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SCANNING ELECTRON MICROSCOPIC OBSERVATION ON THE SURFACE OF CELLS INFECTED WITH VARICELLA-ZOSTER VIRUS

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Abstract

When varicella-zoster virus (VZV) was cultivated in human embryo fibroblast (HE) cells, a focus of rounded cells appeared and the cytopathic change developed radially, namely it was the characteristic of cytopathic effect (CPE) of VZV.

Many particles were seen, ranging from 130 to 350 nm in diameter, attached to the surface of infected cells when observed in detail by scanning electron microscopy (SEM). Furthermore, it was proved that these particles were herpes virions by section technic. And the result of application of immune adherence (IA) by SEM suggested these particles had specific antigen of VZV. With time, the number of virion appeared on VZV infected cells increased. This result seemed to suggest that these virions accumulated on the surface of the cells without releasing from the cells, and this evidence strongly suggested the characteristic of cell associate about VZV.

INTRODUCTION

As VZV has a characteristic of cell associate in vitro, the experiment on the surface of infected cells by SEM was carried out to elucidate this characteristic. The new result about the appearance of virion on the infected cells surface will be discribed.

METARIALS AND METHODS

Strain of VZV: HS-1 strain cordially supplied by Dr. Hondo, Department of Viral Infection, Institute of Medical Science, The University of Tokyo, and two other strains were obtained from vesicle fluid of zoster patients. The trypsinized cell suspension of infected cells or the sup of freezing and thawing cells was used for virus sources.

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HE cells maintained in our laboratory were used and the SEM preparations were done previously described method.[1] For thin sectioning the infected cells were harvested, fixed with 1% glutaraldehyde, spun down in to pellet by low centrifugation and fixed 1% osmium tetroxide. After dehydration in aceton series the pellet was embedded in Vestopal W. The sections obtained on ultratome were stained doubly with uranyl acetate and lead hydroxide solution, and then they were observed in electron microscopy.

IA test: IA test was applied according to the method[2] of Tachibana *et al.* with some modification. For the antigen the VZV infected cells on the coverglass, for the antibody VZV positive sera,[3] and for the complement fresh guinea pig sera are added alternately, and antigen-antibody-complement complexes are thus prepared. After human red blood cells (RBC) were added to the complexes, the specimens for SEM were prepared by the previsouly described method.

RESULTS

Many particles ranged from 130 to 350 nm in diameter were observed on the surface of cytopathic changed cells infected with VZV. Though on the unchanged cells the particles occupied only a small part of the cells or they got scattered, on the swollen cells, which was white under the SEM low magnification, these particles grouped in cluster. And sometimes these particles lined along the cell long axis (Fig. 1). In the late stage of CPE on the rounded cells these particles were seen as a lump (Fig. 2, 3). On the portion which these particles were seen, microvilli were seen rarely. These particles were observed mainly on the rounded cells, the center of the focus, and the further the distance estranged from the focus, the fewer the number of particles got decreased.

The thin section technic of these CPE cells demonstrated that enveloped particles with nucleocapside and aberrant form[4] particles without nucleocapside were layered evenly on the periphery of the cells (Fig. 4).

IA test applied if the particles had virus specific antigen, and the result shown as in Fig. 5, 6 demonstrated that the RBC adhered to the cells at one or two points. As the CPE progressed the cells had more particles, and adherence of RBC were remarkably increased in number.

DISCUSSION

When VZV propagated in monolayer cultures, no infectious virus were found in the culture fluid. Cook *et al.*[5] suggested the labile coat

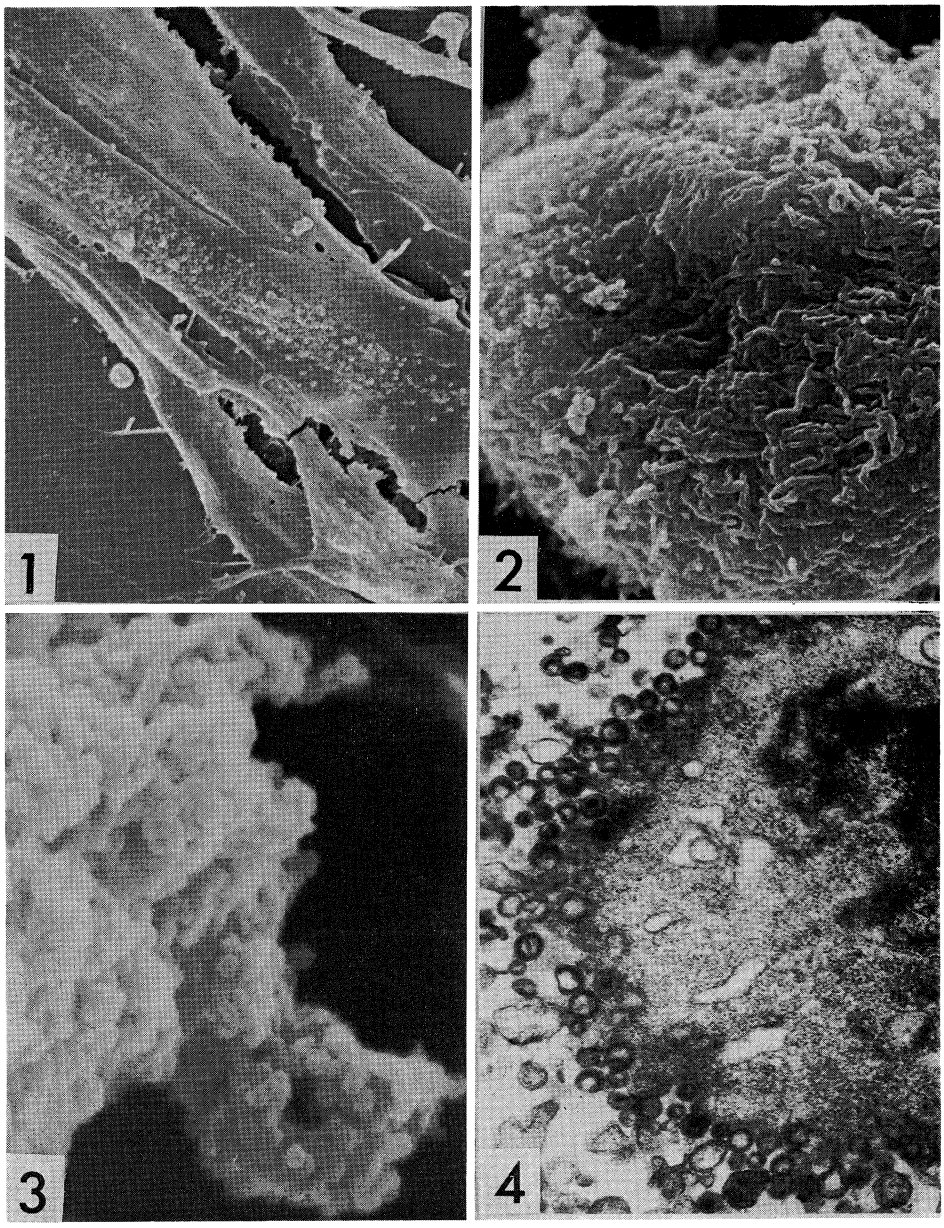
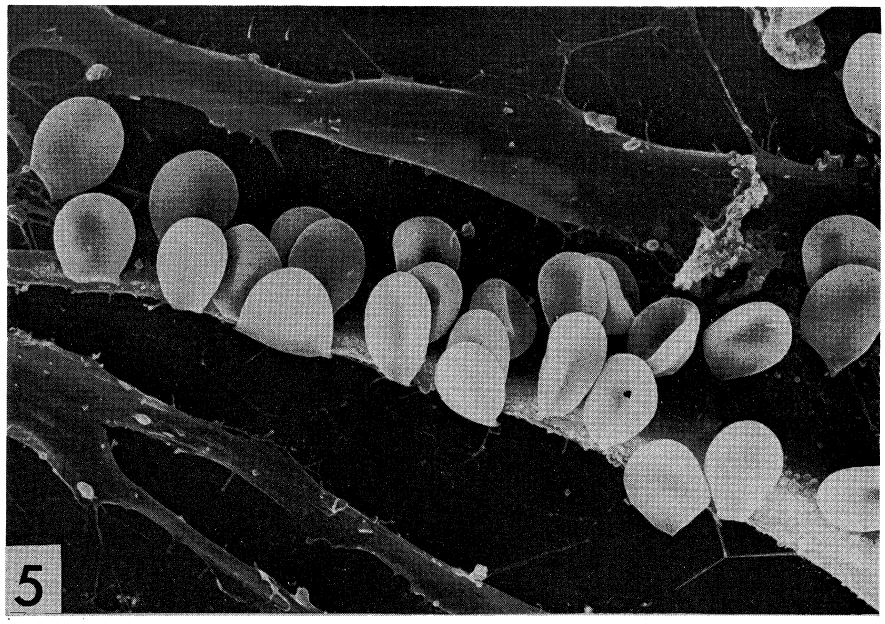


Fig. 1. Many particles lined along the cell long axis on the surface of cells infected with VZV. ×5,000.
Fig. 2. Particles were seen as a lump on the rounded cells. ×6,000.
Fig. 3. A part of Fig. 2 is shown at higher magnification. ×20,000.
Fig. 4. Enveloped particles and aberrant form particles were layered evenly on the periphery of the cells. ×24,000.



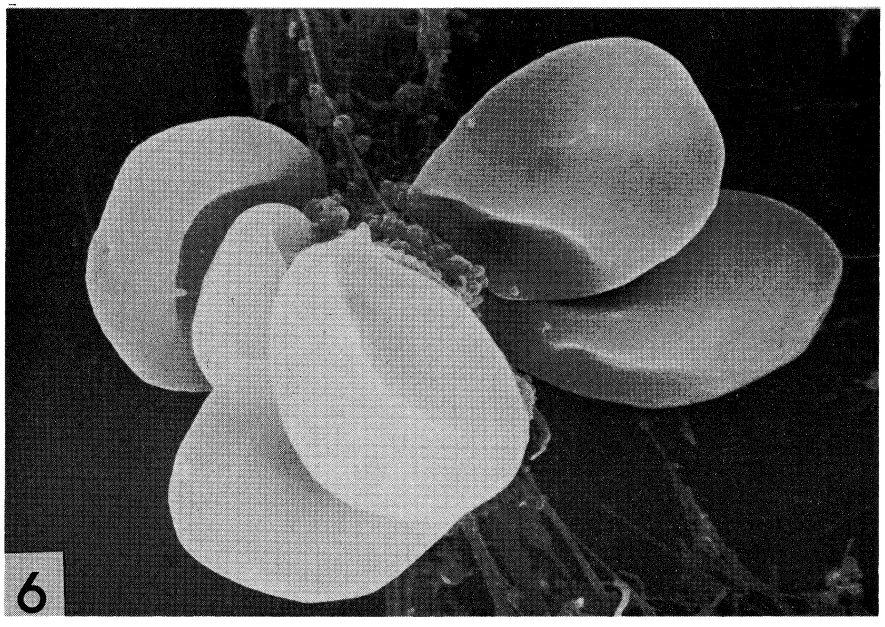


Fig. 5. RBC adhered to the infected cells at one or two points. ×2,000.
Fig. 6. RBC adhered to the cells which have many particles. ×7,000.

was the principal reason for the lack of cell free infectious virus. Gershon *et al.*[6] postulated that presence of lysosomal enzyme and VZV in the same cytoplasmic vacuoles might result in inactivation of VZV during egress of the virus from the cells. We observed on the surface of cells infected with VZV by SEM and the result was obtained; as the cells tranceforming from the flat to the rounded, the particles on the surface increased in number. These particles observed by SEM were prevailed to be enveloped particles and aberrant form particles by thin section technic. These particles might be come off by reverse phagocytosis to outside of the cells and adhere to the cell surface without releasing from the cell.

It was suggested that VZV infection spread from cell to cell by no means contradicted the idea that progeny virus adhered to the host cells which produced the progeny virus and infected only adjacent to neighbouring cells. Under IA test by SEM RBC adhered to the cells which had more particles and this evidence suggested that the more particles the cells had, the more specific antigen increased. These RBC adhered to the cells at one or two points. However it is doubtful whether the RBC adhere to only particles or not, but it may be conceivable the particles have specific VZV antigen.

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