,*
The Research Institute for Microbial Diseases,
Osaka University, Osaka
(*Received for publication, July* **5, 1964**)

EXPLANATION OF FIGURES

Fig. 1. FL cells infected with the Toyoshima strain of measles. A large syncytium having finely distributed fluorescent masses in the cytoplasm. × 1440.

Fig. 2. Only one FL cell containing fluorescent materials is seen, while the other cells in the plate have no fluorescence. × 1440.

Fig. 3. Disseminated fluorescent materials in the lymph follicles of the left axillar lymph node of the monkey (Case No. 23). × 700.

Fig. 4. A higher magnification of the preparation shown in Fig. 3. Reticulum-like cells contain fluorescent materials. × 1440.

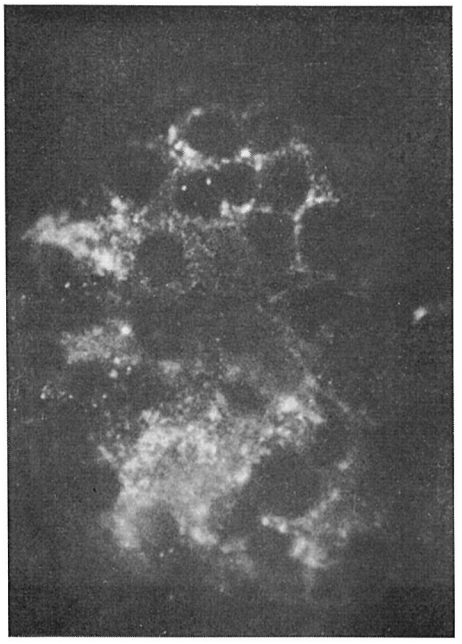

Fig. 1

Fig. 2

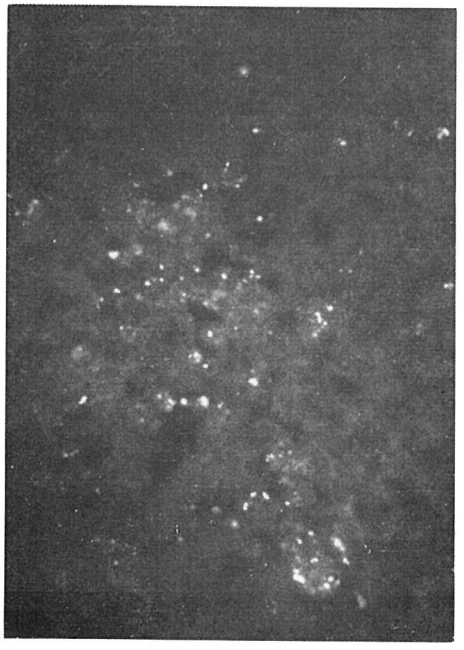

Fig. 3

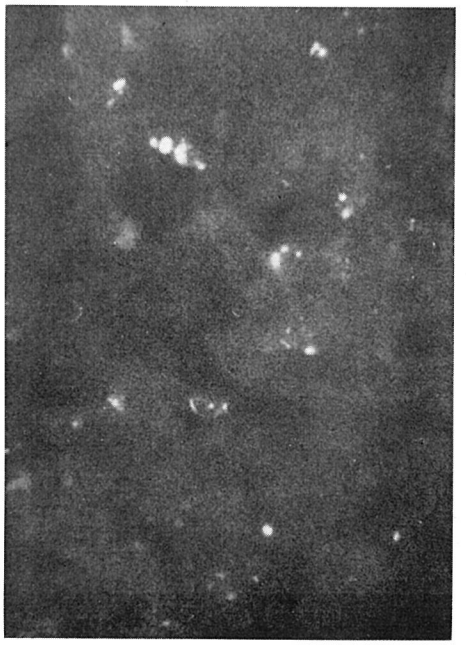

Fig. 4