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Cell-Mediated Cytotoxicity Against Targets Bearing Sendai Virus Glycoproteins in the Absence of Viral Infection

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The glycoproteins HN and F and the lipids were solubilized from Sendai virus envelopes by using the nonionic detergent β-D-octylglucoside. When β-D-octylglucoside was removed by dialysis, the glycoproteins and lipids reassociated to form vesicles. These vesicles displayed hemagglutinating, neuraminidase, and hemolysin activities comparable to those expressed by the intact virus. The vesicles were used as carriers to transfer the glycoproteins to the surface of P815 cells. The recipient cells were tested for the acquisition of the glycoproteins by demonstration of surface neuraminidase, hemadsorption activity, and antigens. The modified cells were used as targets for natural cell-mediated lysis and were found to be sensitive.

Viral infection elicits both humoral and cellular immune responses. In the humoral response, antibodies directed to viral glycoproteins are thought to be the major protective mechanism to subsequent exposure (8). The T cell-mediated responses are directed to the viral glycoproteins displayed on infected cell surfaces. These responses additionally require the compatibility of the major histocompatibility antigens of targets and cells of the immune system (18, 34). This component of the immune response plays a role in recovery from viral infection (25, 33). Another cellular response that is thought to be significant in recovery is mediated by natural killer (NK) cells (10, 19, 24, 32). NK cell activity is augumented in viral infections that induce interferon. Santoli et al. (24) presented evidence that increased susceptibility of virus-infected cultures to NK cell-mediated lysis was due to activation of NK cells by interferon released in the cultures. They concluded that the killing was nonspecific and that virus infection was required only for activation of NK cells. Other investigators (2, 19, 32) have suggested that NK cells may recognize viral or nonviral antigens present on virus-infected cells. Recognition could be associated with viral glycoproteins present on the surfaces of the infected cells or with other virus-induced cell surface modifications which result from alterations in cellular metabolism. In the face of such complexity, the role of viral glycoproteins in target susceptibility is obscured. In this report, a method for approaching an analysis of the role of glycoproteins in the absence of virus infection is presented. The glycoproteins HN and F are removed from Sendai virus and reconstituted into lipid vesicles, and the vesicles are used to transfer the proteins to the surface of uninfected cells. The cells are thus rendered susceptible to natural cell-mediated cytolysis.

MATERIALS AND METHODS

Purification of virus. The Cantell strain of Sendai virus (received from R. Chada, Roswell Park Memorial Institute, Buffalo, N.Y.) was grown in 10-day-old embryonated eggs. Eggs were inoculated with 10^3 embryo infectious doses, 50%, and incubated at 37°C. The allantoic fluid was harvested after 72 h and cellular debris was removed by centrifugation at 3,000 × *g* for 30 min at 4°C. Allantoic fluid samples which had a titer of 1,024 or greater were centrifuged at 46,000 × *g* for 2 h at 4°C. The pelleted virus was suspended in phosphate-buffered saline (PBS), pH 7.2, and purified by the procedure of Haywood (11).

Disruption of virus by OG. Partially purified Sendai virus (0.8 to 1.0 mg/ml) in PBS (pH 7.2) was treated with 30 mM β-D-octylglucoside (OG) (Calbiochem-Behring, La Jolla, Calif.) to solubilize the surface glycoproteins HN and F and the viral lipids. The virus suspension and the detergent were mixed and incubated at room temperature for 1 h. The mixture was centrifuged at 100,000 × *g* for 30 min at 4°C to remove intact virus and other nonsolubilized components.

VLRV. Reconstitution of glycoproteins was performed by the detergent dialysis method described by Petri and Wagner (22). Viral lipid recombinant vesicles (VLRV) were formed with the glycoproteins HN and F and with the viral lipids extracted from Sendai virions with 30 mM OG in PBS (pH 7.2). The detergent was removed by dialysis against a 250-fold volume of 10 mM Tris buffer (pH 7.4) containing 1.0 mM $MgCl_2$ and 0.1 mM dithiothreitol for 36 h at 4°C, with a change of buffer every 8 to 12 h. The retained material was turbid, and only 0.2 mM OG was left as determined by the anthrone test (30). More than 60% of the detergent was removed in the first change of dialysis buffer.

Equilibrium sedimentation. The turbid suspension of VLRV was centrifuged to equilibrium through a 10 to 45% (wt/wt) linear sucrose gradient in 50 mM Tris buffer, pH 7.4, for 24 h at 57,000 × *g* in a Beckman SW27 rotor at 4°C. Fractions of 1.5 ml were collected from the bottom of each tube. Each fraction was dialyzed for 18 h at 4°C against PBS, pH 7.2, containing 0.1 mM dithiothreitol. Before dialysis, the density of each fraction was determined by refractometery. Gradient fractions were flushed with nitrogen and kept at 4°C. Hemagglutination (HA) and neuraminidase (NA) assays as well as protein and phosphate (3) determinations were done on each fraction.

SDS-polyacrylamide gel electrophoresis. Discontinuous sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was performed by the modified Laemmli procedure described by Smith et al. (29). Slab gels 0.75 mm thick were used. The separating gel contained 10% of polyacrylamide, 0.26% bisacrylamide, 0.067% ammonium persulfate, and 0.063% tetramethylenediamine. Gels were run at 15 mA until the tracking dye (bromophenol blue) was approximately 1

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cm from the bottom of the gel. The gels were stained with 2.5% Coomassie blue in 50% methanol–10% glacial acetic acid.

HA. HA titrations were conducted in 96-well microtiter plates with 0.3-ml round-bottom cups (Cooke Engineering Co., Alexandria, Va.). Serial twofold dilutions of samples were made in 100 μl of PBS, pH 7.2, and 100 μl of 0.5% chicken erythrocyte suspension in PBS, pH 7.2, was added. Plates were kept at 4°C for 1 h. Positive HA was recognized by the formation of a characteristic pattern on the bottom of the well. Titers were recorded as the reciprocal of the highest dilution giving positive HA and were expressed as HA units (HAU) per milliliter.

HAD assay. P815 cells treated with VLRV were incubated with 0.5% chicken erythrocyte suspension in PBS for 30 min at 4°C. Samples of these cells were placed on a hemocytometer, and adherence of erythrocytes was observed under a microscope. Cells with three or more adherent erythrocytes were considered hemadsorption (HAD) positive. The degree of HAD was estimated by calculating the mean number of adherent cells per P815 cell.

NA assay. The samples to be tested were diluted with PBS, pH 7.2, in a volume of 100 μl. Fetuin (Calbiochem-Behring) was used as the substrate and was diluted 1:2 in 0.2 M sodium acetate buffer, pH 5.0. The mixture was incubated at 37°C for 30 min. *N*-Acetylneuraminic acid liberated was assayed by the thiobarbituric acid method (1). When 1 mg of fetuin was used as the substrate, the amount of *N*-acetylneuraminic acid liberated was proportional to the enzyme concentration within the range of 0 to 1 absorbance units at 549 nm. One NA unit (NAU) was defined as the amount of enzyme activity that gave an absorbance of 1.0 at 549 nm (27).

Hemolysis assay. Hemolytic activity of partially purified virus and reconstituted vesicles was measured by the method described by Hsu et al. (14). Samples were diluted in PBS, pH 7.2, to a volume of 0.5 ml in glass tubes (12 by 75 mm). Each tube received 0.5 ml of human erythrocyte suspension (0.5% in PBS, pH 7.2). After mixing was performed, the tubes were kept on ice for 45 min and then were incubated at 37°C for 3 h with shaking every 20 min. The mixture was chilled on ice and centrifuged at 2,000 × *g* for 10 min. The amount of hemoglobin in the supernatant was measured by absorption at 454 nm.

IF assay. Cell surface immunofluorescence (IF) assays for the detection of glycoproteins on the P815 cells were done by the procedure of Möller (20) with rabbit antiserum to intact Sendai virus and goat anti-rabbit gamma globulin conjugated to fluorescein isothiocyanate.

Protein determination. The protein concentrations of virus stock, solubilized glycoproteins, and glycoproteins HN and F reconstituted into VLRV (VLRV-HNF) were determined by the method of Bradford (6) using a commercially prepared dye reagent (Bio-Rad Laboratories, Richmond, Calif.).

Interferon assay. Supernatant fluids from cytotoxicity assays were assayed for interferon by an inhibition-of-cytopathic-effect method. Fluids harvested from triplicate assays were pooled, the pH was reduced to 2.0, and the fluids were held for 16 h at 4°C. After readjustment to pH 7.0, the fluids were serially diluted 1:10 to 1:320 and were placed on L-929 cell monolayers for 16 h at 37°C. The fluids were removed, and the monolayers were challenged with vesicular stomatitis virus at a multiplicity of infection of 1.0. NIH standard mouse L-cell interferon containing 2,000 U/ml was run as a positive control. Monolayers were inspected at 48 h after inoculation for the presence of cytopathic effect.

Natural cell-mediated cytotoxicity assay. The method used was similar to that described by Shearer et al. (26). Spleens from BALB/c nude mice 6 to 8 weeks old were used as the source of natural cytotoxic cells. For the purpose of this report, the cell-mediated cytotoxicity of this cell population will be operationally defined as NK activity. The spleens were perfused in Hanks buffered saline solution (HBSS) supplemented with 100 U of penicillin per ml and 100 μg of streptomycin per ml. After standing in a plastic petri dish for 30 min at room temperature, cells were filtered through a nylon mesh, and an equal volume of ACK lysing buffer (8.29 g of NH_4Cl, 1.0 g of $KHCO_3$, and 0.0372 g of disodium EDTA dissolved in 1 liter of deionized distilled water) was added to lyse erythrocytes. After 5 min, HBSS containing 10% heat-inactivated newborn calf serum and antibiotics was added. Cells were centrifuged at 1,000 rpm for 10 min at 4°C, suspended in HBSS, and washed one more time. Cells were then suspended in cold RPMI 1640 medium supplemented with 10% heat-inactivated newborn calf serum and antibiotics. Viable cells were counted by the trypan blue (0.2%) exclusion procedure, and the concentration was adjusted to 2×10^7 cells per ml of medium. Cells were kept on ice.

Ordinary P815 cells, P815 cells modified by the addition of glycoproteins, P815 cells infected with Sendai virus, and Yac-1 cells were used as targets. All cells were suspended in 0.1 ml of HBSS and labeled with 100 to 200 μCi of [^{51}Cr]sodium chromate (New England Nuclear Corp., Boston, Mass.). After incubation at 37°C for 1 h, cells were washed three times with HBSS. For modification, P815 cells were counted, and 5×10^5 viable cells per ml were incubated with appropriate amounts of VLRV-HNF or UV-irradiated intact Sendai virus for 1 h at 4°C. Cells were washed with HBSS three times and suspended in RPMI 1640 medium supplemented with heat-inactivated newborn calf serum and antibiotics. The concentration of viable cells was adjusted to 4×10^5 cells per ml.

NK assays were carried out in 96-round-cup microtiter plates. Fifty microliters of target cells was placed in each well, and various numbers of effector cells were added. Cell suspensions at all the effector-to-target ratios were tested in triplicate. Best results were obtained at an effector-to-target ratio of 100. To determine the spontaneous release, the targets were incubated with the medium alone. Maximum release of ^{51}Cr was determined by adding 0.2 ml of 0.1 N hydrochloric acid. The total volume in each well was 250 μl. Plates were incubated at 37°C with 5% CO_2 for 4 h. Plates were then centrifuged at 1,000 rpm for 10 min, and 100 μl of the supernatant was removed from each well, placed in glass tubes, (12 by 75 mm), and counted in a Packard scintillation counter. The mean count from each triplicate group was used to calculate the value of percent specific lysis according to the formula % specific lysis = [(experimental release − spontaneous release)/(maximum release − spontaneous release)] × 100.

RESULTS

Reconstitution of HN and F into VLRV. Glycoproteins and viral lipids were solubilized from the Sendai virus with OG. In a typical experiment, Sendai virus preparations with 1.0 mg of protein yielded 350 to 380 μg of protein and 780 to 800 μg of viral lipids in the supernatant when disrupted with 30 mM OG. Table 1 shows the HA and NA activities of the supernatant obtained after the disruption. The solubilized material displayed both HA and NA activities comparable with those of intact virus. Analysis of the proteins in the

TABLE 1. HA and NA activities of Sendai virus proteins obtained by treatment of virions with OG[a]

Preparation	HAU/mg (10^3)	NAU/mg
Intact virions	640.0	833.0
Supernatant	864.0	666.6
Pellet after centrifugation (100,000 × g)	80.0	94.0

[a] Measurement of HA and NA activities of a partially purified preparation of Sendai virus (1.0 mg of protein) and the supernatant fluid and pellet from the centrifugation of the OG-disrupted virion preparation conducted as described in the text.

solubilized material by SDS-polyacrylamide gel electrophoresis with 2-mercaptoethanol (Fig. 1) demonstrated the presence of glycoproteins HN, F_1, and F_2. Small amounts of NP protein were often present in these preparations.

Glycoproteins and viral lipids were allowed to reassociate into recombinant vesicles by removal of the detergent. OG was removed by dialysis for 36 h against a 250-fold volume of 10 mM Tris buffer (pH 7.4) containing 1.0 mM $MgCl_2$ and 0.1 mM dithiothreitol. There were four changes of buffer. The concentration of OG in the retained material was reduced from 30 to approximately 0.10 mM, as determined by the anthrone test.

The reconstituted material was centrifuged to equilibrium through a 10 to 45% (wt/wt) sucrose gradient in 50 mM Tris, pH 7.4, for 24 h at 57,000 × g at 4°C in a Beckman SW27 rotor. After centrifugation, a sharp band was seen in the upper half of the gradient. Fractions of 1.5 ml were collected from the bottoms of the tubes and analyzed for phosphate content and density. Each fraction was then dialyzed for 16 h at 4°C against PBS through which nitrogen gas has been bubbled. HA, NA, and protein concentrations of each fraction were determined.

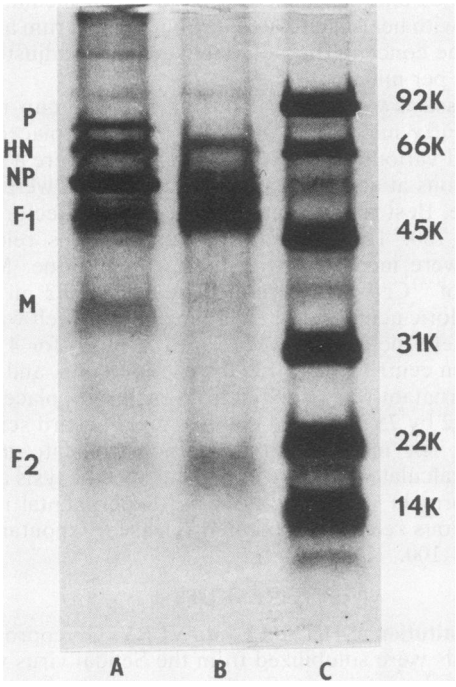

FIG. 1. SDS-polyacrylamide gel electrophoresis in 10% gel with 2% 2-mercaptoethanol. Lane A, partially purified Sendai virus in 30 mM OG; lane B, supernatant from OG treatment; lane C, protein standards in 30 mM OG. P, Polymerase protein; HN, hemagglutinin-neuraminidase; NP, nucleoprotein; F_1, 52,000-molecular-weight fragment of fusion protein; M, matrix protein; F_2, 18,000-molecular-weight fragment of fusion protein.

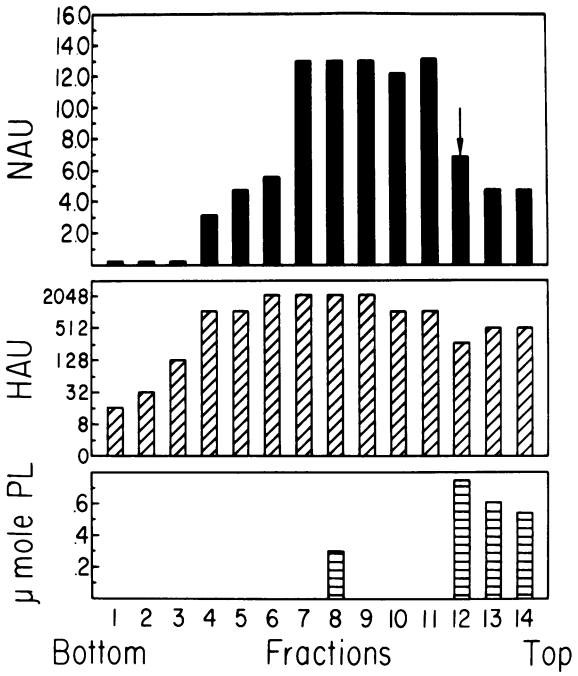

FIG. 2. Analysis of equilibrium sedimentation of VLRV reconstituted from 380 μg of HN and F proteins and 800 μg of viral lipids through a 10 to 45% sucrose gradient. After dialysis to remove OG, the sample was applied to 10 to 45% (wt/wt) sucrose gradient in 50 mM Tris (pH 7.4) and subjected to centrifugation in an SW27 rotor for 24 h at 57,000 × g at 4°C. Fractions were analyzed for NA activity (NAU), HA activity (HAU), and phosphate content. Phospholipid (PL) concentration was estimated by using the molecular weight of 780 assuming phosphatidylcholine. The arrow indicates the fraction containing the visible band.

Figure 2 shows the equilibrium sedimentation pattern of VLRV-HNF reconstituted with 380 μg of HN and F and 800 μg of viral lipids through a 10 to 45% sucrose gradient. Fraction 12 contained a discrete visible band and was selected for further experiments. Although this fraction contained both HA and NA activities and a major component of the phospholipid, biologically active proteins were distributed throughout the gradient. For example, fraction 7 contained 20% of the total protein, and SDS-polyacrylamide gel electrophoresis demonstrated the presence of both HN and F (Fig. 3).

Characteristics of isolated VLRV-HNF. Fraction 12 banded at the density of 1.063 g/cm^3. Although the lipid-to-protein ratio of starting material was 2.1 (wt/wt), the ratio found in the recombinant fraction was 6.6. Of total protein, 12% was reconstituted in the vesicles in fraction 12. VLRV-HNF was biologically active in assays for NA (0.22 NAU/μg) and HA (25,600 HAU/ml). These activities are the expression of functional HN protein. Figure 4 shows the results of hemolyzing assays of VLRV-HNF. The hemolyzing activity of recombinant vesicles was comparable to that of the intact virus.

SDS-polyacrylamide gel electrophoresis of fraction 12 revealed two bands with molecular weights of 69,000 and 53,000 (Fig. 3), corresponding to HN and F_1 polypeptides. A third band corresponding to F_2 was often only faintly visible.

Transfer of Sendai virus glycoproteins HN and F to the plasma membrane of P815 cells by means of VLRV-HNF. To demonstrate that VLRV-HNF can transfer the glycoproteins HN and F to the recipient cells, 5×10^5 P815 cells were incubated with 50 μg of VLRV-HNF for 1 h at 4°C, washed, and assayed for NA activity. In these experiments, NA activity was detected on the cells incubated either with intact virus (0.410 NAU) or with VLRV-HNF (0.194 NAU). Further experiments were conducted to explore the quantitative aspects of transfer of proteins to the recipient cells. Various amounts (protein weight) of VLRV-HNF were incubated with 5×10^5 cells at 4°C for 1 h. Cells were then washed three times with sterile PBS. Samples of 2×10^5 cells were taken, and twofold serial dilutions were made. P815 cells incubated with medium alone were used as controls. All cells were tested for NA activity as described above. The results are summarized in Table 2.

The data shown in Table 2 demonstrate that VLRV-HNF can mediate the transfer of proteins to P815 cells. Protein weights as low as 6.25 μg resulted in detectable surface NA activity. It appeared that the surfaces of the cells were saturated when incubated with VLRV-HNF containing 25 μg (protein weight) or more, as indicated by similar NA activity. This effect was maintained when cells were diluted and tested for NA. Further experiments on the interaction of VLRV-HNF with P815 cells were conducted with 50 μg of VLRV-HNF. The presence of proteins of the recipient cells was determined by other methods, such as IF and HAD assays.

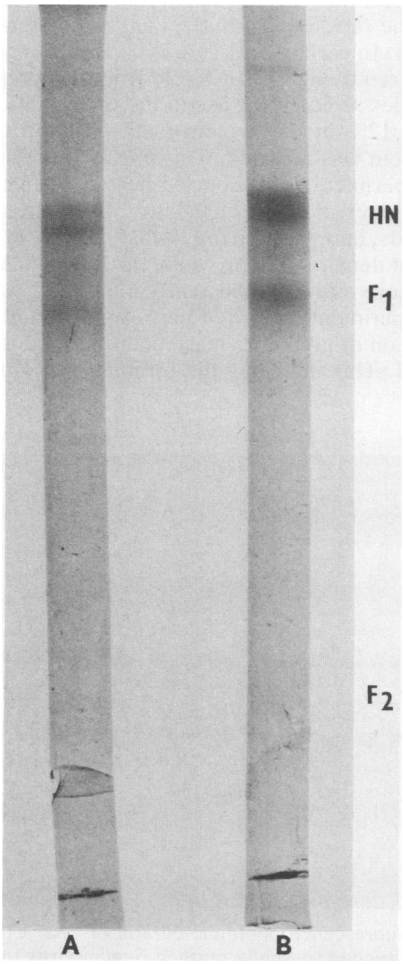

FIG. 3. SDS-polyacrylamide gel electrophoresis of fraction 12 (lane A) and fraction 7 (lane B) in 10% gel with 2% 2-mercaptoethanol.

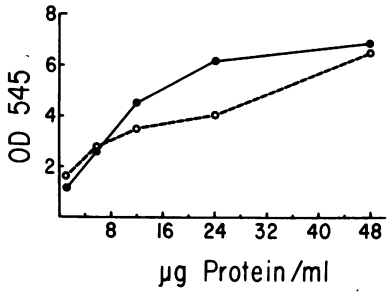

FIG. 4. Hemolyzing activity of VLRV-HNF. Fraction 12 isolated from equilibrium sedimentation of vesicles was reconstituted from 380 μg of Sendai virus HN and F proteins and 800 μg of lipids through 10 to 45% sucrose gradient. VLRV-HNF and partially purified Sendai virus were assayed for hemolyzing activity at the protein concentrations indicated on the abscissa. VLRV-HNF (○) and Sendai virus (●) at various protein concentrations were incubated with 0.5% suspension of human erythrocytes as described in the text. OD 545, Optical density at 545 nm.

Detection of VLRV-HNF on P815 cells by cell surface IF. P815 cells (5×10^5) were incubated with 50 μg (protein weight) of VLRV-HNF. Cells were washed three times with PBS, and samples of 10^5 cells were processed for cell surface IF by using whole Sendai virus antiserum and fluorescein isothiocyanate-labeled goat anti-rabbit globulin conjugate. Cells incubated with UV-irradiated Sendai virus and cells incubated with PBS alone were included as controls. In preliminary experiments, nonspecific staining was observed on cells incubated with PBS alone. To eliminate this nonspecific adherence, serum and conjugate were absorbed with P815 cells and then used in the IF procedure as described above.

Fluorescence was observed on cells incubated with VLRV-HNF or with whole Sendai virus, but the patterns of IF were different. It appeared that VLRV-HNF absorbed to cells in the form of aggregates at certain sites on the cells, thus giving intense staining (Fig. 5). Cells modified by whole Sendai virus showed more uniform staining (Fig. 6). In both preparations, over 90% of cells were positive, although there

TABLE 2. NA activity on P815 cells incubated with various amounts of VLRV-HNF

No. of cells	NAU on cells incubated with μg of VLRV-HNF:					
	6.25	12.5	25	50	100	0 (medium alone)
1×10^5	0.183	0.180	0.215	0.218	0.220	0.004
5×10^4	0.101	0.104	0.160	0.162	0.163	0.003
2.5×10^4	0.017	0.048	0.076	0.083	0.091	0.000

[a] P815 cells (5×10^5) were incubated with VLRV-HNF at 4°C for 1 h and then washed three times with PBS. Samples of 2×10^5 cells were taken, and twofold serial dilutions were made. The NA activity of each sample was measured as described in the text.

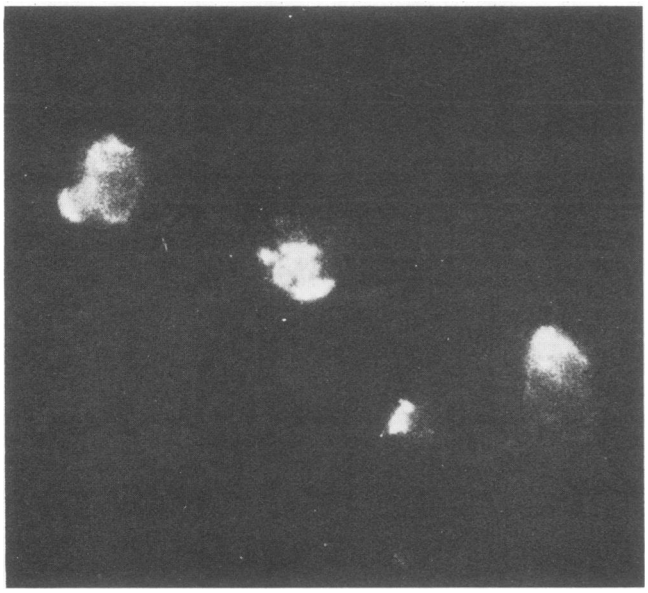

FIG. 5. Fluorescense photomicrograph of P815 cells incubated with VLRV-HNF for 1 h at 4°C. Unfixed cells were stained by indirect IF with rabbit anti-Sendai serum and FITC conjugated goat anti-rabbit antibody. Magnification, ×190.

was variation in the degree of fluorescence among the positive cells.

Characterization of modified recipient cells by HAD. P815 cells (5×10^5) were incubated with 50 μg (protein weight) of VLRV-HNF at 4°C for 1 h and washed three times with PBS, and 0.5% chicken erythrocytes suspension was added. More than 99% of modified cells were HAD positive. The degree of HAD was determined by counting the average number of erythrocytes adhering to each P815 cell. From 15 to 20 P815 cells were counted. Cells incubated with Sendai virus or medium alone were included as controls. Cells treated with the VLRV-HNF adsorbed 7 to 8 erythrocytes, whereas the average for the virion-treated P815 cells was 9 to 10. Controls were negative.

The results of HAD experiments demonstrated that cells incubated with either VLRV-HNF or Sendai virus were HAD positive, although cells coated with Sendai virus had higher degrees of HAD. P815 cells incubated with medium alone did not absorb chicken erythrocytes. It can be concluded that proteins were transferred to the recipient cells by means of VLRV-HNF and were oriented so that they retained function.

NK cell cytotoxicity against P815 cells modified with Sendai virus glycoproteins HN and F. P815 cells incubated with 50 and 100 μg of VLRV-HNF and were tested for susceptibility to NK cell-mediated lysis in 4-h ^{51}Cr release assays. In addition, P815 cells incubated with 200 HAU of intact Sendai virus or cells actively infected with Sendai virus were also used as targets. P815 cells and Yac-1 cells incubated with medium alone were included as controls. Spleens cells from BALB/c nude mice 6 to 8 weeks old were used as a source of effector cells. These experiments were repeated three times with similar results. Results of one such experiment are summarized in Table 3, which indicates that all the P815 targets bearing Sendai virus glycoproteins were more susceptible than unmodified P815 cells. The cells incubated with either 50 or 100 μg of VLRV-HNF had the same degree of susceptibility. Susceptibility of these targets exceeded that of Yac-1 cells, which are known to be very sensitive to NK-cell mediated lysis. These results indicate that the modification of the target cells by the acquisition of Sendai virus glycoproteins (and presumably, viral lipids) in this manner make the cells sensitive targets and that viral protein synthesis is not required for target cell formation.

Interferon assays conducted on supernatant fluids from the cytolytic assays were negative.

DISCUSSION

The experiments presented here document the extraction of Sendai virus glycoproteins HN and F from virions and the subsequent reassociation of the glycoprotein with phospholipid to form vesicles. The vesicles were used to transfer the biologically active glycoproteins to the surface of P815 cells. The P815 mastocytoma is relatively resistant to NK cell-mediated cytolysis (4); however, such cells modified by the acquisition of HN and F were sensitive in the NK assay.

The extraction of the surface glycoproteins and lipids from virions was accomplished by using the nonionic detergent OG. This detergent has been used for the extraction of Semliki Forest virus protein (12), influenza virus glycoproteins (15), and vesicular stomatitis virus G protein (22) as well as other proteins. The detergent has two characteristics that were useful in the investigation. It has a high critical micellar concentration, and it is readily dialyzable (28). In our work, the residual OG in the dialyzed material was less than 0.1 mM. In earlier work, we experienced a great deal of difficulty in removing Triton X-100 from our preparations.

The vesicles selected for use in the investigation incorporated about 12% of the protein available, and the ratio of lipid to protein was around 6.0 despite an initial ratio of 2.1. In other experiments not reported here, we have varied the initial ratio over a range of 0.5 to 2.0 by the addition of phospholipids, and we have found that the visible band floats at a constant density of approximately 1.06 g/ml and that the lipid-to-protein ratio is consistently 6.0.

In the experiments reported here, we found phospholipid in one fraction of greater density than in that containing the visible band. This indicates the formation of other vesicles

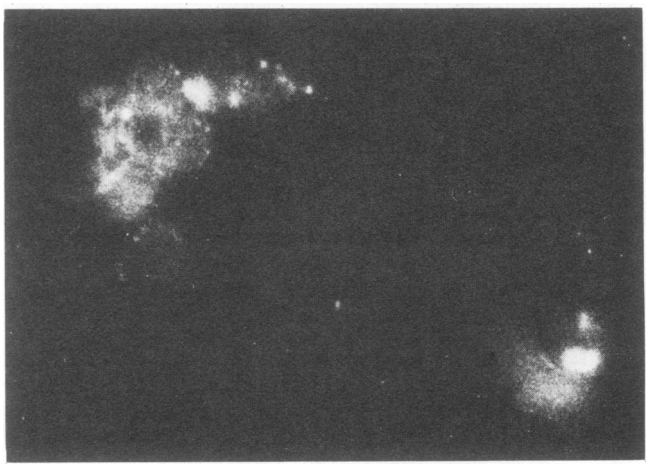

FIG. 6. Fluorescence photomicrograph of P815 cells incubated with UV-inactivated, partially purified Sendai virus for 1 h at 4°C. Unfixed cells were stained by indirect IF with rabbit anti-Sendai serum and fluorescein isothiocyanate-conjugated goat anti-rabbit antibody. Magnification, ×190.

TABLE 3. NK cell-mediated cytotoxicity against P815 cells modified with Sendai virus glycoproteins HN and F

Target cells[a]	Specific lysis (%)[b]
P815 + 50 μg of VLRV-HNF	91.0
P815 + 100 μg of VLRV-HNF	92.3
P815 + 200 HAU of UV-irradiated Sendai virus	35.4
P815 infected with Sendai virus	53.2
P815 + medium	26.9
YAC-1	51.9

[a] Spontaneous release for all of the targets was between 20 and 25%.

[b] Results represent the lytic activity in a 4-h ^{51}Cr release assay at an effector-to-target ratio of 100. Standard deviation was less than 3%. Effector cells were prepared from the spleens of BALB/c nu/nu mice 6 to 8 weeks old.

with different lipid-protein relationships. However, we did not characterize these vesicles further.

We demonstrated the retention of biological function of the HN glycoprotein by measurement of HA and NA activities in the liposome preparation. The demonstration of hemolytic activity was particularly significant since the expression of F glycoprotein function is dependent upon the proper orientation of the protein in a lipid bilayer and a means of anchorage to the target cell (13, 14).

The transfer of glycoproteins HN and F to the surfaces of P815 cells was demonstrated by the acquisition of a surface NA activity that was not removed by repeated washing. In addition, the cells became HAD positive, and the presence of viral antigens was demonstrated by IF assay. The surface appeared to become saturated when amounts of viral protein greater than 25 μg/5 × 10^5 cells were used. Dilution of cell concentration treated with saturating levels of protein suggested that the plateau was a result of saturation and not a steric hinderance of enzyme activity. The latter alternative should have resulted in the retention of the same or higher levels of NA activity in the diluted population than the levels seen in the more concentrated population. Although we did not attempt to measure changes, it seems reasonable to assume that some alteration of the P815 membrane lipids occurred as a result of treatment with the VLRV-HNF.

The modified P815 proved to be very sensitive to cytolysis in the NK assay. Attribution of the cytolysis to NK cells is based on an operational definition of natural killing (23) as the activity resident in nude mouse spleen cell populations remaining after adsorption on plastic and filtering through nylon. The effector-to-target ratio of 100 gave the most consistent results in these experiments. The results of NK assays with other ratios, i.e., 200 and 50, were less efficient and frequently gave inconsistent results. The noninfectious modification of target cells by inactivated virus and the lysis of the cells by cytotoxic T cells has been reported by Sugamura et al. (31) and by Koszinowski et al. (17). These studies used whole virus preparations which would still be capable of inducing interferon. We chose to incorporate the viral glycoproteins into recombinant vesicles to avoid interferon induction. Interferon assays of supernatant fluids from the 4-h NK assays were negative, indicating that within the time limits of the test, stimulation of detectable interferon by the cell-bound glycoproteins did not take place. Ito and Hosaka (16) recently reported the induction of interferon in mouse spleen cells by Sendai glycoprotein HN incorporated in lipid vesicles. The activity was detected after a 16-h incubation period.

Evidence that interferon stimulates NK activity has been presented (19, 24, 32) and is generally accepted. However, Santoli et al. (24) and Welsh and Hallenbeck (32) showed that there is a protective influence of interferon on noninfected targets of NK cytolysis. Fitzgerald et al. (9) presented evidence that interferon present in NK assays did not substantially effect differences in NK lysis of herpes simplex virus-infected targets over noninfected targets. The authors suggested, therefore, that other factors were resposible for the differences observed. Similar lack of correlation between interferon titers and NK-mediated lysis of herpes simplex virus-infected cells was reported by Bishop et al. (5). Casali et al. (7) described the enhancement of cell-mediated cytolysis by peripheral blood lymphocytes by the presence of measles virus glycoproteins in soluble form or incorporated into lipid vesicles. They did not detect interferon in the supernatant fluids from their cytoxicity assay. They also reported that washing of the target cells reduced the cytolysis considerably. Their observation tends to suggest a recognition and attachment role for the glycoproteins between target and effector cells.

Modification of the lipid constitution of the plasma membrane is another factor to be considered in enhancing the NK sensitivity of the P815 targets. It has been reported (21) that there is a significantly higher concentration of sphingomyelin and a lower concentration of phosphatidylcholine in Sendai virions than in the chick embryo cells in which the virus was grown. Transfer of the lipids through the agency of cell recognition by glycoprotein HN and fusion by glycoprotein F may introduce significant amounts of lipid into the P815 plasma membrane and thereby alter the normal relationship among the lipid species. Such an alteration may contribute to the enhanced susceptibility of the liposome-modified P815 cells compared with the Sendai-infected cells. The system described in this report offers the possibility of analyzing the role of individual cell surface viral glycoproteins and lipids in NK cell-mediated lysis in the absence of interferon. It also offers the possibility of quantitating the requirements for target function.

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