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# Isolation and Characterization of Mouse FM3A Cell Mutants Which Are Devoid of Newcastle Disease Virus Receptors

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**A method was developed to select host cell mutants which did not permit the replication of Newcastle disease virus (NDV), and 14 isolates of NDV-nonpermissive mutants of mouse FM3A cells were obtained. All these isolates were judged to be deficient in NDV receptors, since their ability to adsorb $^3$H-labeled NDV virions was markedly decreased. They were tested for genetic complementation in pairs by cell fusion and shown to fall into a single recessive complementation group, which was designated as *Had*-1. Vesicular stomatitis virus was able to replicate in this mutant to produce infectious progeny, but the glycoprotein of the released virion was abnormal in size, suggesting a defective processing of the asparagine-linked carbohydrate chains in the mutant cell. The *Had*-1 mutant was resistant to wheat germ agglutinin, but sensitive to a *Griffonia simplicifolia* lectin, GS-II, which recognizes terminal *N*-acetylglucosamine residues. The altered sensitivity to these plant lectins compared with that of the parental FM3A cells indicates that sialylated sugar chains on the cell surface are almost absent from the *Had*-1 cells, thereby rendering the cells NDV receptor deficient.**

Viruses require for their replication a variety of molecular machineries of host cells which normally serve the purposes of the cells. Thus, analysis of the process of animal virus replication has not only led to the elucidation of the viral life cycle but also quite often provided useful information concerning the functioning of animal cells. Mutant viruses with lesions in various replication steps as well as metabolic inhibitors have been widely utilized as useful tools in analyzing viral replication and interaction with host cells, and an ever-increasing number of cloned virus genomes have also become available. Host cell mutants which are unable to support the production of infectious viral progeny are complementary to the abovementioned tools and would be equally helpful in elucidating the details of virus-host cell interactions. To date, however, only a quite limited number of studies have been done by utilizing mutant host cells in which viral propagation was restricted. Toyama et al. (33, 34) isolated a mutant cell line resistant to Sendai virus-induced cytolysis from human KB cells and later assigned its lesion to sialyltransferase deficiency. More recently, Tufaro et al. (35) identified a cell line defective in glycoprotein transport among a collection of mutant mouse L cells nonpermissive to herpes simplex virus infection.

In view of potential usefulness of mutant cells which restrict viral replication, we started a systematic study for the isolation and the characterization of host cell mutants nonpermissive to Newcastle disease virus (NDV), using mouse FM3A cells as a parental line. Paramyxoviruses, including NDV, require a number of host cell structures and machineries to complete their replication cycle; they are supposed to recognize and attach to specific cell surface receptors containing sialylated sugar chains (32) and enter into the host cells through fusion of viral envelopes with plasma membranes. Transcripts of the viral genome were reported to be associated with cytoskeletal structures (8), although the significance of this remains to be clarified. Envelope proteins most likely share the machinery for intracellular transport and posttranslational modification, such as glycosylation, fatty acylation, and proteolytic cleavage, with many cellular proteins which are also transported to the cell surface by way of the Golgi complexes. Moreover, in the case of Sendai virus, which is closely related to NDV, phosphorylation of the membrane protein and the nucleocapsid protein was suggested to have a role in viral replication or assembly (17). Host cell mutants nonpermissive to NDV may be defective in one of cellular functions mentioned above, and the identification of the defective gene would be useful in the structural and functional analysis of components responsible for these activities, most of which have been only poorly characterized. Furthermore, characterization of such mutant cells might also lead to discovery of new host factors which have so far escaped our attention.

In this article, we describe general principles of isolation, establishment, and genetic grouping of NDV-nonpermissive mutants of mouse FM3A cells.

## MATERIALS AND METHODS

**Cells and viruses.** A subclonal line of mouse mammary carcinoma FM3A (23) cells, F28-7 (13), kindly provided by T. Seno (Saitama Cancer Center Research Institute), was used as a parental cell line. The cells were maintained at 37°C in ES medium (14) (Nissui Seiyaku Co., Tokyo, Japan) containing 2% fetal calf serum (FCS). For colony formation, an agar plate culture technique (15) was used. The agar medium was composed of 0.35% agarose and ES medium containing 2.5% FCS. L cells were grown as monolayers at 37°C in Eagle minimal essential medium supplemented with 5% newborn calf serum. BHK-21 cells were also grown as monolayers at 37°C in Eagle minimal essential medium supplemented with 6% newborn calf serum and 10% tryptose phosphate broth. NDV (Miyadera strain) was a generous gift from Y. Nagai (Nagoya University). Virus was grown in the allantoic sacs of 10-day-old chicken embryos. The titer of viral infectivity was determined by plaque assay on monolayers of chicken embryo cells as described by Maeno et al. (20).

**Selection of mutants by hemadsorption test.** Mouse FM3A cells at the logarithmic growth phase ($1 \times 10^6$ cells) were

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diluted to a density of $2.5 \times 10^5$/ml in a 60-mm plastic dish. They were mutagenized by treatment with 0.6 μg of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) per ml for 3 h and cultured for 6 days in a medium containing 2% FCS. The cells were then inoculated into 90-mm glass dishes ($10^4$ cells per dish) and grown for 3 days to form cell islands. The cells were allowed to settle firmly on the substratum by incubation in a medium containing 0.5% FCS and infected with a minimal saturating dose of NDV ($2.7 \times 10^8$ PFU). At 14 h after infection, the cells were washed once with phosphate-buffered saline (PBS) and incubated with 2 ml of fowl erythrocyte suspension (2% [vol/vol] in PBS) for 0.5 h at 4°C. The cells were then washed thoroughly with ice-cold PBS and examined for hemadsorption with a phase-contrast microscope (magnification, 10 × 10).

**Radioisotopic labeling of viral proteins synthesized in infected cells.** Cells ($2 \times 10^6$) in a 35-mm plastic dish were infected with NDV at a multiplicity of 100 PFU per cell and incubated in a medium containing 0.5% FCS. Cells were then fed with 0.5 ml of methionine-free medium supplemented with [$^{35}$S]methionine (5 μCi/ml, 1,130 Ci/mmol; Amersham Corp., Arlington Heights, Ill.) at 7 h after infection, and labeling was continued for 1 h. To study the synthesis of vesicular stomatitis virus (VSV) proteins, we infected cells at a multiplicity of 10 PFU per cell and incubated them in a medium containing 0.5% FCS. The cells were then fed with 0.5 ml of medium containing [$^3$H]leucine (10 μCi/ml, 56 Ci/mmol; ICN Radiochemicals, Irvine, Calif.) at 6 h after infection, radiolabeled for 1 h, and further incubated in nonradioactive medium for 1 h. When indicated, the cells were treated with 0.5 μg of tunicamycin per ml as described by Leavitt et al. (18). The radiolabeled cells were washed twice with ice-cold PBS and lysed in 0.2 ml of 0.5% Nonidet P-40 in 0.1 M Tris hydrochloride (pH 8.0)–0.1 M NaCl. Viral proteins were immunoprecipitated as described by Kessler (11) by using rabbit anti-NDV serum (kindly supplied by Y. Nagai, Nagoya University) or mouse anti-VSV serum (9). The immunoprecipitates were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (16) followed by fluorography (3).

**Preparation of $^3$H-labeled NDV.** Confluent monolayers of BHK cells ($10^7$ cells per 90-mm plastic dish) were infected with NDV at a multiplicity of 15 PFU per cell. At 3 h after infection, cells were treated with actinomycin D (2 μg/ml) for 30 min and further incubated in a medium containing 6% newborn calf serum and 10% tryptose phosphate broth. The cells were then fed with 4 ml of leucine-free medium containing 2% dialyzed newborn calf serum and [$^3$H]leucine (20 μCi/ml, 56 Ci/mmol; ICN Radiochemicals) at 9 h after infection and further incubated for 8 h. Culture fluid was collected and layered on the top of a 10%-35%-48%-60% discontinuous sucrose density gradient in PBS. After centrifugation at 75,000 × *g* for 4 h at 4°C, $^3$H-labeled NDV virions at the 10%/35% interface were collected.

**Attachment of $^3$H-labeled NDV to cells.** Cells ($2 \times 10^6$) in a 35-mm plastic dish were incubated with 0.25 ml of medium containing $^3$H-labeled NDV ($10^4$ cpm, at an approximate multiplicity of 13 PFU per cell) for 1 h at 37°C. Cells were washed three times with ice-cold PBS, and acid-insoluble radioactivity associated with the cells was measured. Values were corrected for the nonspecific binding, which was measured immediately after the addition of the labeled virions to cells pretreated with unlabeled NDV (fivefold the amount of the unlabeled virions).

**Genetic complementation analysis.** A spontaneous ouabain-resistant clone was isolated from FSthy$^-$21, a thymidine auxotrophic mutant line of FM3A (1) (kindly supplied by D. Ayusawa, Saitama Cancer Center Research Institute) and named Fthy$^-$oua1. To examine whether a particular mutation is dominant or recessive, we fused Fthy$^-$oua1 cells with each mutant and selected cell hybrids on an agar medium containing 1.5 mM ouabain and 2.5% dialyzed FCS (OUA medium). To group isolated hemadsorption-negative (*Had*) mutants by complementation test, either of the two recessive mutations, hypoxanthine-guanine phosphoribosyltransferase deficiency (HGPRT$^-$), or adenine phosphoribosyltransferase deficiency (APRT$^-$), was additionally induced in the mutant clones by mutagenesis with MNNG. *Had* cells to which these mutations were introduced were selected with a medium containing appropriate reagents as follows: 10 μM 6-thioguanine for HGPRT$^-$ (24) or 20 μM 4-carbamoylimidazolium 5-olate for APRT$^-$ (14). Two clones which carried HGPRT$^-$ and APRT$^-$ markers, respectively, were then crossed by somatic hybridization, and cell hybrids were selected by using GAMA medium containing 200 μM guanine, 100 μM adenine, 6 μM mycophenolic acid, and 20 μM azaserine, as described by Liskay and Patterson (19). Somatic cell hybridization was done with polyethylene glycol (molecular weight, 1,540), and the hybrid nature of the selected clones was confirmed by karyotype analysis as described by Koyama et al. (13). Cell hybrids were subjected to the hemadsorption test to judge whether two mutant cell lines complemented each other.

**Lectin sensitivities.** Cells in a 24-well dish ($2 \times 10^3$ cells per well) were grown for 3 days in 1 ml of ES medium containing 2% FCS and various concentrations of lectins, and the number of viable cells was counted. Lectin sensitivities of the cells were expressed in terms of the lowest lectin concentration which reduced the cell density to 0 to 10% of that in wells without lectins, as described by Stanley (30).

**Chemicals and lectins.** Mycophenolic acid and 4-carbamoylimidazolium 5-olate were kindly donated by H. Koyama (Yokohama Municipal University). MNNG, tunicamycin, actinomycin D, 6-thioguanine, fluorodeoxyuridine, and azaserine were obtained from Sigma Chemical Co. (St. Louis, Mo.). Ouabain was obtained from Boehringer GmbH (Mannheim, Federal Republic of Germany). Wheat germ agglutinin (WGA), concanavalin A, leukophytohemagglutinin (L-PHA), and ricin were obtained from Honen Co. (Tokyo, Japan). A lectin from *Griffonia simplicifolia*, GS-II, was obtained from E. Y. Laboratories, Inc., (San Mateo, Calif.).

## RESULTS

**Isolation of mutants.** Cells permissive to NDV infection display hemagglutinin-neuraminidase glycoproteins on their surface during the viral propagation process, which makes them capable of adsorbing erythrocytes (21). Figure 1 shows the time course of the development of the hemadsorptive activity in L cells after NDV infection. Hemadsorption reached a maximum at about 10 h after infection and then decreased gradually as the virus particles were released. The hemadsorption activity of the cells depended on the viral dose and saturated above $2.7 \times 10^8$ PFU/90-mm dish (data not shown). Mutants which were unable to support viral multiplication would become hemadsorption negative, thus providing the basis for mutant selection.

Based on these observations, the following protocol was devised. FM3A cells were mutagenized with MNNG and then subjected to the hemadsorption test by adding fowl erythrocytes at 14 h after infection with a minimum saturating dose of NDV. Under this condition, the cells could

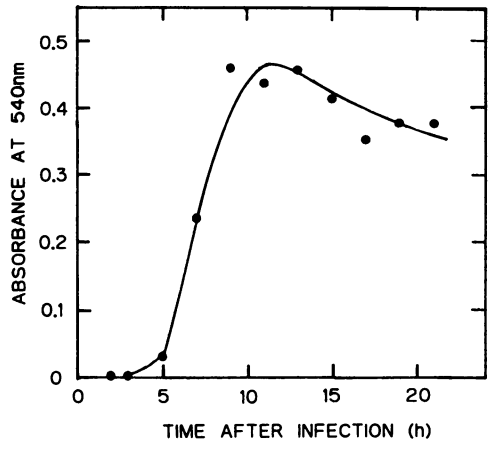


FIG. 1. Time course of the development of hemadsorptive activity in L cells after NDV infection. Cells ($2 \times 10^6$) were infected with NDV at a multiplicity of 15 PFU per cell. At the indicated times after infection, the cells were incubated with 2 ml of a 2% suspension of fowl erythrocytes in PBS for 30 min at 4°C. They were washed with ice-cold PBS, and the adsorbed erythrocytes were lysed by adding 1 ml of 0.5 N ammonium hydroxide. $A_{540}$ was measured.

survive the viral infection. Among $2 \times 10^5$ cell islands derived from a single batch of mutagenized cells, we found 14 negative islands. They were isolated with steel cups and then cloned on agar plates. The mutant clones were definitely less hemadsorptive than the parent (Fig. 2) and were designated as *Had*-1a, -1b, etc. Although *Had*-1b and *Had*-1f were hemadsorptive to some extent (Fig. 2D and E), all other clones were similar to *Had*-1a (Fig. 2C) and almost completely nonhemadsorptive. The growth rate and colony-forming efficiency of the mutant clones were virtually the same as those of the parent (data not shown).

**Absence of viral protein synthesis in NDV-infected *Had*-1 mutant cells.** To identify which step in NDV replication is defective in the mutant cells, we infected them with NDV and 7 h later labeled them with [$^{35}$S]methionine for 1 h. NDV proteins in cell lysates were immunoprecipitated with anti-NDV antiserum and then analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Few, if any, viral proteins were detectable in mutant cells except for *Had*-1b, in which small but definite amounts of viral proteins were synthesized (Fig. 3). Other mutant clones which are not shown here gave almost the same results as *Had*-1a. Synthesis of viral proteins in *Had*-1b cells is consistent with the fact that these cells are partially hemadsorptive. These results suggest that the defects of *Had*-1 mutants lie in earlier stages of NDV propagation preceding the massive accumulation of viral proteins.

**Absence of NDV adsorption to *Had*-1 mutant cells.** Viral attachment onto the host cell surface was measured directly by using [$^3$H]leucine-labeled NDV virions prepared by metabolic labeling in BHK-21 cells. Experiments were done in triplicate for each mutant clone and the results were averaged (Table 1). Clearly, every mutant clone adsorbed many fewer virions compared with the parent, indicating that the mutant clones isolated in this study were almost devoid of NDV receptor activity.

**Genetic complementation test for isolated mutant clones.** To determine whether the isolated mutant clones could be divided into more than one group carrying lesions on different genes, they were subjected to genetic analyses by somatic hybridization. We first tested whether the mutant phenotype of each clone was dominant or recessive. To do this, we fused Fthy$^-$oual cells, which bear both thymidine auxotrophic (recessive) and ouabain-resistant (dominant) mutations, with each *Had*-1 mutant clone. Hybrid cells were selected in OUA medium, and the hemadsorption test was carried out. In every crossing, the hybrid cells were found to be hemadsorptive (data not shown), which indicated that every genetic lesion of *Had*-1 mutants was recessive to the parental allele.

Genetic complementation tests among 14 *Had*-1 mutant clones were then done, and the results are summarized in Table 2. For this purpose, another recessive mutation (HGPRT$^-$ or APRT$^-$) was introduced into the mutants. Cell hybrids between one *Had*-1 mutant marked by HGPRT$^-$ and another having the APRT$^-$ marker were selected with GAMA medium and then examined by the hemadsorption test. *Had*-1a did not complement any one of the *Had*-1 isolates (Table 2). The results were also confirmed by experiments in which HGPRT$^-$ and lack of thymidine kinase (TK$^-$) were introduced as selective markers and cell hybrids were selected in hypoxanthine-amethopterin-thymidine medium (data not shown). These results indicated that all the isolates of *Had*-1 mutants obtained in this study had their defects in the same genetic allele. Based on these results, most of the further analyses were made on *Had*-1a, which was chosen as the representative.

**Synthesis of VSV glycoprotein in *Had*-1 mutant cells.** Carbohydrate chains containing sialic acid (Sia) likely constitute a part of the NDV receptor (32), although the details still remain to be elucidated. To determine whether glycosylation occurs normally in *Had*-1 mutant cells, we examined the synthesis of the glycoprotein (G protein) of VSV in virus-infected parental and mutant cells. Cells were infected with VSV and labeled with [$^3$H]leucine. The viral proteins were immunoprecipitated with anti-VSV antiserum and then analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Unglycosylated viral proteins (L, NS, N, and M) synthesized in both cell types were indistinguishable with respect to their electrophoretic mobilities (Fig. 4A). On the other hand, the mobility of the G protein produced in *Had*-1a cells was greater than that of the one in the parental cells. *Had*-1b and *Had*-1f gave the same results. Treatment with tunicamycin further reduced the size of G protein, suggesting that the asparagine-linked sugar chains were only incompletely synthesized in *Had*-1 cells. The size of the G protein synthesized in the presence of tunicamycin was the same in both parental and mutant cells.

Nearly the same amounts of VSV virions were produced from both FM3A and *Had*-1a cells, but G protein in the purified VSV virions harvested from *Had*-1a cells was again definitely smaller in size than the normal one (Fig. 4B). VSV progeny from *Had*-1a cells were infective (data not shown). It was previously shown that G protein of VSV with altered mobility was also produced in several mutant cell lines (10, 12, 26, 27) which have defects in the glycosylation system.

**Lectin sensitivities of FM3A and *Had*-1 cells.** If we assume that *Had*-1 mutants have some defect in the system of glycoconjugate synthesis, then we would expect them to have altered lectin sensitivities compared with those of the parental cells. Toxic plant lectins bind to the cell surface by recognizing specific sugar residues in the surface carbohydrate components as follows: WGA, terminal Sia residues; concanavalin A, mannose residues; L-PHA, *N*-acetyllactosamine in β1→6 branches of complex-type asparagine-linked sugar chains; ricin, terminal galactose (Gal) or *N*-acetylgalactosamine residues; and GS-II, terminal *N*-acetylglucos-

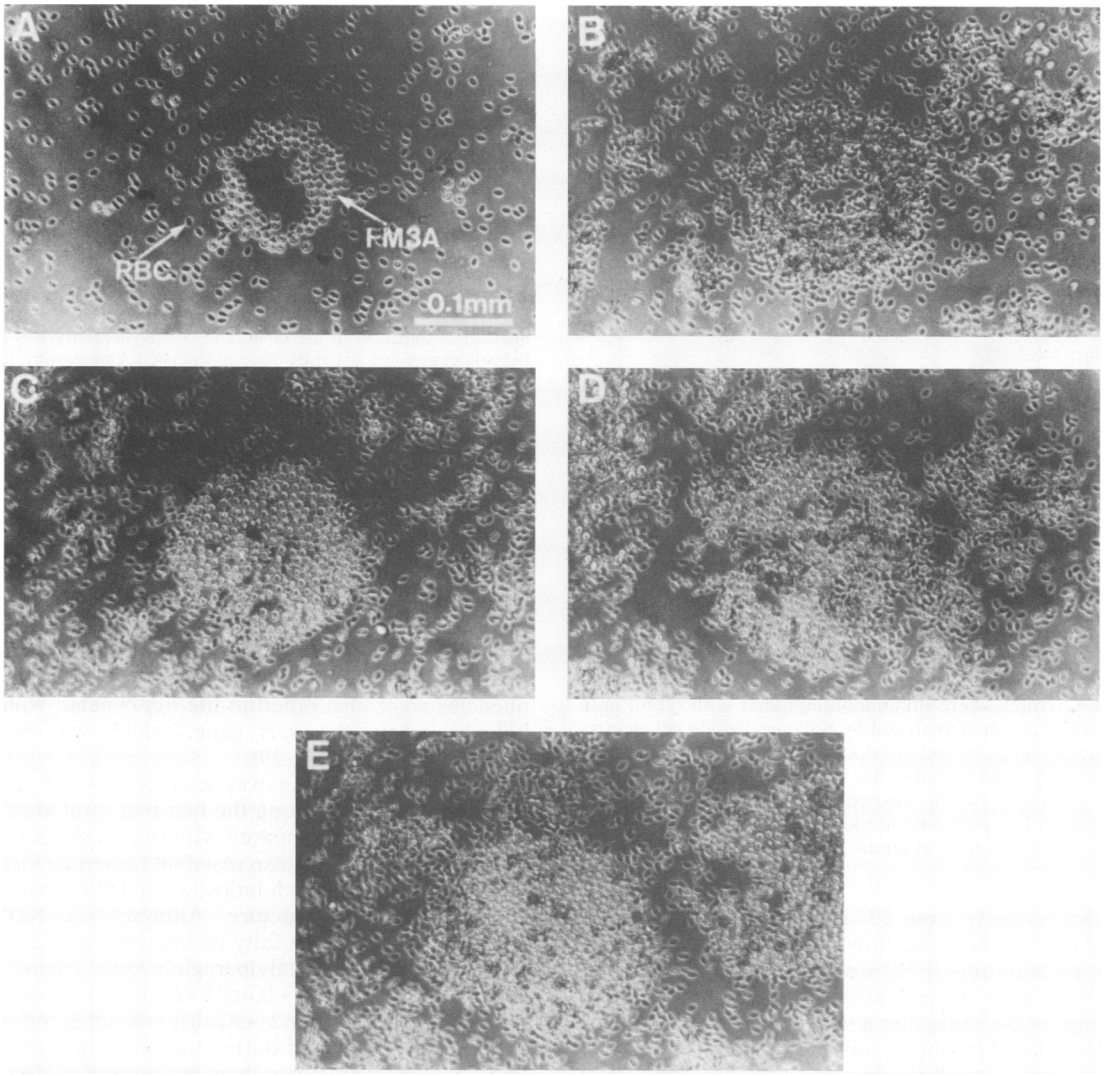


FIG. 2. FM3A and *Had*-1 mutant cells in the hemadsorption test. Cells ($10^4$/90-mm dish) were inoculated and grown for 3 days in a medium containing 1% FCS to form cell islands. After being allowed to settle firmly on the substratum by incubation for 10 h in a medium containing 0.5% FCS, the cells were infected with $2.7 \times 10^8$ PFU of NDV, and 14 h later the hemadsorption test was carried out. (A) Mock-infected FM3A cells. Arrows indicate FM3A cells (FM3A) and erythrocytes (RBC); (B to E) NDV-infected FM3A, *Had*-1a, *Had*-1b, and *Had*-1f cells, respectively. The cells were examined with a phase-contrast microscope (magnification, 10 × 10).

amine (GlcNAc) residues (4, 6, 31). *Had*-1a was 25-fold more resistant to WGA and 10-fold more resistant to L-PHA, but markedly more sensitive to GS-II, as compared with FM3A (Table 3). This strongly suggests that sialylated sugar chains on the cell surface are altered as a result of the *Had*-1 mutation.

## DISCUSSION

We started a systematic study of mouse cell mutants which are nonpermissive to NDV multiplication to gain a deeper insight in the virus-host cell interactions. A new procedure for the selection of mutants which is based on the hemadsorptive activity of NDV-producing cells was devised. Virus-resistant cells were selected in previous studies by picking up survivors after exposure to an excessive dose of viruses sufficient to cause the lysis of the parental cells. In our present protocol, cells are infected with a minimum dose of virus, which makes parental cells fully hemadsorptive but eventually allows their recovery and survival. If the cells were heavily infected to kill the parental cells completely, cytotoxic effect due to the excessive viral dose might cause the cells to lyse during the earlier stages of the viral replication cycle, thereby masking some mutants defective in supporting later stages. Therefore, the present screening may potentially provide us with a more comprehensive set of mutant cells.

The mouse cell-NDV system is suitable for our present protocol since the virus is less cytotoxic and a good recovery of cells from NDV infection is noted. The efficient interferon induction by NDV (22) may be partly responsible for this recovery. FM3A cells, among others, were chosen as the parental cell line because of their favorable characteristics for genetic experiments; they have the normal diploid karyotype (36), and hence the isolation and the genetic analysis of recessive mutants are easier compared with other polyploidic cell lines such as L cells. In fact, we were able to isolate

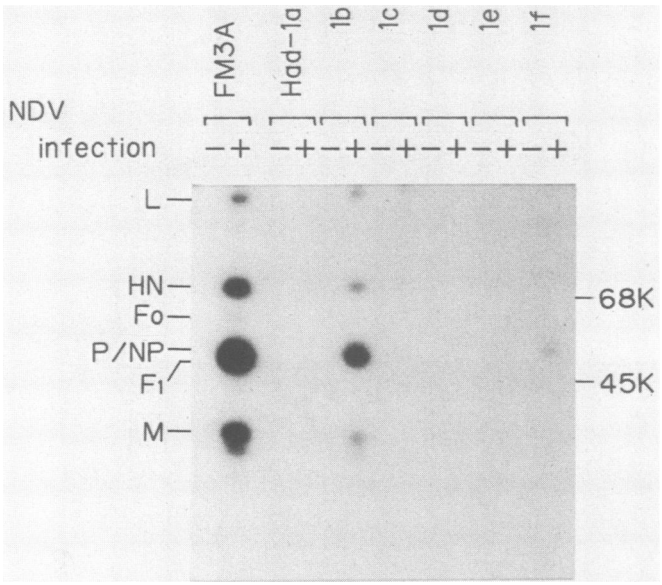


FIG. 3. NDV protein synthesis in FM3A and *Had*-1 mutant cells. Cells ($2 \times 10^6$) were infected with NDV at a multiplicity of 100 PFU per cell. At 7 h after infection, cells were labeled with [$^{35}$S]methionine for 1 h, and cell extracts were prepared. NDV proteins in the extracts were immunoprecipitated with rabbit anti-NDV serum and were then analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (12% polyacrylamide) followed by fluorography. L, HN, $F_0$, P, NP, $F_1$, and M indicate the positions of the respective NDV proteins on the electropherogram. The positions marked by 68K and 45K indicate bovine serum albumin and ovalbumin, respectively.

HGPRT$^-$ clones easily from $10^5$ mutagenized FM3A cells but not from L cells (data not shown). Furthermore, depending on culture conditions, FM3A cells can form colonies on an agar medium, grow in suspension in a medium containing 1 to 2% serum, or be settled on a substratum at 0.5% serum concentration. By choosing a suitable condition, we were able to easily carry out cell cloning on a soft agar, large-scale culture for preparative purpose in suspension, or virus infection and metabolic labeling with cells attached to a substratum.

In the present study, 14 hemadsorption-negative isolates were obtained and characterized. The genetic complementation test revealed that they had defects in the same genetic locus. The hemadsorption-negative phenotype was recessive and was maintained for generations without any selective pressure. *Had*-1 mutation occurred at a considerably high frequency ($7 \times 10^{-5}$) for some reason. We have isolated very recently a distinct class of NDV receptorless mutant whose occurrence was some 40-fold less frequent than the *Had*-1 mutant (T. Hara, unpublished data). High mutational frequencies were also noted in the *hgprt* gene, which was sex linked, and in the *aprt* gene, which was present as a hemizygous locus (28, 29).

TABLE 1. Adsorption of $^3$H-labeled NDV virions to FM3A and *Had*-1 mutant cells[a]

| Cell type | $^3$H-labeled NDV bound (cpm/2 × $10^6$ cells) |
|---|---|
| *Had*-1a | 10 |
| *Had*-1x[b] | <100 |
| FM3A | 490 |
| L | 480 |

[a] NDV was metabolically labeled with [$^3$H]leucine in BHK-21 cells. Purified $^3$H-labeled virions ($10^4$ cpm) were allowed to adsorb to FM3A and *Had*-1 mutant cells ($2 \times 10^6$) at 37°C for 30 min at a multiplicity of 13 PFU per cell. After being washed with ice-cold PBS, cells were scraped off the plate into 4% trichloroacetic acid, and the radioactivity of the insoluble precipitates was measured. The results were corrected for the nonspecific binding (approximately 400 cpm) to each cell type, and the averaged values from three independent experiments are presented in the table. The standard deviations were within the range of ±20%. The value 0 was given when the uncorrected binding was equal to or less than the nonspecific binding.

[b] *Had*-1x represents any of the *Had*-1 mutant clones other than *Had*-1a.

TABLE 2. Complementation analyses between *Had*-1 mutants and the parental line[a]

| Cross | Selection medium | Complementation |
|---|---|---|
| Fthy$^-$ oua1 × *Had*-1a | OUA | + |
| Fthy$^-$ oua1 × *Had*-1x[b] | OUA | + |
| *Had*-1a APRT$^-$ × *Had*-1x HGPRT$^-$ | GAMA | − |

[a] Fthy$^-$ oua1 cells (a thymidine auxotrophic and ouabain-resistant mutant of FM3A) were fused with each of the *Had*-1 mutants. Crosses between *Had*-1 mutants were done with a suitable derivative of each mutant line in which another recessive mutation (HGPRT$^-$ or APRT$^-$) was introduced additionally as a genetic marker. Cell hybrids were selected by using an appropriate medium as indicated in the table, and complementarity was judged by the hemadsorption test. At least three independent clones were tested for each cross. +, All the hybrid clones tested were hemadsorptive; −, all hybrid clones were nonhemadsorptive.

[b] *Had*-1x represents any of the *Had*-1 mutant clones other than *Had*-1a.

The *Had*-1 mutation was assigned to the deficiency in NDV receptors, based on the fact that viral attachment was markedly reduced compared with that in the parental FM3A cells. The mutant was also resistant to Sendai virus infection (data not shown), which indicates that these viruses share a common receptor structure. Although the NDV receptor molecule has not been fully characterized yet, Suzuki et al. (32) showed that sialylparagloboside (Siaα2→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1-ceramide) (where Glc is glucose) and $GM_3$ (Siaα2→3Galβ1→4Glcβ1-ceramide) were specifically recognized during the NDV-induced hemolysis. In fact, a defective sugar chain processing in *Had*-1 cells was demonstrated by examining the VSV G protein produced in these cells. Thus, the sugar chains of the G protein produced in the mutant seemed to be significantly smaller in size than those in the parental G protein. The structure of the sugar chains on the VSV glycoprotein was determined by Reading et al. (25), and it is reported to be rather invariable irrespective of host cell lines (7). As can be easily noted, the sugar chain of G protein shares the structure of its tail-end moiety (Siaα2→3Galβ1→4) in common with sialylparagloboside and $GM_3$. It is therefore strongly suggested that the *Had*-1 mutant is defective in one of the steps involved in the synthesis of the above structure. Our recent studies on the extensive structural analysis of the sugar chains present in FM3A and *Had*-1a cells have clearly indicated that *Had*-1 cells are unable to transfer galactose residues either to the terminal GlcNAc residues in asparagine-linked sugar chains or to the glucose residues in lipid-linked sugar chains (T. Hara et al., manuscript in preparation). Increased sensitivity of *Had*-1a cells to a plant lectin (GS-II) is consistent with the exposure of GlcNAc at the end of asparagine-linked sugar chains of the mutant. WGA resistance of Had-1a cells is also quite reasonable if we assume that NDV recognizes *N*-acetylneuraminic acid, since the same sugar residue is also recognized by the lectin. The pattern of lectin sensitivities of *Had*-1a resembles that of CHO-derived *Lec*-8, 1d1B mutant,

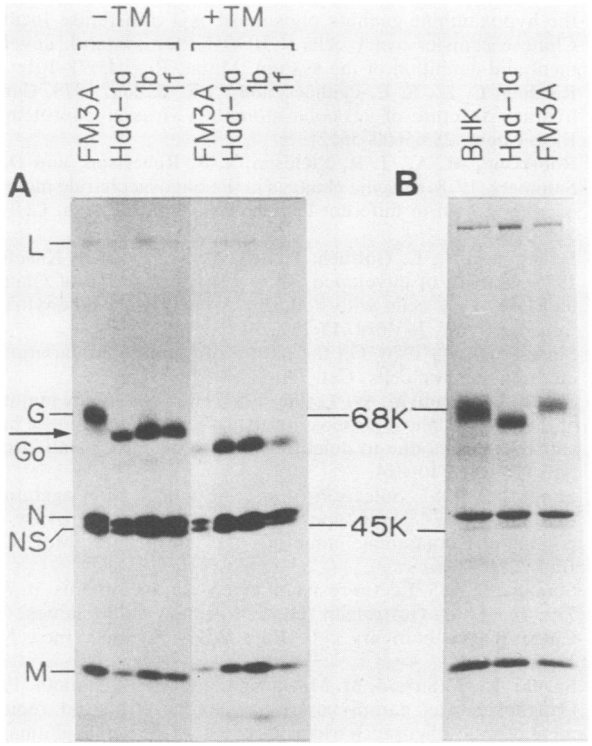


FIG. 4. (A) VSV protein synthesis in *Had*-1 mutant cells. Cells ($2 \times 10^6$) were infected with VSV at a multiplicity of 10 PFU per cell. At 6 h after infection, cells were pulse-labeled with [$^3$H]leucine for 1 h, chased for 1 h, and then immunoprecipitated with mouse anti-VSV serum. +TM indicates that the samples were treated with tunicamycin (0.5 μg/ml) from the beginning of infection to the end of the chase. The immunoprecipitates were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (10% polyacrylamide) followed by fluorography. G and $G_0$ represent the glycosylated and unglycosylated forms of the VSV glycoprotein, respectively. L, N, NS, and M are unglycosylated viral proteins. The arrow indicates the position of the G protein derived from *Had*-1a cells. The positions of bovine serum albumin (68K) and ovalbumin (45K) are also indicated. (B) VSV virions produced in *Had*-1a cells. Cells ($5 \times 10^8$) were infected with VSV at a multiplicity of 1 PFU per cell. At 24 h after infection, culture fluid was collected and VSV particles were purified by the method of Barenholz et al. (2). Virion proteins were analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (10% polyacrylamide) and stained with Coomassie brilliant blue.

and MDAY-D2-derived MDW4 (5, 12, 31). Genetic and biochemical analyses of the relationship of *Had*-1 with these cells are currently in progress. Mutants of sugar chain synthesis would be a valuable tool in analyzing not only virus-cell interaction but also the function of glycoconjugates in a variety of cellular activities. The *Had*-1 phenotype may also serve as a readily available recessive genetic marker in complementation analyses and other genetic studies making use of the FM3A system.

It was rather unexpected to find that all of the 14 isolates of mutant cells belonged to a single complementation group. Mutants of other complementation groups must have been masked and escaped our detection because of the highly frequent occurrence of the *Had*-1 mutation. In this respect, we admit that the outcome of our first attempt has fallen rather short of our aim, since we attempted to have a collection of as many mutant alleles involved in the virus propagation process as possible. Because of the finding that *Had*-1 cells are extremely sensitive to GS-II, however, we would be able to eliminate mutants of this complementation group easily in subsequent trials. This would certainly increase the chance of obtaining mutants with lesions in other genetic loci. Further attempts to isolate mutants whose genetic lesions reside in some steps in the intracellular phase of viral multiplication are in progress in this laboratory.

TABLE 3. Lectin sensitivities of FM3A and *Had*-1a cells[a]

| Cell type | $LD_{10}$ | | | | |
|---|---|---|---|---|---|
| | WGA (μg/ml) | ConA (μg/ml) | L-PHA (μg/ml) | RCA-60 (ng/ml) | GS-II (μg/ml) |
| FM3A | 2 | 6 | 10 | 60 | >200 |
| *Had*-1a | 50 | 6 | 100 | 15 | 4 |

[a] The lectin concentrations which reduced the cell density to 0 to 10% of that of untreated cells ($LD_{10}$) were determined by a semiquantitative 3-day growth test as described in Materials and Methods. Lectins used were WGA, concanavalin A (ConA), L-PHA, ricin (RCA-60), and GS-II.

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