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THE COMPARATIVE STUDIES ON THE SURFACE OF CELLS INFECTED WITH TYPE 1 AND TYPE 2 HERPES SIMPLEX VIRUS BY SCANNING ELECTRON MICROSCOPY

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Abstract

The differences between in the surface of type 1 and type 2 herpes simplex virus (HSV) infected cells were noticeable, when these cells were observed in detail by scanning electron microscopy (SEM).
1. Whereas many 100-250 nm particles on the type 1 HSV infected human embryo fibroblast (HE) cells surface were seen, a few on the type 2 infected cells. Therefore we can easily distinguish these two strains.
2. The evidence is applicable in the laboratory strains and the freshly isolated strains from the patients. The result obtained from this observation might indicate that these differences are significant as biological markers and provide an useful evidence in the laboratory diagnosis of herpes infection.

INTRODUCTION

A few investigators having reported[1,2] that they can distinguish these two types of HSV by observing the cytopathic effects (CPE) with the light microscopy, it is not always easy. Our study was undertaken to examine the surface of HE cells infected with two types of HSV by scanning electron microscopy and to exploit the differences between two types as markers for identification and characterization.

MATERIALS AND METHODS

Strains of HSV: Takeuchi strain of type 1 and Ogiwara strain of type 2 were kindly supplied by Dr. U. Aoyama, Department of Pathology, Institute of Medical Science, The University of Tokyo. Other six strains which consisted of three strains (type 1) and three strains (type 2) used in this study were obtained from our patients. HE cells maintained in

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our laboratory were prepared on a 5×5 mm coverglass in Leighton tubes and then infected with HSV. When the CPE appeared the cells were harvested and fixed doubly with 1% glutaraldehyde and 1% osmium tetroxide, dehydrated in the ethanol series and amylacetate, and then dried by the critical point drying technic. After coating with gold-palladium alloy, the preparations were examined with the Hitachi HHR-2R electron microscope.

RESULTS

Under the SEM, HSV infected cells were shown round and swollen at the low magnification. On a little rounded cells and the flat cells of the foregoing part infected with type 1 the particles, ranging from 100 to 250 nm in diameter, were spread in the wide areas or grouped in many clusters (Fig. 1, 2). The cells formed a spider web like net on the coverglass and the particles covered all over the surface (Fig. 3). On the other hand, in type 2 infected cells these particles were rarely spread on the rounded and flat cells (Fig. 4, 5). As shown in Fig. 6 on the net like cells these are no particles. These particles were different from the bulbing of the cells. Some cells infected with type 1 were covered with these particles (Fig. 7). Furthermore, Fig. 8 shows the particles are connected by themselves. The microvilli were rare on such infected cells on which many particles could be seen. Therefore there were less microvilli on type 1 than type 2 infected cells.

This difference between type 1 and type 2 HSV infected cells were shown both in the laboratory strains and the freshly isolated strains.

DISCUSSION

The spectrum of differences in biologicalactivities of these two types of HSV has expanded, and certain properties have been proposed as biologic markers to separate type 1 from type 2 HSV.[3-5] Nii[1] and Yoshino[2] reported that they could distinguish two type strains of the freshly isolated HSV by observing the cytopathic effect. Couch *et al.*[6] and Schwartz *et al.*[7] reported that microtubules appeared in mainly nucleus type 2 infected cells by thin section technic and this properties have been proposed as biologic markers to discriminate type 2 from type 1. In this study, we examined the surface of cells infected with type 1 and type 2 HSV by SEM and got the above result, which indicates that there are many 100-250 nm particles on the type 1 infected cells but a few on type 2 infected cells.

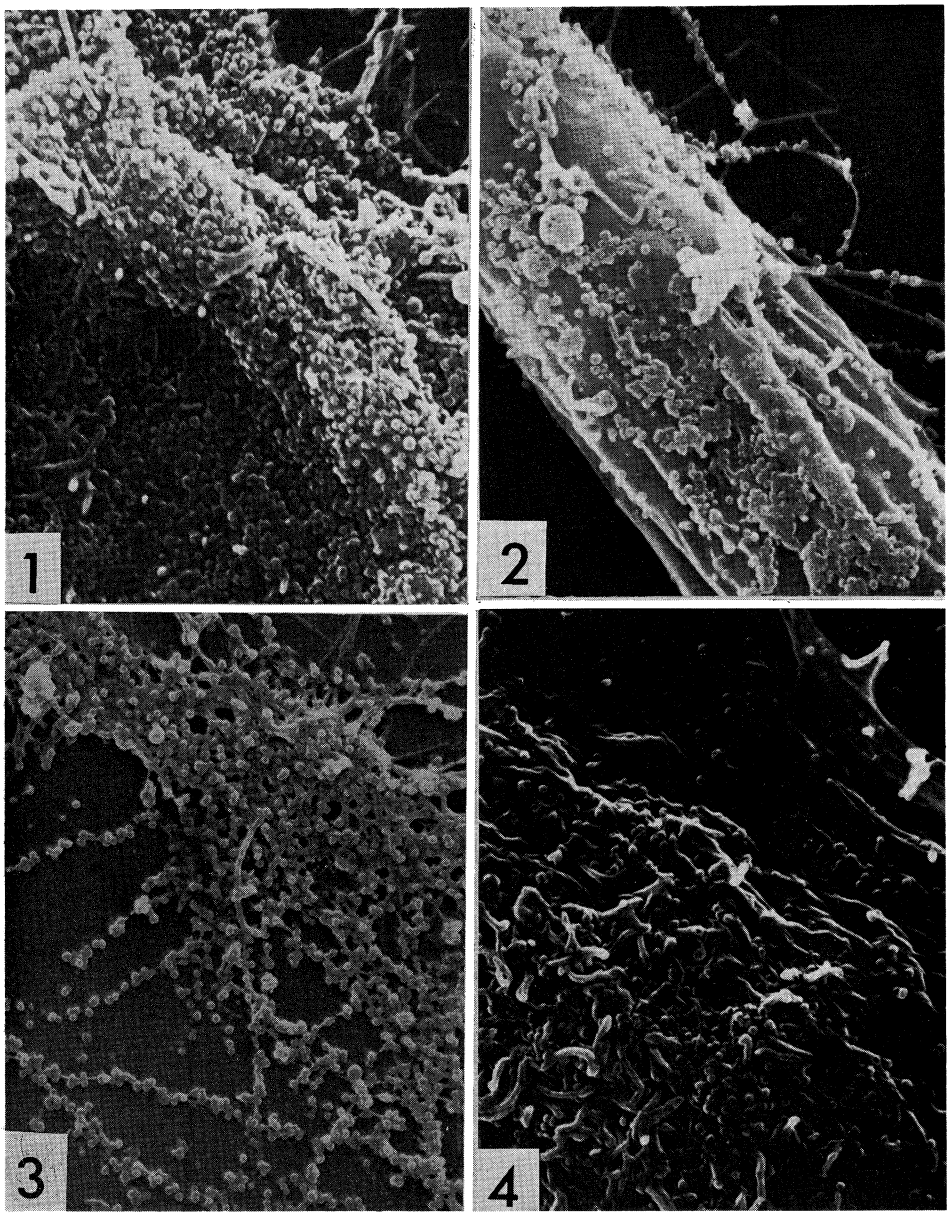

Fig. 1. A part of a little rounded HE cells infected with type 1 HSV. Many particles were seen. ×7,000.
Fig. 2. Flat cells infected with type 1 HSV. ×7,000.
Fig. 3. Net like cells infected with type 1 HSV the particles covered all over the surface. ×7,000.
Fig. 4. A part of a little rounded cells infected with type 2 HSV particles were rarely. ×7,000.

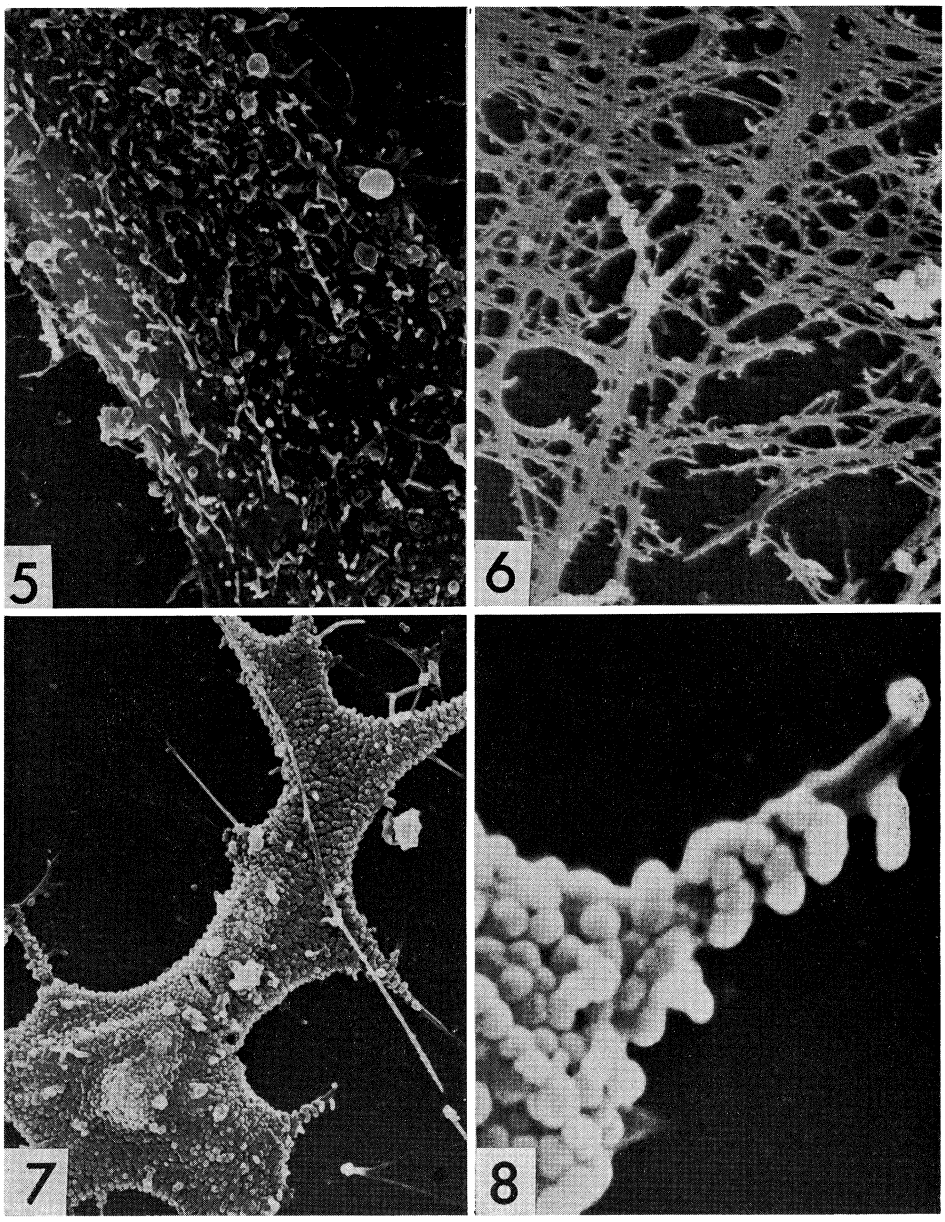

Fig. 5. Flat cells infected with type 2 HSV. ×3,500.
Fig. 6. Net like cells infected with type 2 HSV. There is no particle. ×8,000.
Fig. 7. Virions covered all over the surface of the HE cell infected with type 1 HSV. ×7,000.
Fig. 8. A part of Fig. 7 is shown at higher magnification. ×30,000

The result of thin section technic revealed that the particles were herpes virions at the periphery of the host cells. Plummer *et al.*[8] reported that in the growth curves of the two types of HSV in human fibroblast cultures, type 2 strain attained lower titer of infectivity. Their report showed that the infectivity of type 2 in the culture fluid was higher than the cell associated. Our results showed that type 1 infected cells had more virions on the surface than type 2, suggesting that type 1 produced more virion than type 2 infected cells and type 2 released virion easier than type 1.

Acknowledgment

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