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Abstract

The surface of Gross virus-induced murine lymphoblasts and C-type virus particles budding from these cells were investigated under the scanning electron microscope (SEM). The cells appeared spindle-shaped or roughly-rounded with extensive surface features consisting of microvilli, blebs and ruffled membranes. C-type virus particles were detected on the cell membrane as small spherical particles, distinguishable from the microvilli. Clustered virions were observed in some cases. However, the distribution of virions appeared to be random. The surface of the virion was smooth and had no globular units at high magnification. These morphological observations were confirmed in ultrathin sections.

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MORPHOLOGICAL STUDIES OF GROSS VIRUS-INDUCED LYMPHOBLASTS BY SCANNING ELECTRON MICROSCOPY

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Abstract. The surface of Gross virus-induced murine lymphoblasts and C-type virus particles budding from these cells were investigated under the scanning electron microscope (SEM). The cells appeared spindle-shaped or roughly-rounded with extensive surface features consisting of microvilli, blebs and ruffled membranes. C-type virus particles were detected on the cell membrane as small spherical particles, distinguishable from the microvilli. Clustered virions were observed in some cases. However, the distribution of virions appeared to be random. The surface of the virion was smooth and had no globular units at high magnification. These morphological observations were confirmed in ultrathin sections.

Neoplastic cells transformed *in vitro* by x-irradiation appear pleomorphic and display surface excrescences, such as microvilli, blebs and ruffles (1). Similar observations were reported by chemically transformed mouse embryo cells (2). Porter, Todaro and Fonte (3) have employed SEM to study the topography of virally and spontaneously derived transformants of BALB/3T3 cells and reported that all transformants showed morphological differences from the parent cell lines. These changes included increased numbers of microvilli, blebs and ruffles. In these reports budding virus were not observed on cell surfaces.

Recently, high resolution SEM was used to study and identify the budding particles on the plasma membrane. Holmes (4) showed maturation of vesicular stomatitis virus (VSV) in cultured cells and pointed out that the major topological changes in the cell surface, such as microvilli, filipodia and ruffled membrane, result from virus infection. Another application of SEM was to examine the production and release of oncogenic RNA virus. Wong and MacLeod (5) used temperature sensitive mutants and demonstrated the budding process of the C-type virus. They calculated the budding particles on the surface with the aid of SEM. Panem and Kirsten (6) showed budding of murine oncornavirus at the plasma membrane of productively infected cells. Moreover, they pointed out that the surface of the virion was composed of globular unit 20–28 nm in di-

ameter and that the distribution of the budding virus appeared to be random over the cell surface. In this communication we report on the budding virion and surface morphology on Gross virus-induced murine lymphoblasts using high resolution SEM. These observations were confirmed in ultrathin sections.

MATERIALS AND METHODS

Cell cultures. Gross virus-induced lymphoblasts (7) were kindly provided by Dr. M. Nakai (Osaka Medical College, Osaka, Japan). These lymphatic leukemia cells were maintained in our laboratory and were grown in suspension culture in RPMI 1640 supplemented with 20% inactivated fetal calf serum or Eagle's medium as modified by Baluda and Goetz (8) supplemented with 5% inactivated fetal calf serum. The medium contained 200 units/ml penicillin and 200 μg/ml streptomycin. Cells were fed every 3 days, subcultured twice a month and incubated at 37°C in an atmosphere of 5% CO_2.

Preparation of specimens for SEM. Cells were seeded on glass coverslips in 6 cm plastic petri dishes and cultivated for 2–3 days. The coverslips were removed from the petri dishes, immediately fixed for 20 min with 2.5% glutaraldehyde, rinsed twice with 0.1 M cacodylate buffer, postfixed for 15 min with 1% osmium tetraoxide, rinsed twice with the same buffer and then dehydrated in a graded series of ethanol followed by critical point drying using liquid CO_2 (9). The resulting specimens were lightly coated with platinum-palladium by a sputter coating method and were observed by field emission type SEM (HFS-2, Hitachi Ltd., Japan).

Preparation of specimens for ultrathin sections. Cells were harvested at exponential growth. The collected cells were fixed with 2.5% glutaraldehyde for 30 min and postfixed with 1% osmium tetraoxide for 15 min. The specimens were dehydrated through a graded series of ethanol and embedded for ultrathin sectioning. Thin sections were poststained with uranyl acetate and lead citrate.

RESULTS

Morphology of lymphatic leukemia cells by SEM. The cells were attached to the glass substrates and to each other by rigid filipodia. On a few days incubation, the cultured cells grew as a monolayer and detached from the glass into the media. Fig. 1 shows the lymphoblasts as spindle-shaped and rounded. Ruffled membranes were observed at the margins of the fusiform cell. Small rounded projections, which have been called blebs, were present on the surface of some cells. The cell surface was studded with curving, cylindrical, unbranched microvilli with rounded tips. Fig. 2 demonstrates a rounded cell with extensive surface features: microvilli, ruffled membranes, blebs and some clustered virus particles. These microstructures could be resolved at low magnification as illustrated in Figs. 1 and 2.

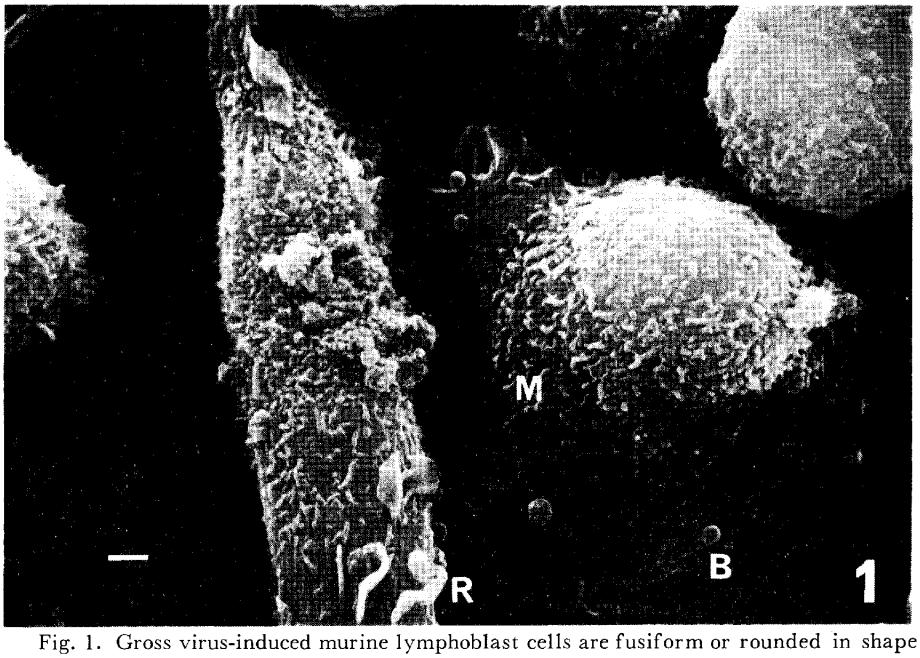

Fig. 1. Gross virus-induced murine lymphoblast cells are fusiform or rounded in shape with numerous microvilli (M), some blebs (B) and a few marginal ruffles (R). Bar: 1 μm. $\times$6,300.

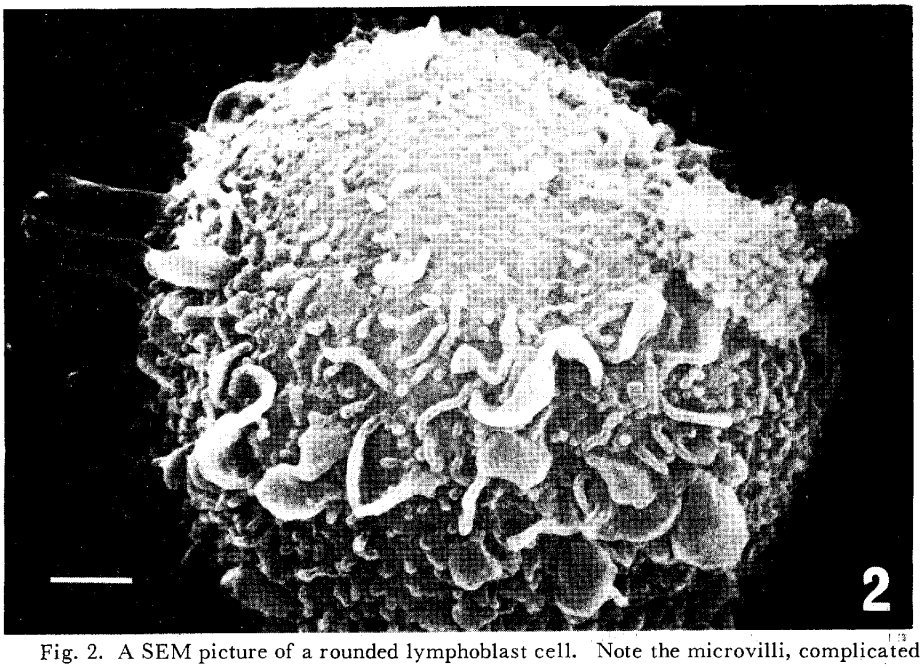

Fig. 2. A SEM picture of a rounded lymphoblast cell. Note the microvilli, complicated ruffles and some clustered virions on the surface of the cell. Bar: 1 μm. $\times$12,600.

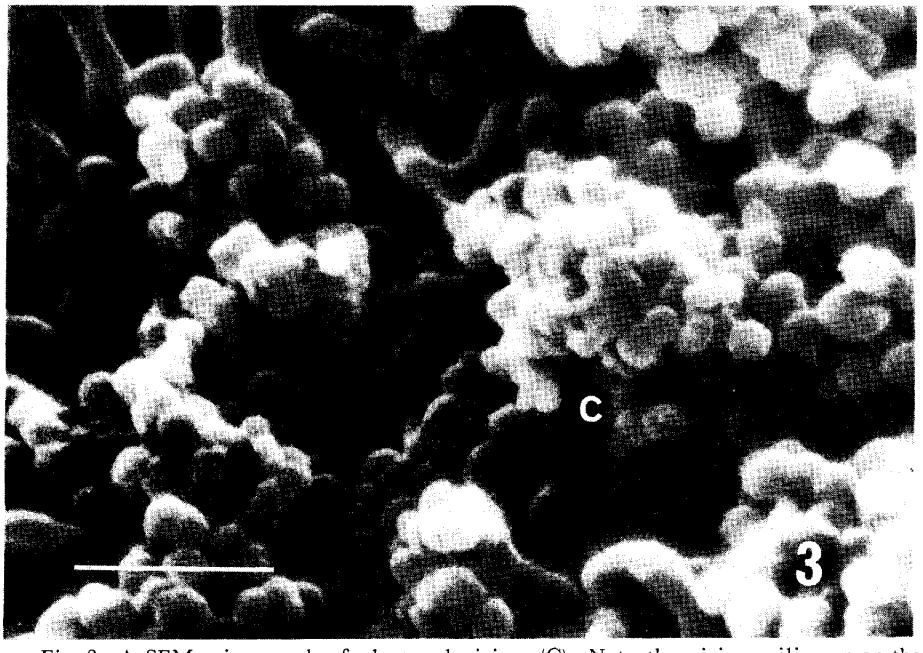

Fig. 3. A SEM micrograph of clustered virions (C). Note the virions piling up on the cell surface. The surface of the virion is smooth and has no globular units. Bar: 0.5 μm. ×63,000.

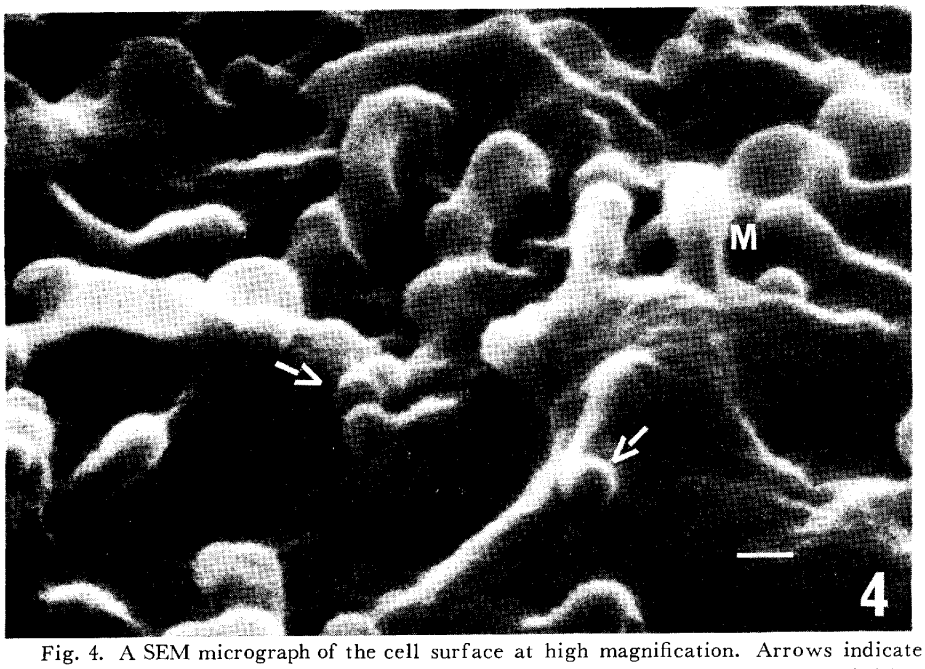

Fig. 4. A SEM micrograph of the cell surface at high magnification. Arrows indicate virions budding from the cell membrane. Virions are distinguishable from microvilli (M) by their surface features. Bar: 0.1 μm. ×86,000.

Budding of C-type virus. Structures which were considered to represent virions were identified at high magnification (Figs. 3 and 4). Virions were observed as small uniform spherical particles attached to the cell surface, distinguishable from microvilli (Fig. 4). The diameter of the virion was about 100 nm. Microvilli were of varying lengths but most had a diameter of about 100–120 nm; therefore, some microvilli were confused with virions, especially when the microvilli were viewed as short structures.

Frequently, C-type virus were observed as clusters piling up on the surface of cells (Fig. 3). Clusters of three to five or more virions were observed by high resolution SEM. The surface of the virion was smooth and had no globular unit. We could not observe the budding virion attached on the ruffled membranes or blebs. Therefore, these excrescences appeared not related to the budding process of the C-type virus. Fig. 5 illustrates clusters of C-type virus particles with di-

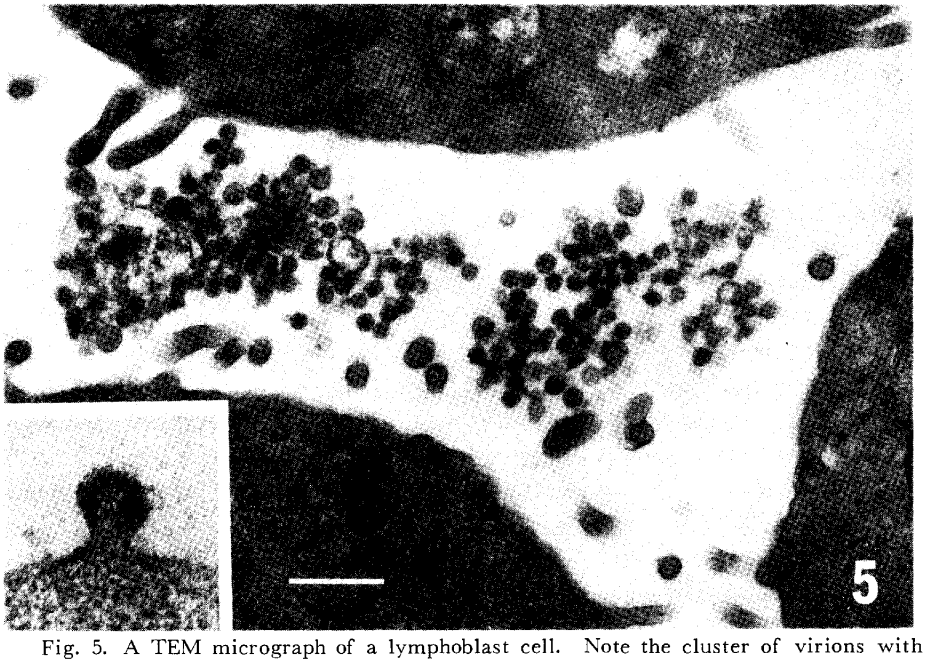

Fig. 5. A TEM micrograph of a lymphoblast cell. Note the cluster of virions with dense nucleoid. Bar: 0.5 μm. ×3,000. Inset shows a budding virus with a diameter of 100 nm, from the plasma membrane. ×100,000.

ameters of 100 nm and the electron dense nucleoid by transmission electron microscope (TEM). The inset of Fig. 5 shows an enveloped budding virion from the plasma membrane. No figures were observed budding from the microvilli or other surface projections. Ultrathin sections confirmed the budding virus observed by SEM.

DISCUSSION

SEM permits the study of a large number of whole cells *in situ* and is a valuable tool for investigating the surface morphology of tissue culture cells. We studied the morphology of the Gross virus-induced murine lymphoblasts with the budding C-type virus. The surface of the cell was covered with microstructures, such as microvilli, blebs and ruffles. These microstructures on the cell surface have been observed on neoplastic cells transformed *in vitro* by x-irradiation (1) or by chemical carcinogens (2). These excrescences were not a characteristic features of the transformants. The surface of cells infected with VSV but not oncogenic virus showed the same microstructures (4). Other observations pointed out that established cell lines synchronized in culture changed surface structures at different stages of the cell cycle with large numbers of microvilli, blebs and ruffles being displayed in the G1 stage (10).

The present SEM study demonstrated the budding virions on the plasma membrane. We found virions revealed as small spherical particles associated with the cell surface, distinguishable from microvilli. Virus budding from microvilli and from surface folds could not be observed in our experiments. Clusters of the virions were observed on the murine lymphoblasts surface. The clustered virions were the most striking features and were absent on cells infected with murine oncornavirus (6). The virion was smooth and we could not observed the globular unit. Therefore, we could not agree with Panem and Kirsten (6), who pointed out that the virion was composed of symmetrically arrayed globular unit 20–28 nm in diameter. Wong and MacLeod (5) counted the virus particles, and an average of 1600 virions was observed on a single cell surface during the production of Moloney murine leukemia virus. They suggested that the distribution of virions appeared to be random. In the case of Gross virus-induced leukemia cells, however, the determination of the number of virus particles per cell was difficult to estimate because of the clustered virions. Therefore, some form of cell treatment, such as trypsinization seems to be necessary to harvest the extracellular virions successfully from these leukemia cells. The release of Friend leukemia virus was almost completely inhibited in low-ionic strength medium (11). On the other hand, the production of C-type virus is significantly enhanced by the addition of dimethylformamide and bromodeoxyuridine (BUDR) on murine leukemia cells (12) or by adrenocorticoid in iododeoxyuridine (IUDR)-treated cells (13). IUDR and its bromo-analog have been useful as an inducer of murine leukemia virus from AKR mouse embryo cells (14, 15).

From a practical standpoint, the SEM technology demonstrated here may represent a screening method for virus-producing cell lines by examining cell surfaces for budding particles, rather than the extracellular supernatants for free virions.

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