Monensin-resistant Mouse Balb/3T3 Cell Mutant with Aberrant Penetration of Vesicular Stomatitis Virus

MAYUMI ONO,* KUMATO MIFUNE,* AKIHIKO YOSHIMURA,‡ SHUN-ICHI OHNISHI,‡ and MICHIHIKO KUWANO*

* *Department of Biochemistry and Microbiology, Oita Medical School, Oita 879-56; and* ‡*Department of Biophysics, Faculty of Science, Kyoto University, Kyoto 606, Japan.*

ABSTRACT A mutant (MO-5) resistant to monensin (an ionophoric antibiotic) derived from the mouse Balb/3T3 cell line, was a poor host for vesicular stomatitis virus (VSV) or semliki forest virus (SFV) multiplication. The yield of VSV particles in MO-5 is one 100-fold reduced as is VSV-dependent RNA synthesis. In contrast to a pH-remedial mutant, the abortive production of infectious VSV particles in MO-5 cells was not restored by low pH treatment. The pH values in the endosome and the lysosome of MO-5 cells were 5.2 and 5.4, respectively, values that were comparable to the pH value in Balb/3T3 cells. Assays with [^{3}H]uridine-labeled VSV indicated similar binding of VSV in MO-5: percoll gradient centrifugation analysis of [^{35}S]-methionine-labeled VSV-infected Balb/3T3 showed accumulation of VSV in the lysosome fraction 20 min after VSV infection, whereas VSV can be found mainly in endosome/Golgi fraction of MO-5 cells after 40 to 60 min on the percoll gradients. Degradation of [^{35}S]-methionine-labeled VSV was observed at a significant rate in Balb/3T3 cells, but not in MO-5 cells. The monensin-resistant somatic cell may thus provide a genetic route to study the mechanism of endocytosis or transport of enveloped viruses.

Cellular recognition and uptake of bioactive macromolecules such as nutrients, plasma transport proteins, peptide hormone, and lysosomal enzymes are mediated through receptor-dependent binding and endocytosis (1–4). Enveloped animal viruses also penetrate plasma membranes of mammalian cells through endocytosis or fusion (5, 6). Viruses are then seen at later times in an acidic compartment, the lysosome, where uncoating of viral genome was supposed to occur (7, 8). However, recent study shows that before reaching the lysosomes, fusion and decoating of influenza virus (9) or of Semliki forest virus (SFV)[1] (10) proceeds in the endosome, another acidic compartment.

To further understand the biochemistry of endocytosis, isolation of somatic cell mutants with aberrant response to viruses can be invaluable. Moehring and Moehring (11) have isolated human KB cell lines resistant to diphtheria toxin which were also cross-resistant to viruses. Mento and Siminovitch (12) have isolated variants from Chinese hamster ovary cells resistant to sindbis virus which were concomitantly resistant to diphtheria toxin. Moehring and Moehring (13) have further isolated many mutants of the Chinese hamster ovary cell resistant to both *Pseudomonas* exotoxin A and enveloped viruses. The failure of virus RNA synthesis in the toxin-resistant mutant was found to be completely overcome by exposure to low pH (13). Similar mutants were independently isolated by Robbins et al. (14).

In our laboratory, we have isolated Chinese hamster ovary cell mutants resistant to an ionophoric antibiotic, monensin, which showed reduced uptake of ricin and insulin (15–17). We have further isolated monensin-resistant (Mon^r) clones from the mouse Balb/3T3 cell line, and a Mon^r clone, termed MO-5, was found to be low in the endocytosis of low density lipoproteins (18). In this study, we report that MO-5 might yield a novel type of vesicular stomatitis virus (VSV) resistance.

MATERIALS AND METHODS

Cell Line, Culture Medium, and Virus: Mouse Balb/3T3 cell line, Balb/3T3 A3-1-1, which was obtained from Dr. T. Kakunaga (National Cancer Institute, USA), was the parental clone from which drug-resistant cells

[1] *Abbreviations used in this paper:* FITC, fluorescein isothiocyanate; MEM, Eagle's minimal essential medium; moi, multiplicity of infection; MO-5, a monensin-resistant clone; Mon^r, monensin-resistant; pfu, plaque-forming units; SFV, semliki forest virus; VSV, vesicular stomatitis virus.

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were isolated. The Balb/3T3 cells showed a saturation density of 1–2 × 10^6 cells per ml at 37°C (19, 20). Balb/3T3 cells were cultured in minimal essential medium (MEM) containing 0.1% Bactopeptone (Difco Laboratories, Detroit, MI), 10% newborn calf serum (Flow Laboratories, Inc., McLean, VA), and penicillin G (100 U/ml) (21, 22). VSV and SFV grown in human embryonic lung cells were used as previously described (23). The infectivity of viruses was assayed in L-929 cells by plaque formation as previously described (23).

Isolation of Mon^r Mutant and Colony Formation: Balb/3T3 cells were treated with 300 μg/ml of ethyl methane sulfonate for 18 h at 34°C to a survival level of 50% of the initial number, and then cultured for 7 d in the absence of any drug (15). The cells were treated with 10 μg of monensin per milliliter and both the medium and the drug were changed every 3 or 4 d. Colonies appearing in the presence of the drug after incubation for ~3–4 wk at 34°C were purified and six Mon^r clones were independently isolated. The Mon^r clone termed MO-5 was used in this study. Mutagenesis with ethyl methane sulfonate increased the frequency of Mon^r clones ~10-fold higher than that observed without mutagen. To determine cellular sensitivities to chemicals, we plated 700 cells in replicate 60-mm plastic dishes in the absence of any drug for 18 h, and further exposed the cells to chemicals and incubated them for 10 d (15, 21). Colonies were stained with Giemsa and scored. Plating efficiency was ~50% in the absence of any drugs.

Chemicals and Isotopic Compounds: Radioisotopic compounds and chemicals were obtained from following sources: [^{35}S]methionine (1,235 Ci/mmol) and [^{3}H]uridine (6 Ci/mmol), New England Nuclear, Boston, MA; colchicine, Boehringer Mannheim Biochemicals, Federal Republic of Germany); monensin and nigericin, Calbiochem-Behring Corp., San Diego, CA; and chloroquine, Sigma Chemical Co., St. Louis, MO.

Virus Infection Test and Viral RNA Synthesis: Cell monolayers were infected with VSV at a multiplicity of infection (moi) of 5 to 10 plaque-forming units (pfu) per cell and incubated for 1 h at 37°C in a CO_2-incubator for adsorption. Then the monolayers were washed once with phosphate-buffered saline (PBS) and further incubated for 5 h at 37°C. To assay virus yield, we titrated extracellular virus.

Cell monolayers were infected with VSV at a moi of 5 in the presence of 3 μg/ml actinomycin D in 1 ml MEM for 1 h at 37°C, and then the cells were labeled with 2 μCi/ml [^{3}H]uridine. After incubation for various times, the cells were lysed in H_2O and suspended in trichloroacetic acid to a final concentration of 10%. The TCA-insoluble fractions were collected on glass fiber filters and counted. We subtracted from each value activities in RNA of uninfected cells treated with actinomycin D.

Binding Assay of VSV: Binding of [^{3}H]VSV was carried out at 4°C (24). [^{3}H]VSV were prepared from HEL-R66 cells infected with VSV which were incubated with 5 μCi/ml [^{3}H]uridine and 1 μg/ml of actinomycin D in 10 ml of MEM for 6 h, and specific activities of [^{3}H]VSV were 10,000–12,000 cpm/μg virus protein. Some 5 to 10 × 10^5 cells per dish were incubated with 10–15 μg of [^{3}H]VSV in 1 ml of MEM at 4°C for 100 min, and binding activity was measured for cell-associated radioactivity after two washings with PBS.

Assay for Effect of Low pH Treatment: Balb/3T3 or Mon^r cells (2.8 × 10^5/ml) in 5 ml of medium were treated without or with 0.1 mM chloroquine for 15 min at 37°C, and the cells were incubated at a moi of 5 with VSV for 60 min at 4°C. The drug-containing medium was then removed by washing once with PBS, and 1 ml of Eagle's minimal essential medium at pH 7.5 or pH 5.5 was added without chloroquine. The cells were then incubated for 3 min at 37°C. The PBS was removed and the prewarmed MEM with or without 0.1 mM chloroquine was added to follow incubation for an additional 6 h at 37°C. Virus yield was assayed as previously described (23).

Analysis of VSV Distribution by Colloidal Silica Gradients: [^{35}S]Methionine-labeled VSV ([^{35}S]VSV) was prepared from HEL-R66 cells in 100 mm of methionine-free medium infected with 5 μCi/ml of [^{35}S]methionine and 1 μg/ml of actinomycin D for 6 h. [^{35}S]VSV was purified by sucrose density gradient centrifugation and ~3 × 10^4 cpm/μg virus protein was obtained. Some 5 to 10 × 10^6 cells per dish were incubated with 10 μg of [^{35}S]VSV in MEM medium at 37°C for 90 min. After the incubation, half of the sample (three dishes per each assay) was washed and analysed by density gradient centrifugation, and the other half was further incubated at 37°C for 60 min with fresh medium containing 10% newborn calf serum. After the incubation, the cells were removed from plates with a rubber policeman. Cell suspensions on 1 ml of a buffer containing 10 mM triethanolamine, 1 mM EDTA, 0.25 M sucrose (pH 7.5) were placed in a nitrogen cavitation bomb and pressurized to 35 psi for 15 min (18, 25). The cells were then homogenized in a Dounce homogenizer (Kontes Institute, Vineland, NJ) with ~10 strokes. This procedure enabled us to obtain more than 90% lysis. After centrifugation of the homogenate at 3,000 *g* for 10 min to pellet nuclei and unbroken cells, the supernatant was layered over 8 ml of 25% isoosmotic percoll (Pharmacia Fine Chemicals, Piscataway, NJ) in 10 mM triethanolamine, 1 mM EDTA, 0.25 M sucrose (pH 7.5). The bottom of the tube contained a 0.5-ml cushion of 2.5 M sucrose. After centrifugation at 40,000 *g* for 60 min, the density gradient was collected from the top and the fractions were assayed for density using density marker beads (Pharmacia Fine Chemicals). Radioactivities were counted in Scintisol EX-H (22) by an Aloka gamma counter. β-Hexosaminidase as a marker enzyme for lysosomes was assayed as previously described (25, 26).

Degradation of VSV in Mouse Cells: Some 3.4–7.1 × 10^5 cells per dish were infected with [^{35}S]methionine-labeled VSV at a moi of 50 at 37°C for 90 min. Then the dishes were washed twice with PBS and followed incubation with fresh MEM at 37°C. At the indicated time, 1 ml of medium was removed and trichloroacetic acid was added to a final concentration of 10%. After spin down at 2,000 rpm for 10 min, 1 ml of supernatant fraction in 10 ml of scintisol EX-H was measured. At time 0, 250–261 cpm was found in the medium, and we subtracted the background activity from each radioactivity.

Measurement of Lysosomal and Endosomal pH: Fluorescein isothiocyanate (FITC)-dextran (60,000–90,000-mol-wt, Wako Chemical Industries, Osaka, Japan) was synthesized according to the procedure of Straubinger et al. (27). A standard curve relating to the ratio of fluorescence intensities at 520 nm with excitation at 495 nm and 450 nm was constructed according to the method of Ohkuma and Poole (28) using FITC-dextran (20 μg/ml) in 0.2 M sodium citrate (pH 4.5 to 6.0), sodium phosphate (pH 6.5 to 7.5), or Tris-HCl (pH 8.0) buffer. To load FITC-dextran on to the lysosome, cells were incubated with FITC-dextran (2 mg/ml in MEM) at 37°C overnight and chased for 2 h in MEM containing 2 mg/ml of nonlabeled dextran. To load FITC-dextran onto the endosome, cells were incubated with FITC-dextran (40 mg/ml in MEM) at 20°C for 2 h and chased for 30 min. Cells were separated from dishes by trypsin treatment, washed three times by centrifugation, and suspended in Hanks' buffered salt solution–10 mM HEPES (pH 7.4 containing 0.2% bovine serum albumin and 1 mM ATP). Fluorescence intensities of ~1 × 10^6 cells were measured by an Hitachi 850 fluorescence spectrophotometer. Autofluorescence was determined using the cells not exposed to FITC-dextran. Fluorescence intensities from single cells were estimated by quantitative fluorescence microscopy as previously described (9).

RESULTS

Production of Enveloped Viruses and Viral RNA Synthesis in a Mon^r Clone Derived from the Mouse Balb/3T3 Cell Line

A Mon^r clone, MO-5, was found to show fivefold higher resistance to monensin and threefold-resistance to nigericin, an Na^+/K^+ ionophoric antibiotic, than the parental Balb/3T3 cell line (Table I). Our recent study suggested that MO-5 cell is low in the endocytosis of low density lipoproteins (18), and many ligands often enter the cells through common pathways (2, 4). We could ask whether MO-5 is resistant to enveloped viruses, VSV, or SFV. As seen in Table I, in comparison with the parental clone, relative virus yield of VSV was 10^{-2} and that of SFV was 0.05 in MO-5. Cytopathic effect of MO-5 cells required about 100-fold higher amounts of VSV than

TABLE I. *Relative Resistance To Monensin and Yield of Enveloped Viruses in Mon^r Clone*

Cell lines	Relative resistance to monensin*	Relative resistance to nigericin*	Relative virus yield‡ VSV	Relative virus yield‡ SFV
Balb/3T3	1.0	1.0	1.0	1.0
MO-5	5.0	3.0	10^{-2}	5 × 10^{-2}

* Relative resistance to various agents is expressed when D_{10} dose of each drug at each cell line is divided by that of the parental Balb/3T3 cell line. D_{10} values of each drug for Balb/3T3 were 20 ng/ml of monensin, which inhibited 90% of colony formation of initial number.

‡ Cells (4 × 10^4 cells/well) were infected with VSV or SFV at a moi of 5 and then incubated for 2 d. Titers of virus released from Balb/3T3 cells into medium were 6 × 10^5 pfu/ml in VSV and 8 × 10^5 pfu/ml in SFV.

the virus dose to cause similar cytopathic effect against Balb/3T3 cells (Table II).

Production of specific viral RNA was also compared between VSV-infected Balb/3T3 and MO-5 cells. The cells were infected with a moi of 10 at 37°C and VSV-specific RNA synthesis was monitored by measuring incorporation of [^{3}H]uridine in the presence of actinomycin D at 37°C. In comparison with viral RNA synthesis in Balb/3T3, the RNA synthesis was greatly reduced in MO-5 (Fig. 1).

Effect of Low pH Treatment on VSV Production

It is known that acidification of endosomes is a necessary process for endocytosis of enveloped viruses (6, 29). Lysosomotropic amines like chloroquine or ammonium chloride interfere with the endocytotic process possibly through raising the endosomal pH (30), as well as the lyososomal pH (28). In addition, after a brief exposure to low pH, uncoating of enveloped viruses is supposed to proceed through fusion of the viruses to their plasma membranes of enveloped virus-resistant mutants with acidification-negative endosomes (14, 31). We thus examined whether the decreased yield in MO-5 is remedied by exposure to low pH. The production of infectious VSV particles in MO-5 was one hundredth or much less of that in the parental Balb/3T3 cells when briefly exposed to pH 5.5 or pH 7.5 (Table III). The treatment by low pH could not overcome the failure in VSV production in the resistant clone. The presence of 0.1 mM chloroquine during the incubation after the pH treatment at pH 7.5 significantly inhibited the VSV production in both Balb/3T3 and MO-5, whereas chloroquine inhibited the virus production only slightly by 30–35% of the control after the treatment at pH 5.5 (Table III).

TABLE II. *Cytopathic Effect of VSV on Balb/3T3 and MO-5**

VSV samples	Balb/3T3	MO-5
10^{-1}	100%	100%
10^{-2}	100%	50%
10^{-3}	80%	0%
10^{-4}	20%	0%
10^{-5}	0%	0%

* Balb/3T3 or MO-5 (4×10^4 cells/well) were infected with various diluted samples of VSV (10^{-1} dilution corresponding to 2×10^6 virus particles per ml), and after 48 h, cytopathic effects were tested.

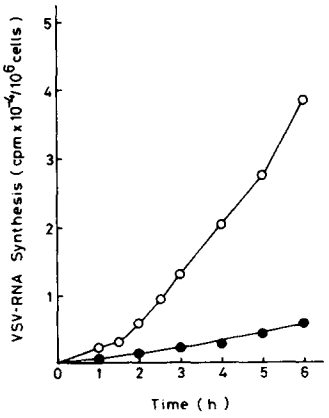

FIGURE 1 Production of VSV RNA by Balb/3T3 and MO-5 cells. The cells were exposed to a moi of five plaque-forming units of virus for 1 h at 37°C in the presence of 3 μg/ml of actinomycin D, washed with PBS, and incubated in 1 ml of MEM containing actinomycin D (3 μg/ml) and [^{3}H]uridine at 2 μCi/ml. At indicated times, radioactivities of [^{3}H]uridine incorporated into acid-insoluble fractions were determined. O, Balb/3T3; ●, MO-5.

pH of Endosomes and Lysosomes

FITC-dextran was loaded to lysosomes or endosomes by incubation at different temperatures. At 20°C for 2 h, most FITC-dextran was loaded in endosomes as demonstrated by Dunn et al. (32). The amount of pinocytosed FITC-dextran was only slightly different between the parent and mutant cells. We estimated the pH value in these two compartments from the ratio of the fluorescence intensities according to Ohkuma and Poole (28). The fluorescence intensities with two different excitation wavelengths and the relative rate of I_{495}/I_{450} are indicated in Table IV. The pH values were estimated from standard curve of the relative rate against pH as previously described (9). The pH values of lysosomes of the parent Balb/3T3 cells and the mutant MO-5 cells, were about 5.1–5.2, and those of endosomes were 5.4–5.5 (Table IV). There was no significant difference in the pH values between the two cell lines. These pH values were sufficiently low for the envelope fusion of VSV to occur. The pH measured from single cells also showed little difference between the two cell types. Addition of 10 μM monensin caused an increase in the pH values of these two compartments to higher than 6.7 (data not shown).

Binding and Intracellular Distribution of VSV in Balb/3T3 and MO-5

Several independent assays so far showed that MO-5 was resistant to VSV, and thus one might argue whether the

TABLE III. *Effect of Low pH Treatment on VSV Production in Balb/3T3 and MO-5**

pH	0.1 mM chloroquine	Balb/3T3	MO-5
5.5	–	6.8×10^5 (100)	3.0×10^3 (100)
5.5	+	5.0×10^5 (74)	2.1×10^3 (70)
7.5	–	8.0×10^5 (100)	5.0×10^3 (100)
7.5	+	5.6×10^3 (0.7)	1.0×10^3 (20)

* Cell (5.6×10^4/well) were adsorbed with VSV at a moi of 5 for 60 min at 4°C and washed once with PBS to follow exposure for 3 min at 37°C to pH 5.5 or pH 7.5 MEM medium. Then the cells were further incubated in the absence or presence of 0.1 mM chloroquine for 6 h at 37°C, and titer of VSV in medium was measured as described in Materials and Methods. In parenthesis, relative virus yield (%) was presented as average data from two independent assays.

TABLE IV. *Comparison of pH in the Endosomes and the Lysosomes of Balb/3T3 and MO-5 Cells**

Cell line	Acidic compartment	I_{495}	I_{450}	$\frac{I_{495}}{I_{450}}$	pH
Balb/3T3	Lysosome	0.118	0.056	2.638	5.1
Balb/3T3	Endosome	0.231	0.078	3.586	5.5
MO-5	Lysosome	0.147	0.062	2.826	5.2
MO-5	Endosome	0.325	0.110	3.276	5.4

* Measurement of pH in endosome and lysosome was done as described in Materials and Methods. Background of I_{495} and I_{450} is 0.023 and 0.020 in Balb/3T3, and 0.017 and 0.016 in MO-5, respectively.

resistance in MO-5 results from a failure in binding to the cell surface. To test this possibility, binding activities of [^{3}H]-uridine-labeled VSV were compared in Balb/3T3 and MO-5 cells. Binding was performed at 4°C for 100 min, and then binding activity was assayed from two washings with PBS. Balb/3T3 and MO-5 showed similar binding activity; binding activities (cpm per 10^6 cells) were 3,820 ± 260 in Balb/3T3 and 3790 ± 225 in MO-5.

We compared intracellular distribution of VSV in Balb/3T3 and MO-5 by using density gradient centrifugation. Balb/3T3 and MO-5 cells infected with [^{35}S]methionine-labeled VSV were followed for various times at 37°C, and then the cells were washed and analysed on colloidal silica gradients (Fig. 2). Radioactivities associated with VSV were enhanced mainly in the endosome/Golgi region with a density of 1.03 to 1.04 g/ml in Balb/3T3 cells 10 min after the infection, and almost all the radioactivities moved in lysosomal fractions with densities of 1.05 to 1.06 g/ml 20 min after the infection (Fig. 2, *A* and *B*). The complete shift of the radioactive VSV into the lysosomal fraction of Balb/3T3 cells was observed 60 min after the infection or after a 3-h chase of the 60-min incubation sample with [^{35}S]VSV (Fig. 2, *D* and *E*). In contrast, in MO-5 cells, the main peak of the radioactive VSV could be observed at regions of endosome/Golgi after 20–40 min incubation, and half the radioactivities of VSV appeared in the lysosomal fraction 60 min after the incubation with [^{35}S]VSV (Fig. 2, *F–I*). After a 3-h chase of the 60-min incubation sample of MO-5 cells, most of the radioactivities now appeared as a main peak in the lysosomal fraction (Fig. 2*J*). Movement of VSV into the endosome and the lysosome was found to be very slow in MO-5 cells in comparison with the parental cell line.

Degradation of [^{35}S]VSV in Balb/3T3 and MO-5

Enveloped virus like SFV during endocytic pathway is degraded in the lysosome (7). Degradation of virus was compared between Balb/3T3 and MO-5 cells to follow acid-soluble radioactivity in the medium (Fig. 3). In virus-infected Balb/3T3, significant amounts of radioactivity in acid-soluble form appeared 2 h after the [^{35}S]VSV infection. In comparison with the Balb/3T3 cells, greatly reduced amounts of acid-soluble radioactivities were found in VSV-infected MO-5 cells: degradation activity of VSV in MO-5 cells was ~20% of that in Balb/3T3 cells (Fig. 3).

DISCUSSION

In this study we have isolated mutants resistant to monensin, and the Mon^r clone termed MO-5 was found to be resistant to enveloped viruses. Monensin inhibits receptor-mediated endocytosis of many ligands, possibly through raising the pH of the endocytic vesicles (30). Acidification of the endocytic vesicles appears to be essential for the uptake of ligands (33). Selection of mutants resistant to monensin is thus expected

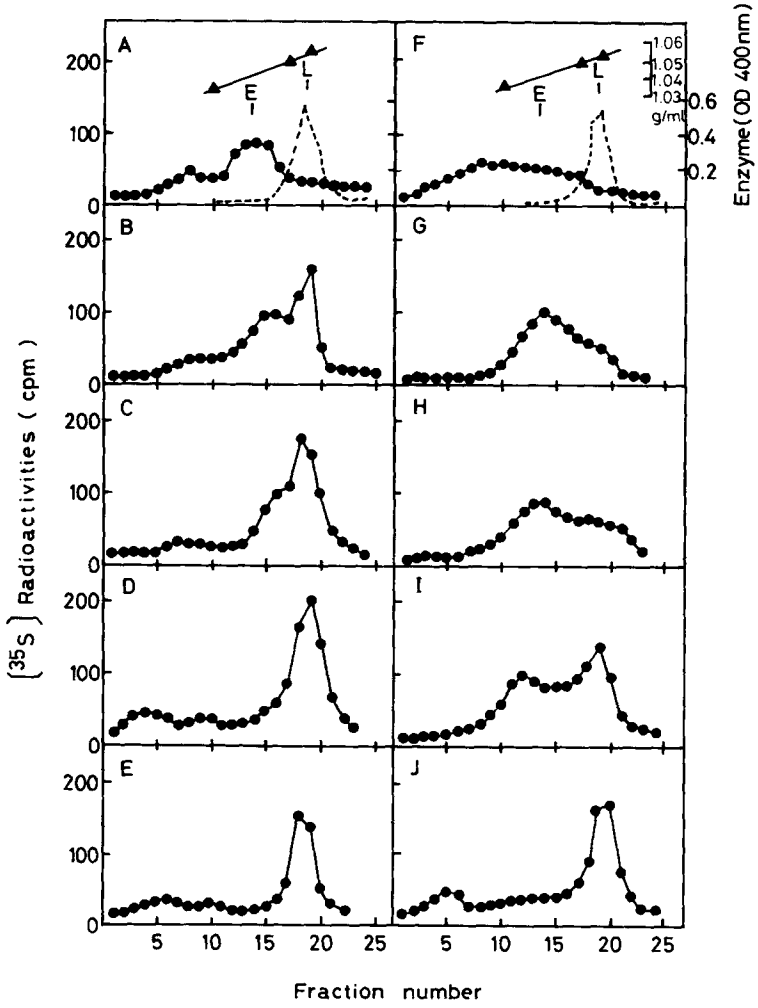

FIGURE 2 Comparison of intracellular localization of [^{35}S]VSV in Balb/3T3 and MO-5 as determined by equilibrium density gradient centrifugation. Cells of Balb/3T3 (*A–E*) and MO-5 (*F–J*) were incubated for 10 (*A* and *F*), 20 (*B* and *G*), 40 (*C* and *H*), and 60 (*D* and *I*) min at 37°C with [^{35}S]VSV, and the homogenates were analyzed by Percoll gradient centrifugation. In *E* and *J*, the cells incubated for 60 min with [^{35}S]VSV were then washed, further incubated for 3 h at 37°C, and the homogenates were analyzed by Percoll gradient centrifugation. Position of the lysosome (*L*) was determined by assaying β-hexosaminidase and the region corresponding to the endosome or/and Golgi was indicated by *E*, estimated from density. Density marker beads were used to determine the gradient density.

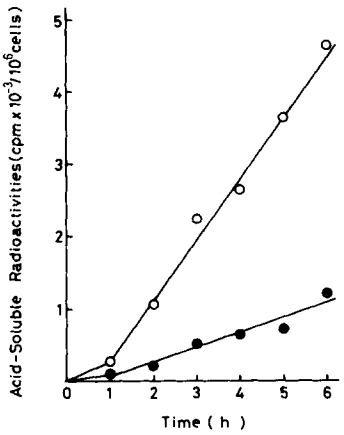

FIGURE 3 Degradation of [^{35}S]methionine-labeled VSV in Balb/3T3 and MO-5. After infection of Balb/3T3 cells (○) and MO-5 cells (●) with [^{35}S]VSV, acid-soluble radioactivity in media was followed at various times as described in Materials and Methods. From each value, background radioactivity (150–200 cpm) at time 0 was subtracted and the corrected values were presented.

to obtain clones with aberrant endocytosis systems or with aberrant acidification. An acidification-defective mutant has been previously isolated and identified as a toxin-resistant clone (14). A brief exposure of the mutant resistant to diphtheria toxin and enveloped viruses to pH 5.0 increased subsequent production of VSV (14), and thus the mutant appears to be altered in the acidification process. The mutant also showed failure to release iron from the transferrin possibly through the low acidification activity (34). Similarly, *Pseudomonas* exotoxin A–resistant clones isolated from human cancer KB cells by Moehring and Moehring (13) were cross-resistant to sindbis virus and only slightly to VSV, and they were found to be highly sensitive to ricin. Abortive synthesis of sindbis virus RNA in the toxin-resistant mutant was completely remedied by low pH treatment (13). Further study has suggested that ATP-dependent acidification of endosome is altered in the toxin-resistant mutant (31). Somatic cell mutant resistant to enveloped viruses thus far appears to be due to deficient acidification of endosome.

Uncoating process of SFV (10) or influenza virus (9) during development of viruses in cells is expected to proceed in the acidic compartment known as the endosome. We have yet to determine which step in the endocytotic process is mutated in MO-5: acidification-defective mutation as described by other workers (11, 14) or membrane fusion–defective mutation, as expected. In MO-5, binding activity of VSV to the cell surface was comparable to the parental cell line, but movement of VSV from the cell surface into the endosome and lysosome was extremely slow in comparison with the parent cell (Fig. 2). Measurements of pH in the endosomes and the lysosomes of MO-5 cells showed acidic pH similar to those in Balb/3T3 (Table IV). Our VSV-resistant clone, MO-5, appears not to alter in the acidification activity. The poor production of VSV in MO-5 could not be remedied by a brief exposure to low pH (Table III): the low pH is necessary for enveloped viruses to fuse the cell membrane (35, 36). Membranous lipid components are recently suggested to be closely linked with the endocytosis. Mahoney et al. (37) suggested that altered ratio of unsaturated fatty acid to saturated fatty acid in membranes affects endocytotic activity of the macrophage. In addition, cholesterol molecules are found to be a prerequisite for the fusion of SFV and membranes (38). In MO-5 cells, retardation of the intracellular transport of low density lipoproteins (18) as well as VSV (Fig. 2) was observed, suggesting alteration of membranous lipids. Endocytosis of viruses is supposed to proceed as does that for other ligands: adenovirus and epidermal growth factor (39) or α_2-macroglobulin and VSV (40) are found respectively in the same vesicles. Our present data suggest that any alteration in the membrane constituents affects a common pathway, plausibly an earlier step during the endocytosis process of various ligands in MO-5 cells.

A Chinese hamster ovary cell mutant with a dominant trait of ricin-internalization defect has been isolated (41) and the mutant was found to carry two aberrant fatty acyl proteins (42). The compactin (ML236B)-resistant clone of Chinese hamster V79 cell line with defective endocytosis of low density lipoproteins (43) showed altered components of membranous fatty acid (44). Since MO-5 showed similar sensitivity to ricin or compactin (ML236B) as the parental clone (Ono, M., unpublished data), an altered lesion of MO-5 appears to be different from these mutants defective in ricin-internalization (15, 40) or in low density lipoprotein–internalization (43). Mutants with defective endocytosis of several ligands have different properties, suggesting involvement of many factors in the endocytosis.

In comparison with Balb/3T3 cells, degradation of VSV in MO-5 cells was found to be very low (Fig. 3). Proteins of enveloped viruses are degraded in lysosomes of the virus-infected cells (7). Intracellular transport of VSV was more inhibited in MO-5 cells than Balb/3T3 (Fig. 2). VSV thus reaches the lysosomal compartment at a much slower rate in MO-5 than the parental clone, which might result in the low degradation of VSV in the resistant clone. One could also argue that VSV particles themselves internalized in MO-5 cells are resistant to degradation enzyme(s). Enveloped proteins of VSV after the possible decoating in the endosomes are supposed to be highly susceptible to degradation enzyme(s) in the lysosomes of Balb/3T3 cells. By contrast, if VSV moves into the lysosomes without decoating in the endosomes of MO-5 cells, the intact VSV particles might not be susceptible to degradation enzyme(s) in lysosomes. Further study of whether decoating of VSV actively proceeds in the resistant clone is in progress.

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