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Restricted Replication of Two Alphaviruses in Ricin-Resistant Mouse L Cells with Altered Glycosyltransferase Activities

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Two mouse L cell variant lines (CL 3 and CL 6) selected for resistance to the toxic plant lectin ricin were restricted in their ability to replicate the two alphaviruses Sindbis virus and Semliki Forest virus. CL 3 cells have been shown to exhibit increased CMP-sialic acid:glycoprotein sialyltransferase and GM_3 synthetase activities, whereas CL 6 cells have been shown to contain decreased UDPgalactose:glycoprotein galactosyltransferase and UDP-*N*-acetylglucosamine: glycoprotein *N*-acetylglucosaminyltransferase activities. The adsorption of Sindbis virus to CL 6 cells was considerably reduced, suggesting that the loss or inaccessibility of the receptors for Sindbis virus accounted for a major defect in virus production in these cells. In contrast, CL 3 synthesized Sindbis viral RNA and proteins but were unable to convert the precursor glycoprotein PE2 to the structural protein E2. The cleavage of PE2 to E2 was also blocked in both CL 3 and CL 6 cells infected with Semliki Forest virus.

Sindbis virus and Semliki Forest virus are closely related enveloped RNA alphaviruses (27). The genome of these virions is a 42-49S plus-strand RNA which is translated to produce polypeptides involved in RNA synthesis and replication. Both virions contain three structural proteins: two glycoproteins, E1 and E2, and the internal capsid protein, C. Semliki Forest virus also contains an additional glycoprotein, E3, derived from the precursor to E2 (9). These proteins are translated from a 26S mRNA, a subgenomic species of the virion RNA found in infected cells. There is a single initiation site for translation on the 26S mRNA, and during translation there are a series of proteolytic cleavages to generate the capsid protein, PE2 (a precursor of E2), and E1. One of the last steps in the assembly of these alphaviruses is the cleavage of PE2 to E2. This step is essential for virion formation (2-4, 13, 24, 25).

We have been investigating the role of glycosylation in the structure and replication of Sindbis virus and the rhabdovirus vesicular stomatitis virus (VSV). There is considerable evidence that the structures of the oligosaccharide chains on viral glycoproteins are determined by host cell enzymes (8, 15, 17). In a previous study we analyzed the structure and replication of these viruses in a variant of CHO cells deficient in a specific UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosaminyltransferase activity (20). These cells produce normal amounts of infectious virus, although the viral glycoproteins contain incomplete oligosaccharide chains. Robertson et al. have grown VSV on other CHO variants and obtained similar results (19).

In contrast to the results obtained previously, we now report that two mouse L cell ricin-resistant variant clones with altered membrane carbohydrate contents fail to support productive infection by either Sindbis virus or Semliki Forest virus. Both, however, are capable of producing infectious VSV. One of the variant clones, termed CL 3, has been shown to have an increased content of membrane sialic acid, whereas the contents of galactose, mannose, and hexosamines are not significantly altered relative to those of the parent L cells. These cells were found to have increased CMP-sialic acid: glycoprotein sialyltransferase and GM_3 synthetase activities, which could account for the observed changes in carbohydrate content. The other variant, CL 6, was found to have decreased amounts of membrane sialic acid, galactose, and hexosamine but an increased content of mannose. This variant was shown to have decreased UDP-*N*-acetylglucosamine:glycoprotein *N*-acetylglucosaminyltransferase and UDPgalactose: glycoprotein galactosyltransferase activities. The detailed characterization of these cells has been reported elsewhere (11). The steps involved in Sindbis and Semliki Forest virus production

have been examined in each cell line to locate the block in virus production. The findings are reported below.

MATERIALS AND METHODS

Cells. The variant clones 3 and 6 were selected from a mouse fibroblast L cell line by using ricin, a highly toxic galactose-binding lectin, as described previously (11). The parent and variant cells were grown both as monolayers and as suspension cultures in alpha minimal essential medium supplemented with 10% fetal calf serum, 50 U of penicillin, and 50 μg of streptomycin per ml.

Growth of viruses. Sindbis virus used in these experiments was a substrain selected for its ability to grow in the mouse plasmacytoma line MOPC 315 (28). It also exhibits enhanced infection of mouse L cells relative to the wild-type virus. Semliki Forest virus was obtained from S. I. T. Kennedy (University of California at San Diego, La Jolla, Calif.), and the Indiana strain of VSV was obtained from Alice Huang (Harvard University, Cambridge, Mass.). Nearly confluent monolayers of cells were infected with Sindbis and Semliki Forest viruses at multiplicities of infection of 100 to 200 PFU per cell or VSV at 10 PFU per cell. The virus was allowed to adsorb for 1 h at 37°C, followed by removal of the unadsorbed inoculum and addition of fresh growth medium. The medium was harvested 12 to 16 h after infection. Radioactive labeling of viruses with [^{35}S]methionine ([^{35}S]Met), [^{3}H]glucosamine, or [^{14}C]glucosamine was performed as previously described (20) with the exception that only methionine was omitted from the medium rather than all amino acids when [^{35}S]Met was used for labeling.

Virus purification. Harvested virus was purified by equilibrium centrifugation in a sucrose gradient containing 0.1% bovine serum albumin as previously described (10).

Determination of PFU. For PFU determination, samples were diluted and titered on monolayers of chicken embryo fibroblasts as described previously (20).

Measurement of viral RNA synthesis. Cells were infected as described above, and uninfected dishes were treated identically. At the end of the adsorption period the monolayers were washed twice, and 2 ml of alpha minimal essential medium containing 1 μg of actinomycin D per ml was added. [^{3}H]-uridine (2 μCi) was added to each dish 1 h later. After various periods of incubation at 37°C, duplicate dishes were washed three times with cold phosphate-buffered saline (PBS). Each dish was then scraped with a rubber policeman in 1 ml of cold 10% trichloroacetic acid and washed with 1 ml of acid. The precipitate from each dish was collected on a membrane filter (Millipore), dried, and counted in scintillation fluid.

Indirect immunofluorescence studies. Cells grown as monolayers on sterile glass cover slips for 24 h were infected as described above. At 2 and 12 h after infection, cover slips were washed with PBS. Half the cover slips were air dried and then fixed by soaking in acetone for 10 min, whereas the other half were processed directly, without drying or fixation. Uninfected cells were treated identically except for the absence of virus in the adsorption step. Rabbit anti-Sindbis antiserum (1:200 dilution) was layered onto the cover slips. Incubation was carried out in a humidified atmosphere for 20 min at room temperature. After extensive washing with PBS, the cover slips were layered with fluorescein-conjugated goat anti-rabbit antiserum (1:50 dilution; Gateway Immunosera Co., St. Louis, Mo.) and incubated in a humidified atmosphere for 30 min at room temperature. The cover slips were again washed extensively and then mounted onto microscope slides with glycerol for examination with a Zeiss fluorescence microscope.

Transmission electron microscopy. Cells infected with either Sindbis virus or Semliki Forest virus were washed with PBS and fixed with glutaraldehyde. Cells were scraped with a rubber policeman, stained, and embedded in Spurr low-viscosity resin by standard methods (18, 26). Uninfected cells were prepared in an identical way.

Virus binding assay. Gradient-purified [^{35}S]Met-labeled virus was dialyzed against PBS before use in virus binding assays. In method 1, suspension culture cells were harvested in log-phase growth (5×10^5 to 8×10^5/ml) and washed twice with PBS. Cells were then suspended in PBS containing 1% fetal calf serum (PBS-FCS) and incubated with virus in test tubes presoaked in PBS-FCS. After various periods of incubation at either 25 or 37°C, 0.2-ml samples (0.3×10^6 to 2×10^6 cells) from the mixture were removed and injected into 3 ml of ice-cold PBS. The cells were centrifuged at top speed in a clinical centrifuge and washed once more with 3 ml of cold PBS. The pellets were dissolved in tissue solubilizer and counted in scintillation fluid. Controls consisted of incubation mixtures without cells. In method 2, monolayers of cells were grown in 15-mm-diameter glass vials overnight (2×10^5 to 5×10^5 cells). Control blank vials were similarly incubated with growth medium. All vials were then washed with 2 ml of PBS-FCS and then incubated with radioactive virus in 0.2 ml of PBS-FCS at either 25 or 37°C. After various periods of incubation, duplicate vials were washed twice with 2 ml of cold PBS each time. The contents of the vials were dissolved in tissue solubilizer and counted directly in scintillation fluid. In both methods the background binding in the absence of cells was usually less than 0.5% of the maximum binding to L cells.

Pulse-chase radioactive labeling of intracellular alphavirus proteins. Monolayers in 35-mm petri dishes were infected with Sindbis virus or Semliki Forest virus as described above. Actinomycin D (1 μg/ml) was added to the dishes after the adsorption period to decrease host cell protein synthesis. Five hours after infection the incubation medium was changed to Met-free medium containing 10% fetal calf serum. Then 1 h later 10 μCi of [^{35}S]Met was added to each dish. After 1 h of further incubation, one dish of each cell line was harvested, and the duplicate dishes were washed three times with PBS and reincubated for an additional 1 h in medium containing a fourfold excess of Met and 10% fetal calf serum. These chase plates were then also harvested by washing with PBS and scraping the monolayers. The extracts were then analyzed by polyacrylamide gel electrophoresis.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Samples were reduced before sodium dodecyl sulfate-polyacrylamide gel electrophoresis on discontinuous slab gels. The procedure for electrophoresis was described previously (20).

Preparation and analysis of Sindbis virus PE2 glycopeptides. Monolayers of L and CL 3 cells (5×10^6 to 1×10^7 cells in 100-mm dishes) were infected with Sindbis virus and incubated with medium containing 1 μg of actinomycin D per ml. At 4 h postinfection the cells were labeled with 20 μCi of [^{14}C]-glucosamine and 100 μCi of [^{3}H]glucosamine, respectively, in medium containing 1 μg of actinomycin D per ml and 1/10 the normal amount of glucose. Incubation was terminated 4 h later. The cells were scraped from the plates in 10% trichloroacetic acid, sedimented by centrifugation, and washed with ether to remove the acid. They were then solubilized in 4% sodium dodecyl sulfate. PE2 was purified on cylindrical polyacrylamide gels as described previously (21). The PE2 fractions eluted from the gel slices were precipitated with 9 volumes of acetone after the addition of 0.1 mg of bovine gamma globulin as carrier. A portion of each sample was reanalyzed by gel electrophoresis to ascertain that there was no contamination with the E1 Sindbis glycoprotein. The remainder of the samples were then combined and subjected to Pronase digestion as described by Gottlieb and Kornfeld (11). One-half of this sample was chromatographed on Bio-Gel P-6 (1.2 by 100 cm) in 0.1 M Tris-hydrochloride, pH 8 (23). The other half was treated with 25 U of neuraminidase (Behring Diagnostics, Somerville, N.J.) per ml at pH 5.2 for 16 h at 37°C under a toluene atmosphere. This fraction was then chromatographed on the Bio-Gel P-6 column.

RESULTS

Restricted growth of two alphaviruses in ricin-resistant L cell variants. CL 3 and CL 6, the two ricin-resistant clones derived from mouse L cells, did not support the growth of either Sindbis virus or Semliki Forest virus. Virus yields on these clones were reduced more than 10^3-fold compared with those obtained on L cells (Table 1). When infections were carried out in the presence of [^{35}S]Met and the radioactive medium from each cell line was analyzed by sucrose gradient centrifugation, no peak of radioactivity at the density of virus could be detected from the medium harvested from CL 3 or CL 6 cells (data not shown). Thus, the decrease in infectious units corresponded to a decrease in particle formation.

In contrast to the results obtained with these alphaviruses, the yields of VSV from all three cell lines did not show any major differences (Table 1). Therefore, the lack of formation of Sindbis virus and Semliki Forest virus cannot be due to nonspecific mechanisms, such as constitutive interferon production or nonspecific virus inactivation either in the medium or intracellularly. In addition, we have previously reported that the VSVs synthesized by the two variant cell lines contained the carbohydrate alterations characteristic of their respective host cells (11).

TABLE 1. *Production of alphaviruses and VSV in L cells and the two ricin-resistant cell lines*

Cell line	Virus (PFU/cell)		
	Sindbis	Semliki Forest	VSV
L	3,200	3,600	525
CL 3	14	14	225
CL 6	1.7	0.6	450

Sindbis virus infection of CL 6 cells. In our initial observations we noted that CL 6 cells exposed to Sindbis virus did not show any cytopathic effects, whereas in all of the other infections cell death was apparent. Furthermore, indirect immunofluorescent assays demonstrated that CL 6 cells infected with Sindbis virus did not synthesize virus-specific antigens under conditions in which infected L cells and CL 3 cells both showed positive fluorescence. These results indicated that the restricted replication of Sindbis virus in CL 6 cells was due to a mechanism distinct from that restricting Sindbis virus replication in CL 3 cells or Semliki Forest virus replication in both cell lines. A comparison of Sindbis viral RNA synthesis in CL 6, CL 3, and L cells provided further evidence for this distinction (Fig. 1). In this experiment the cell monolayers were treated with actinomycin D to suppress host DNA-dependent RNA synthesis. L and CL 3 cells exposed to Sindbis virus actively incorporated [^{3}H]uridine into acid-precipitable material markedly above the background level of incorporation found in their respective uninfected controls. In contrast, there was no difference in the amounts of radioactivity incorporated by infected and uninfected CL 6 cells. These results demonstrated that the block in the replication of Sindbis virus in CL 6 cells occurs at some point before genome replication and messenger amplification.

All three cell lines showed marked stimulations of RNA synthesis after infection with Semliki Forest virus (data not shown).

One possible explanation for the failure of CL 6 cells to synthesize Sindbis virus components is that specific virus adsorption does not occur. To test this possibility, we examined the adsorption of radioactive Sindbis virus to L cells and to the two variant clones. For comparison we also tested the adsorption of VSV to these cells. The results of adsorption studies with suspension culture cells are shown in Fig. 2. Similar results were obtained with cell monolayers. Relative to the parent L cells, the adsorption of Sindbis virus to CL 6 cells was markedly reduced,

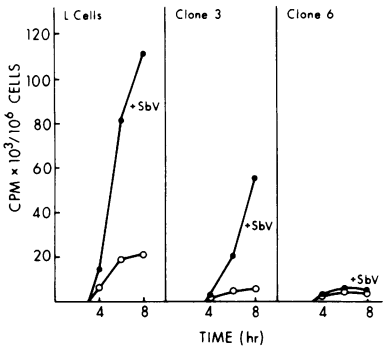

FIG. 1. *Synthesis of virus-specific RNA in Sindbis-infected (+SbV) L, CL 3, and CL 6 cells. [³H]uridine incorporation into acid-precipitable material was measured in cell monolayers treated with actinomycin D. Open circles represent monolayers not exposed to Sindbis virus. The cell lines used are as marked for each panel.*

whereas that of VSV was not significantly different. In this experiment adsorption of Sindbis virus to CL 3 cells was moderately reduced. Although the decreased adsorption to CL 3 cells was not observed in all experiments, we noted that in some instances not all of the CL 3 cells exposed to Sindbis virus became productively infected. We measured the percentage of cells infected by Sindbis virus by using an indirect immunofluorescent assay as described in Materials and Methods. Twelve hours after exposure to virus, virtually all of the L cells and 50 to 80% of CL 3 cells showed positive immunofluorescence, whereas CL 6 cells were negative and essentially indistinguishable from uninfected cells.

To rule out the possibility that CL 6 cells selectively inactivated Sindbis virus during incubation, we preincubated radioactive virus with CL 6 cells and then used the preincubated virus for adsorption to L cells. The adsorption was equal to that of an equivalent amount of virus not preincubated with cells. In addition, nonradioactive virus was preincubated with CL 6 cells and then titrated on chicken embryo fibroblasts. The plaque-forming ability was not diminished by such preincubation (data not shown).

The low level of adsorption of Sindbis virus to CL 6 cells may represent nonspecific binding. This adsorption did not initiate viral replication, since essentially no stimulation of viral RNA synthesis was detected in these cells after exposure to Sindbis virus. However, we cannot rule out the possibility that there are also additional blocks in viral replication before RNA synthesis.

Sindbis virus infection of CL 3 and Semliki Forest virus infection of CL 3 and CL 6. The formation of nucleocapsids represents an intermediate stage in the maturation of alphavirions (1). These particles acquire their envelope as they bud from the host cell plasma membrane. The transmission electron micrographs shown in Fig. 3 demonstrate the presence of nucleocapsids within the cytoplasm of L and CL 3 cells infected with Sindbis virus and all three cell lines infected with Semliki Forest virus. Budding and mature virions, however, are seen at the periphery of infected L cells, but not at the surface of infected CL 3 or CL 6 cells.

In L and CL 3 cells the nucleocapsids of both Sindbis and Semliki Forest viruses appear to concentrate around membranous vacuoles. Similar results have been observed previously in Semliki Forest virus-infected L cells as well as in other cell lines (1, 12). This particular pattern was seen much less frequently in CL 6 cells infected with Semliki Forest virus.

A comparison of the virus-specific proteins synthesized in infected L, CL 3, and CL 6 cells was carried out by analyzing the radioactively labeled intracellular proteins on polyacrylamide gels. In CL 3 cells infected with Sindbis virus and in both CL 3 and CL 6 cells infected with Semliki Forest virus the viral glycoprotein E2 was not found, although its precursor, PE2, was. PE2 is normally an intracellular virus-specified glycoprotein which is converted by specific proteolytic cleavage to E2, one of two virion envelope glycoproteins (21). The failure to convert PE2 to E2 was further documented in a pulse-chase experiment (Fig. 4). The major intracellular viral proteins labeled by the pulse in L cells consisted of the capsid protein, C, the precursor,

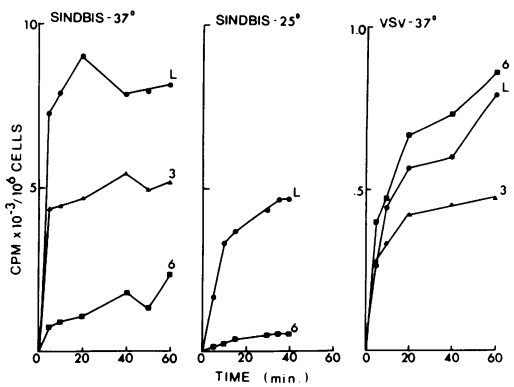

FIG. 2. *Sindbis virus and VSV binding to L, CL 3, and CL 6 cells. Gradient-purified radioactive virus was incubated with suspension culture cells. Samples of the incubation mixture were taken at the indicated times to measure radioactivity sedimenting with the cells which was not removed by washing with buffer. The virus and temperature used for each incubation are as marked for each panel. Symbols: ●, L cells; ▲, CL 3 cells; ■, CL 6 cells.*

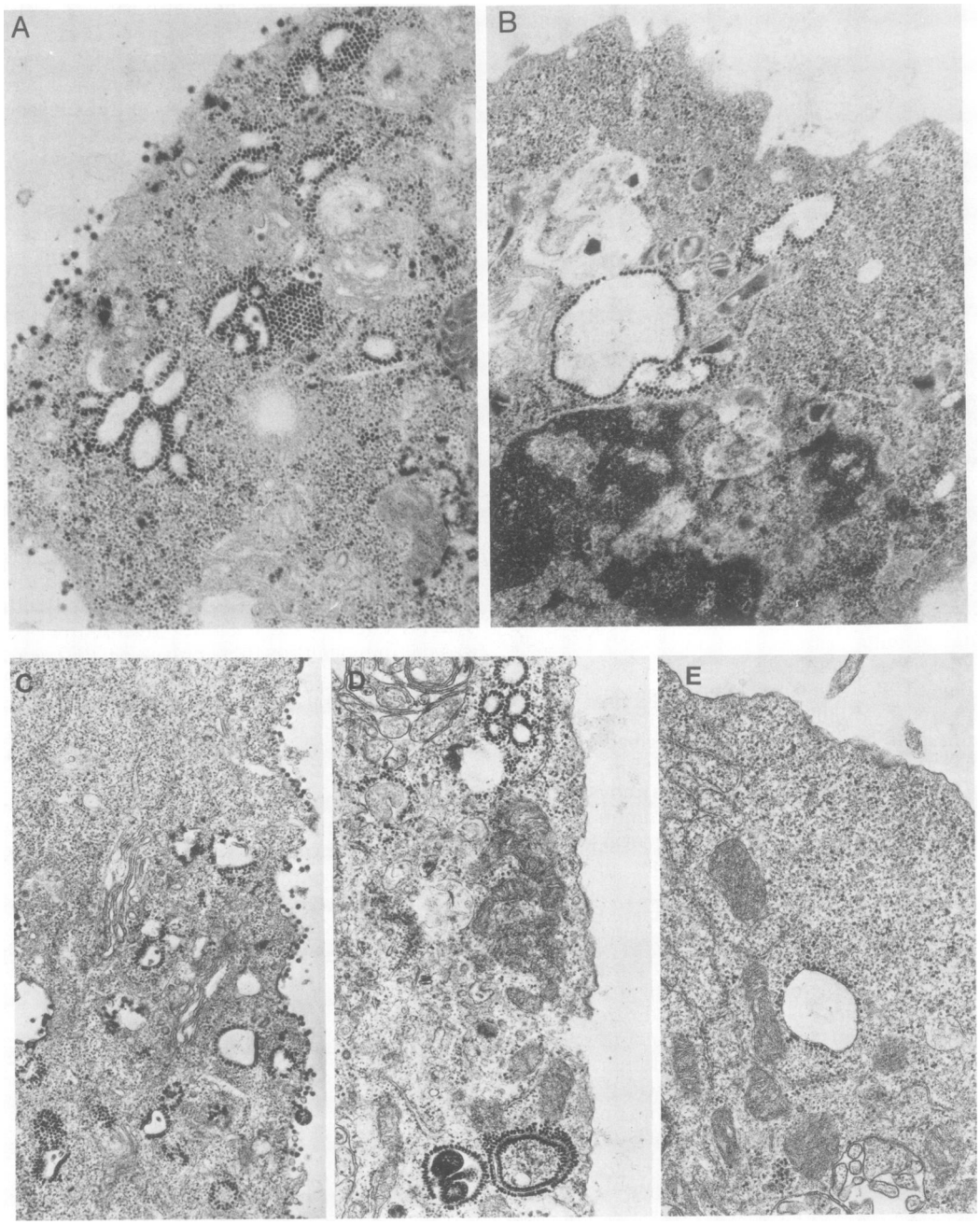

FIG. 3. *Transmission electron micrographs of alphavirus-infected L, CL 3, and CL 6 cells. L and CL 3 cells infected with Sindbis virus were harvested at 10 h postinfection. L, CL 3, and CL 6 cells infected with Semliki Forest virus were harvested at 7.5 h postinfection. The samples were prepared for electron microscopy by standard methods. (A) L cells infected with Sindbis virus; (B) CL 3 cells infected with Sindbis virus (×22,500). (C) L cells infected with Semliki Forest virus; (D) CL 3 cells infected with Semliki Forest virus; (E) CL 6 cells infected with Semliki Forest virus (×18,000).*

PE2, and both virion glycoproteins, E1 and E2. After the chase period the gel pattern of the L-cell proteins showed an increase in labeled E2 and a concomitant decrease in labeled PE2. In contrast, both the pulse and the chase gel profiles of infected variant cells showed virtually no radioactivity corresponding to the position of E2. Since E1, PE2, and C are synthesized from

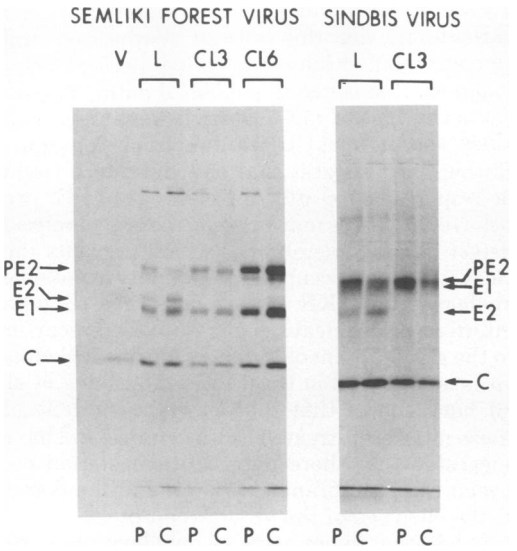

FIG. 4. *Pattern of intracellular viral proteins from alphavirus-infected L, CL 3, and CL 6 cells labeled in a pulse-chase experiment. Monolayers of cells were infected with either Sindbis or Semliki Forest virus in the presence of actinomycin D. [^{35}S]Met was added at 7 h postinfection. One hour later, one dish of each infected culture was harvested (P) and the other was incubated for an additional 1 h in medium containing excess unlabeled Met (C). Samples were analyzed on sodium dodecyl sulfate-polyacrylamide slab gels. V refers to a sample of Semliki Forest virions that was included to identify the virion proteins.*

the same mRNA and cleaved during synthesis into the individual proteins, it is clear that both CL 3 and CL 6 cells can perform the initial proteolytic steps; it is only the cleavage of PE2 that is specifically blocked.

Characterization of glycopeptides of PE 2 isolated from L cells and CL 3 infected with Sindbis virus. Since CL 3 cells have been shown to oversialylate glycoproteins, one possible explanation for the failure of PE2 to be cleaved in CL 3 is that it is also oversialylated. Although there are other explanations for the block in cleavage, to consider oversialylation as a possibility it was necessary to determine if the amounts of sialic acid found in PE2 synthesized in CL 3 and that synthesized in L cells were different. We used radioactive glucosamine to label the sialic acid, *N*-acetylglucosamine, and *N*-acetylgalactosamine residues of the complex carbohydrates (11) and purified PE2 from L and CL 3 cells infected with Sindbis virus by preparative polyacrylamide gel electrophoresis. The samples from each cell line were mixed and digested with Pronase, and one aliquot was chromatographed on a Bio-Gel P-6 column (Fig. 5). The CL 3 PE2 glycopeptides were shifted toward the higher-molecular-weight species relative to those of the L PE2. Such a shift was compatible with an increase in the sialic acid content of the CL 3 PE2. Further evidence that the larger size was due to sialic acid was obtained by chromatography of the second aliquots of the glycopeptides of CL 3 PE2 and L PE2 after pretreatment with neuraminidase. The resulting elution profile of treated CL 3 PE2 glycopeptides showed the disappearance of peaks 1 and 2 as well as an increase in radioactivity eluting with authentic sialic acid. In addition, peak 4 radioactivity increased and became more heterogeneous. Since further studies were not carried out to define these glycopeptides, we cannot relate them to the four glycopeptides obtained from Sindbis virions (23). Our chromatography results, however, established that CL 3 PE2 is more heavily sialylated than L PE2.

DISCUSSION

The two L cell variants used in these studies were selected by their ability to grow in the presence of the lectin ricin and were shown to have altered patterns of glycosylation (11). CL 3 has an increased level of sialyltransferase activity; CL 6 is defective in both *N*-acetylglucosaminyltransferase and galactosyltransferase activities. These variants are also unable to support the replication of the two alphaviruses Sindbis virus and Semliki Forest virus. In both cases CL 3 cells carry out most of the steps of replication except the conversion of the precursor, PE2, to the virion glycoprotein E2. This same step is blocked in CL 6 cells infected with Semliki Forest virus, but in this cell line the replication of Sindbis virus is prevented at a very early step in infection.

It has been well established that the failure to cleave PE2 to E2 is sufficient to prevent virion formation. In previous studies, cleavage has been prevented by using temperature-sensitive mutants in the genes coding for either PE2 or E1 (3, 13, 24, 25), inhibitors of glycosylation (7, 14, 16, 22), or protease inhibitors (2). Furthermore, treatment of cells with antibody directed against E1 will block this cleavage (3). Our finding that PE2 isolated from CL 3 cells infected with Sindbis virus contains more sialic acid than PE2 from Sindbis virus-infected L cells is consistent with the hypothesis that an excess of sialic acid on PE2 may be inhibitory to the proteolytic cleavage. It has been reported that the presence of sialic acid on glycoproteins can block proteolytic activity (30). However, it is also possible that the cleavage enzyme is defective in CL 3, and this may or may not be related to the alteration in glycosylation.

Although it seems reasonable to suggest that oversialylation of PE2 inhibits the cleavage of PE2 to E2, an analogous explanation for the

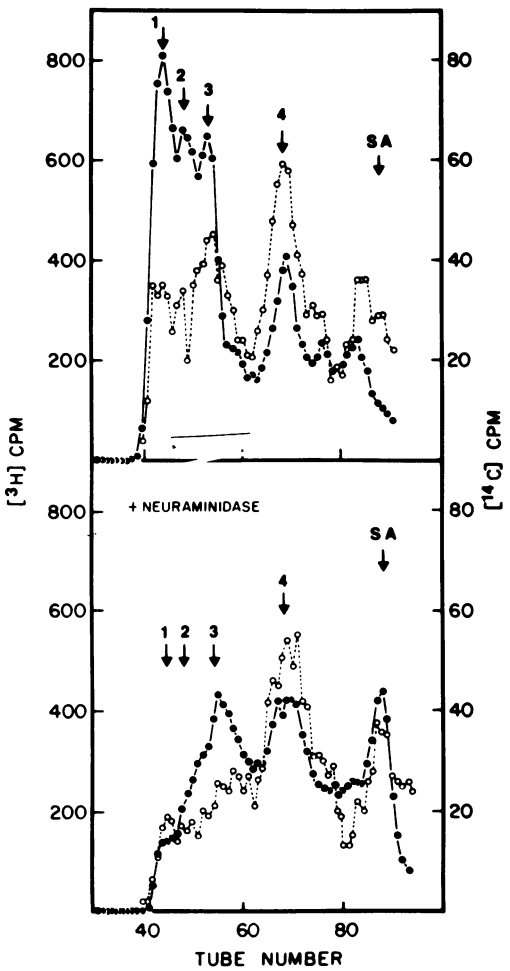

FIG. 5. *Bio-Gel P-6 chromatography of glycopeptides of PE 2 synthesized by L and CL 3 cells. [^{3}H]glucosamine- and [^{14}C]glucosamine-labeled PE2s were purified from infected L and CL 3 cells as described in the text. Glycopeptides were prepared from the PE2s. Half of each sample was chromatographed on a Bio-Gel P-6 column. The other half was digested with neuraminidase and then applied to the same column. The L-cell PE2 glycopeptides were labeled with ^{14}C (○). The CL 3 PE2 glycopeptides were labeled with ^{3}H (●). The upper panel shows the chromatography profile of the untreated glycopeptides, and the lower panel shows the results after neuraminidase treatment. SA denotes the elution position of free sialic acid.*

failure of this reaction to occur in CL 6 cells infected with Semliki Forest virus is less plausible. The oligosaccharide chains on viral glycoproteins in CL 6 are incompletely processed high-mannose chains (S. Kornfeld, unpublished data). It is unlikely that this would affect the cleavage reaction. Both Sindbis and Semliki Forest viruses produce normal yields in the CHO variant 15B, and this cell line synthesizes viral glycoproteins with incompletely processed high-mannose chains (29; unpublished data). The oligosaccharides of 15B contain five mannose residues, and those of CL 6 contain from six to nine. Rather than suggest that this difference could be responsible for affecting cleavage of PE2, we believe that there may be some more generalized defect in CL 6 membranes which prevents the cleavage from occurring. There is a possible analogy with AKR mouse cells treated with interferon. The cleavage of a putative precursor to the glycoprotein of Moloney murine leukemia virus is inhibited in these cells (5). Chang et al. (6) have shown that the plasma membrane of these interferon-treated cells is altered and have suggested that there may be a correlation between this membrane alteration and the defect in the cleavage of the viral glycoprotein.

In addition to not carrying out the proteolytic cleavage of PE2, CL 6 cells are blocked at a very early step in the replication of Sindbis virus. CL 6 cells exposed to Sindbis virus do not synthesize virus-specific RNA and do not exhibit cytopathic effects after infection. Although we cannot rule out the possibility that there are additional blocks in the initial steps in replication, our data showing decreased binding of Sindbis virus to CL 6 cells suggest that there is a block at the level of adsorption. At present very little is known about the chemical nature of the receptors for Sindbis virus. One explanation for the inability of Sindbis virus to attach to CL 6 cells is that there is a specific oligosaccharide structure that is an essential part of the receptor and that it is lacking or inaccessible in CL 6 cells.

We know that CL 6 cells have several phenotypic defects. In addition to the specific alterations we have observed with Sindbis virus and Semliki Forest virus, our electron micrographs suggest that in CL 6 there may be some aberration in the interaction between Semliki Forest virus nucleocapsids and membrane vacuoles. Furthermore, two glycosyltransferase activities are defective (11). The different phenomena that have been observed could reflect a single specific alteration in the cells leading to pleiotropic effects, or they could be indicative of several independent mutations in these cells. The present work, however, demonstrates that cells can be altered in a specific way, presumably in only one or a very few biochemical steps, such that they acquire resistance only to certain viruses. In the case of Sindbis virus and CL 6, the cells have become completely protected against virus infection; in the other cases, although infected cells are killed, the spread of virus is prevented. It will be interesting to explore the idea that

alterations such as those in CL 6 or CL 3 can be associated with genetic resistance or susceptibility to specific viruses, such as is found in certain strains of mice.

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LITERATURE CITED

1. **Acheson, N. H., and I. Tamm.** 1967. Replication of Semliki Forest virus: an electron microscopic study. Virology **32**:128-143.
2. **Bracha, M., and M. J. Schlesinger.** 1976. Inhibition of Sindbis virus replication by zinc ions. Virology **72**:272-277.
3. **Bracha, M., and M. J. Schlesinger.** 1976. Defects in RNA$^+$ temperature-sensitive mutants of Sindbis virus and evidence for a complex of PE2-E1 viral glycoproteins. Virology **74**:441-449.
4. **Brown, D. T., and J. F. Smith.** 1975. Morphology of BHK-21 cells infected with Sindbis virus temperature-sensitive mutants in complementation groups D and E. J. Virol. **15**:1262-1266.
5. **Chang, E. H., and R. M. Friedman.** 1977. A large glycoprotein of Moloney leukemia virus derived from interferon-treated cells. Biochem. Biophys. Res. Commun. **77**:392-398.
6. **Chang, E. H., F. T. Jay, and R. M. Friedman.** 1978. Physical, morphological, and biochemical alterations in the membrane of AKR mouse cells after interferon treatment. Proc. Natl. Acad. Sci. U.S.A. **75**:1859-1863.
7. **Duda, E., and M. J. Schlesinger.** 1975. Alterations in Sindbis viral envelope proteins by treating BHK cells with glucosamine. J. Virol. **15**:416-419.
8. **Etchison, J., and J. Holland.** 1974. Carbohydrate composition of the membrane glycoprotein of vesicular stomatitis virus grown in four mammalian-cell lines. Proc. Natl. Acad. Sci. U.S.A. **71**:4011-4014.
9. **Garoff, H., K. Simons, and O. Renkonen.** 1974. Isolation and characterization of the membrane proteins of Semliki Forest virus. Virology **61**:493-504.
10. **Gibson, R., R. Leavitt, S. Kornfeld, and S. Schlesinger.** 1978. Synthesis and infectivity of vesicular stomatitis virus containing nonglycosylated G protein. Cell **13**:671-679.
11. **Gottlieb, C., and S. Kornfeld.** 1976. Isolation and characterization of two mouse L cell lines resistant to the toxic lectin ricin. J. Biol. Chem. **251**:7761-7768.
12. **Grimley, P. M., J. G. Levin, I. K. Berezesky, and R. M. Friedman.** 1972. Specific membranous structures associated with the replication of group A arboviruses. J. Virol. **10**:492-503.
13. **Jones, K. J., M. R. F. Waite, and H. R. Bose.** 1974. Cleavage of a viral envelope precursor during the morphogenesis of Sindbis virus. J. Virol. **13**:809-817.
14. **Kaluza, G.** 1975. Effect of impaired glycosylation on the biosynthesis of Semliki Forest virus glycoproteins. J. Virol. **16**:602-612.
15. **Keegstra, K., B. Sefton, and D. Burke.** 1975. Sindbis virus glycoproteins: effect of the host cell on the oligosaccharides. J. Virol. **16**:613-620.
16. **Leavitt, R., S. Schlesinger, and S. Kornfeld.** 1977. Tunicamycin inhibits glycosylation and multiplication of Sindbis and vesicular stomatitis viruses. J. Virol. **21**: 375-385.
17. **Lenard, J.** 1978. Virus envelopes and plasma membranes. Annu. Rev. Biophys. Bioeng. **7**:139-165.
18. **Muller, L. L., and T. J. Jacks.** 1975. Rapid chemical dehydration of samples for electron microscopic examination. J. Histochem. Cytochem. **23**:107-110.
19. **Robertson, M. A., J. R. Etchison, J. S. Robertson, and D. F. Summers.** 1978. Specific changes in the oligosaccharide moieties of VSV grown in different lectin-resistant CHO cells. Cell **13**:515-526.
20. **Schlesinger, S., C. Gottlieb, P. Feil, N. Gelb, and S. Kornfeld.** 1976. Growth of enveloped RNA viruses in a line of Chinese hamster ovary cells with deficient *N*-acetylglucosaminyltransferase activities. J. Virol. **17**: 239-246.
21. **Schlesinger, S., and M. J. Schlesinger.** 1972. Formation of Sindbis virus proteins: identification of a precursor for one of the envelope proteins. J. Virol. **10**: 925-932.
22. **Schwarz, R. T., J. M. Rohrschneider, and M. F. G. Schmidt.** 1976. Suppression of glycoprotein formation of Semliki Forest, influenza, and avian sarcoma virus by tunicamycin. J. Virol. **19**:782-791.
23. **Sefton, B. M., and K. Keegstra.** 1974. Glycoproteins of Sindbis virus: preliminary characterization of the oligosaccharides. J. Virol. **14**:522-530.
24. **Simons, K., S. Keranen, and L. Kaariainen.** 1973. Identification of a precursor for one of the Semliki Forest virus membrane proteins. FEBS Lett. **29**:87-91.
25. **Smith, J. F., and D. T. Brown.** 1977. Envelopment of Sindbis virus: synthesis and organization of proteins in cells infected with wild type and maturation-defective mutants. J. Virol. **22**:662-678.
26. **Spurr, A. R.** 1969. A low-viscosity epoxy resin embedding medium for electron microscopy. J. Ultrastruct. Res. **26**:31-43.
27. **Strauss, J. H., and E. G. Strauss.** 1977. Togaviruses, p. 111-166. *In* D. P. Nayak (ed.), The molecular biology of animal viruses, vol. 1. Marcel Dekker, Inc., New York.
28. **Symington, J., and M. J. Schlesinger.** 1975. Isolation of a Sindbis virus variant by passage on mouse plasmacytoma cells. J. Virol. **15**:1037-1041.
29. **Tabas, I., S. Schlesinger, and S. Kornfeld.** 1978. Processing of high mannose oligosaccharides to form complex type oligosaccharides on the newly synthesized polypeptides of the vesicular stomatitis virus G protein and the IgG heavy chain. J. Biol. Chem. **253**:716-722.
30. **Yamashina, I.** 1956. On the carbohydrate components of the α_1-acid glycoprotein of human plasma. Acta Chem. Scand. **10**:1666-1669.