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Effect of Vesicular Stomatitis Virus Infection on the Histocompatibility Antigen of L Cells

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When mouse L cells are infected for 22 hr with vesicular stomatitis virus (VSV), a ribonucleic acid-containing enveloped virus, greater than 70% of the major histocompatibility antigen (H-2), is no longer detectable by the method of inhibition of immune cytolysis. Infected cells prelabeled with ^{14}C-glucosamine also show a correspondingly greater loss of trichloroacetic acid-insoluble radioactivity than uninfected cells. The loss of H-2 antigenic activity is not due to the viral inhibition of host cell protein synthesis since cells cultured for 18 hr in the presence of cycloheximide have the same amount of H-2 activity as untreated controls. Also, cells infected with encephalomyocarditis virus, a picornavirus, show no loss of H-2 activity at a time when host cell protein synthesis is completely inhibited. VSV structural proteins associated in vitro with uninfected L-cell plasma membranes do not render H-2 sites inaccessible to the assay. Although antibodies may not combine with all the H-2 antigenic sites on the plasma membrane, anti-H-2 serum reacted with L cells before infection does not prevent a normal infection with VSV. H-2 activity can be detected in virus samples purified from the medium of infected L cells; this virus purified after being mixed with L-cell homogenates shows greater H-2 activity than virus purified after being mixed with HeLa cell homogenates. However, VSV made in HeLa cells shows no H-2 activity when mixed with L-cell homogenates.

Vesicular stomatitis virus (VSV) is an enveloped ribonucleic acid (RNA)-containing virus which matures at the cell surface. During this process, there is a modification of host membranes which includes the insertion of virus envelope proteins into the host cell membrane, forming an altered site. It is at this site that the virus finally "buds" off from its host cell, forming an intact virion (5, 9, 18, 25, 30). It is believed that the protein components of the viral envelope are entirely virus-specific, whereas the lipid, glycolipid, and possibly the carbohydrate components are host specified (3, 8, 12, 13, 17). It is therefore important to establish the manner in which virus-specific envelope proteins are inserted into the preformed host cell membrane and also to follow the fate of host cell membrane protein molecules as virus proteins become associated with the host surface. The host cell protein we have studied is the major histocompatibility antigen of the mouse, the H-2 locus gene product, since this cell glycoprotein is localized almost entirely in the plasma membrane and can be assayed readily (22, 23, 29). We have shown that L cells, of H-2^k haplotype, lose over 70% of the H-2 alloantigenic activity upon infection with VSV.

MATERIALS AND METHODS

Cell culture. Suspension cultures of L cells and HeLa S_3 cells were grown in Eagle's medium (MEM) (Schwartz Research, Inc.) plus 6% fetal calf serum (Grand Island Biological Co., Inc.) at a concentration of 5×10^5 to 10×10^5 cells/ml and 3×10^5 to 6×10^5 cells/ml, respectively.

Virus. VSV of the Indiana serotype was grown either in L cells or HeLa cells by a method similar to that of Kang and Prevec (11). Cells at 37 C were concentrated to 10 times that of growing conditions in MEM without serum and infected with 10 plaque-forming units (PFU) of VSV per cell in the presence of 14 mM HEPES buffer (*N*-2-hydroxyl-ethylpiperazine-*N*-2-ethane sulfonic acid; Nutritional Biochemical Corp.). Where indicated, actinomycin D (a gift of Merck and Co., Rahway, N.J.) was added to a concentration of 10 μg/ml at 30 min postinfection (PI). At 90 min PI, cultures were diluted 10-fold with MEM containing 6% fetal calf serum, and the temperature was lowered to 32 C. At various times PI, samples of cells were removed from the culture, washed twice with cold Earle's solution, and assayed for H-2 antigenic activity.

Purification of VSV from both L cells and HeLa cells followed a modification of the procedure of McSharry and Benzinger (15) and was routinely performed at 18 to 24 hr PI. Cells were collected by

centrifugation for 15 min at 700 × *g* and were discarded. For each 100 ml of virus-containing supernatant fluid, 2.2 *g* of NaCl and 6.0 *g* of solid polyethylene glycol (PEG) 6000 (Union Carbide Corp.) were added, and the solution was stored overnight at 4 C. The precipitate which formed was pelleted by centrifugation for 15 min at 700 × *g* and resuspended in about 35 ml of ET [1 mM tris(hydroxymethyl)-aminomethane(Tris)-hydrochloride; 1 mM ethylenediaminetetraacetic acid, *p*H 7.6] buffer. The virus suspension was then centrifuged in a Spinco SW27 rotor at 27,000 rev/min for 2 hr at 4 C. The virus pellet was suspended in ET buffer, sonically treated for 2 min in a Branson sonic oscillator at a setting of three, layered onto a 30 to 50% (w/w) sucrose gradient prepared in ET buffer, and centrifuged in a Spinco SW27 rotor for 18 hr at 27,000 rev/min at 4 C. The isopycnic virus band was collected, and the density was determined by measurements with a Bausch and Lomb refractometer. The virus was either made 10% with respect to dimethyl sulfoxide (DMSO) and stored at −70 C, or pelleted in ET buffer as above, suspended in a small amount of ET buffer made 10% DMSO, and stored at −70 C (26).

Plaque assays were performed on L-cell monolayers by the method of Jimenez et al. (10).

Infection with encephalomyocarditis (EMC) virus. L cells, at 6 × 10^6 cells/ml, were infected with 10 PFU of EMC virus/cell (kindly provided by Robert Bases) in the absence of serum and in the presence of 10 μg of actinomycin D per ml. At 1.5 hr PI, 5% fetal calf serum was added, and infection was monitored by the incorporation of ^{3}H-uridine (2 Ci/mmole) (New England Nuclear) into trichloroacetic acid-precipitable material (6). At 7 hr PI cells were centrifuged, washed with cold Earle's solution, and assayed for H-2 antigenic activity.

Radioactive labeling of VSV proteins. L cells were infected with VSV as above but in MEM containing one-fourth the usual concentration of amino acids. ^{14}C-L-amino acid (U) mixture or ^{3}H-L-amino acid (G) mixture (New England Nuclear) was added to a concentration of 2 or 20 μCi/ml, respectively, at 4 hr PI for a duration of 3 hr. The radiolabeled infected cells were then washed twice with cold Earle's solution, suspended in 10 mM Tris-hydrochloride buffer (*p*H 8.0), and homogenized with a tight-fitting Dounce homogenizer. The cytoplasmic fraction was precipitated with 10% trichloroacetic acid, washed twice with cold 5% trichloroacetic acid, and finally washed with cold acetone before solubilizing the sample in 0.2 ml of 0.01 M phosphate buffer (*p*H 7.4) containing 2% sodium dodecyl sulfate (SDS) and 1% 2-mercaptoethanol. The sample was then subjected to electrophoresis on a 20-cm 7.5% polyacrylamide-SDS gel as detailed previously (19, 24).

Where association of radioactive VSV proteins with uninfected plasma membranes was required, the infected cytoplasmic fractions described above were treated as previously reported (4); membrane fragments were removed from the cytoplasm by centrifuging one volume of this fraction over two volumes of 30% (w/w) sucrose in 10 mM Tris-hydrochloride buffer (*p*H 8.0) at 6,000 *g* for 30 min, three times. The resulting radiolabeled cytoplasm called the "de-membraned cytoplasmic fraction" was collected and used to associate with uninfected plasma membranes prepared from L cells.

Plasma membranes prepared from L cells. The procedure used was that of Atkinson and Summers (1) except that L cells were swollen in a saturated solution of fluorescein mercuric acetate (27) made up in 10 mM Tris-hydrochloride, *p*H 8.0. Total protein was determined by the method of Lowry with bovine serum albumin (Calbiochem) as standard.

Assay for H-2 alloantigen and production of alloantiserum. L cells and VSV were assayed for H-2 activity by the inhibition of immune cytolysis measured by ^{51}Cr (as sodium chromate) (Amersham/Searle) released from target lymph node cells (22, 23, 29) from B10.BR (H-2^k) mice in the presence of alloantiserum and guinea pig complement (Grand Island Biological Co.). The dilution of infected cells was plotted against the percent lysis of the target lymph node cells. The reciprocal, times 10, of the dilution which results in lysis of 50% of the target cells is defined as the number of H-2 units per milliliter of antigen. Protein determinations were made on the samples, and specific activity was obtained by dividing H-2 units per milliliter by the milligrams of protein per milliliter of sample. Alloantisera were made by immunizing BALB/c (H-2^d)mice with L cells (H-2^k). In this paper, the term H-2 antigenic activity refers to the activity of the set of specificities which make up the H-2^k haplotype and which are detectable by the assay using antisera produced in H-2^d mice. (For a review of nomenclature see reference 21.) Inbred mice were purchased from Jackson Laboratories, Bar Harbor, Maine.

RESULTS

H-2 assay of VSV-infected cells. L cells were infected with VSV and assayed for H-2 antigenic activity at 6.5, 10, and 22 hr PI. Figure 1 is a typical experimental curve showing inhibition of immune cytolysis by infected and uninfected cells at various times PI. By 6.5 hr PI infected cells lost 40% of the antigenic activity compared with uninfected cells, and by 22 hr PI about 75% of the H-2 activity is no longer detectable by the immune assay (Fig. 2, Table 1). Uninfected cells, in the presence of actinomycin D, showed no decrease in H-2 activity up until 22 hr when they were no longer viable (Fig. 2).

The fate of glucosamine in VSV-infected cells. Since glucosamine is a major constituent of H-2 antigens and other cell surface components (20), the fate of trichloroacetic acid-precipitable glucosamine radioactivity in L cells was measured after infection with VSV. L cells were grown in the presence of 0.5 μCi of ^{14}C-glucosamine (53.1 mCi/mmole) (New England Nuclear) per ml for 23 hr, during which time incorporation into trichloroacetic acid-insoluble material was linear. The cells were then washed twice with cold Earle's solution and infected with VSV. Samples were taken at intervals, washed with cold Earle's solution, lysed with water, and precipitated with trichloro-

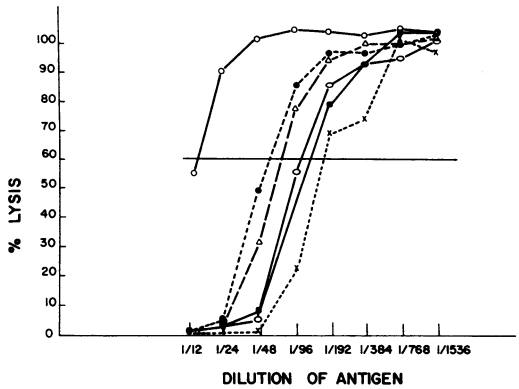

FIG. 1. *Inhibition of immune cytolysis by VSV-infected and uninfected L cells. At 6.5, 10, and 22 hr, 10^8 VSV-infected and control L cells, both in the presence of actinomycin D, were washed twice with cold Earle's solution and concentrated to 0.5 ml. Cell suspensions (antigen) were serially diluted 1:2 and reacted with appropriately diluted anti-H-2^k antiserum, 1:10 dilution of guinea pig complement, and ^{51}Cr-labeled lymph node cells from a B10.Br mouse. The reaction was stopped after 1 hr at room temperature with 10 mM EDTA in phosphate-buffered saline, cells were centrifuged, and samples of the supernatant fluid were counted for radioactivity. Per cent lysis of lymph node cells was calculated for each dilution, and the reciprocal times 10 of the dilution which caused 50% lysis was taken as H-2 units/ml. When the full release control, containing diluted antiserum but no inhibiting antigen, was not 80% of the total counts released by undiluted antiserum, the 50% lysis level for determining H-2 units/ml was adjusted. Symbols: △, 6.5-hr infected cells; ■, 6.5-hr control cells; ●, 10-hr infected cells; ×, 10-hr control cells; ○, 22-hr infected cells; and ⊂, 22-hr control cells. The horizontal line is the adjusted 50% lysis level in this experiment. See Table 1 for specific activity of these cells.*

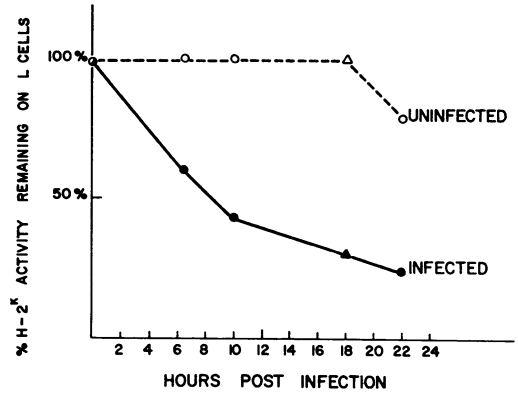

FIG. 2. *H-2 activity on the surface of L cells after infection with VSV. Symbols: ●, VSV-infected cells (from data presented in Fig. 1); ○, uninfected cells; ▲, VSV-infected cells assayed at 18 hr from a second experiment; and △, uninfected cells assayed at 18 hr from a second experiment.*

TABLE 1. *Calculations of H-2 units/ml and specific activity from data presented in Fig. 1*

Sample	H-2 Units/ml	H-2 Units/mg of protein
6.5-hr control	1,333	121
10-hr control	1,724	120
22-hr control	1,099	94
6.5-hr infected	769	72
10-hr infected	606	51
22-hr infected	149	29

acetic acid as described (6). Infected cells showed about a 45% loss of ^{14}C radioactive prelabel, whereas uninfected controls showed only about a 15% loss (Fig. 3).

The effect of cycloheximide on the H-2 assay. To determine whether the loss of H-2 activity in VSV-infected cells is due to turnover without replacement in the plasma membrane as a result of viral inhibition of cellular protein synthesis (19, 28), L cells were cultured for 18 hr in the presence of 100 μg of cycloheximide per ml. This concentration causes greater than 99% inhibition of cellular protein synthesis almost immediately after addition (T. T. Hecht, *unpublished observation*). When assayed for H-2 activity, treated cells showed the same level of H-2 activity as untreated cells (Fig. 4).

Infection with EMC virus. EMC virus is a picornavirus which, unlike VSV, does not mature at host cell surfaces but does shut off cellular protein synthesis by 4 hr PI (Fig. 5). At 7 hr PI, when cells infected at 10 PFU/cell have produced maximal yields of virus but have not completely lysed, cells were washed with cold Earle's solution, and the viable cell concentration was determined by the exclusion of trypan blue dye (Fig. 5). Cells were then assayed for H-2 activity. Table 2 shows that antigenic activity of infected and noninfected cells was nearly identical.

Infection of antibody-coated cells. It has been demonstrated that during virus entry into, as well as exit from, L cells, VSV envelope proteins are inserted into the host cell plasma membrane (7). Since the above experiments might indicate that there could be a specific association between VSV maturation and H-2 antigenic sites, an experiment was performed to determine whether antibodies directed against H-2 specificities on the L-cell plasma membrane would prevent a normal infection with VSV. Prior to infection, L cells, in serum-free MEM, were treated with anti-H-2^k

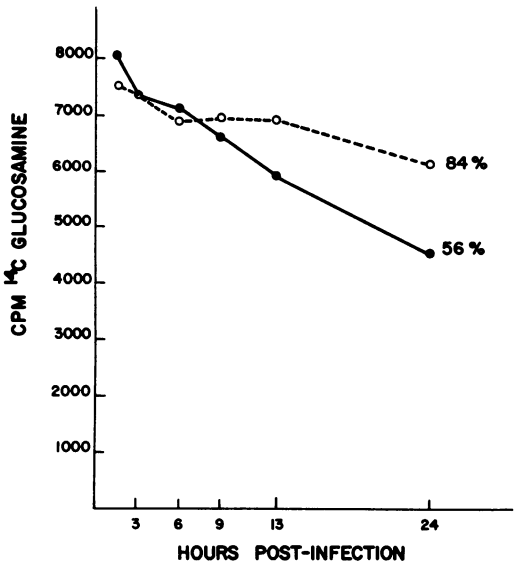

FIG. 3. *Fate of steady-stable glucosamine prelabel in VSV-infected cells. Symbols: ●, VSV-infected L cells; and ○, uninfected L cells.*

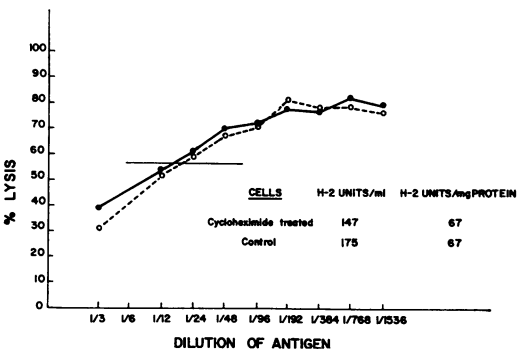

FIG. 4. *H-2 activity of cycloheximide-treated L cells. L cells at 10^7 cells/ml were treated with 100 μg of cycloheximide per ml for 18 hr. Cells were washed twice with cold Earle's solution prior to the assay. Symbols: ●, cycloheximide-treated cells; and ○, untreated cells. The horizontal line is the adjusted 50% lysis level.*

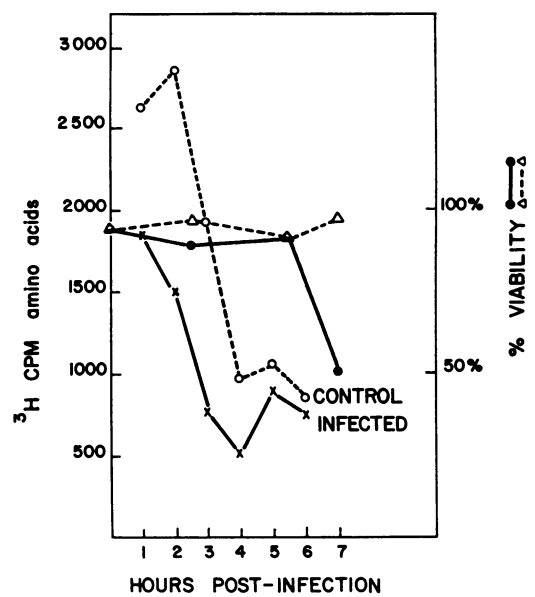

FIG. 5. *Rate of amino acid incorporation in EMC-infected cells. L cells, at 6 × 10^6 cells/ml, were infected in the presence of actinomycin D which was also added to uninfected control cells. At various times PI, 0.5 ml of the cultures was removed into a tube containing 5 μCi of ^{3}H-L-amino acid (G) mixture (New England Nuclear) in a volume of 50 μliters. Zero time points were immediately taken by removing 0.1 ml into 1 ml of cold Earle's solution. At 10 min, samples were also taken. All samples were centrifuged and lysed in 1 ml of water, and the macromolecules were precipitated with 10% trichloroacetic acid. Samples were filtered and counted for radioactivity. Zero time point samples counts were subtracted from those of the corresponding 10-min samples. Viability of L cells was examined at various times PI by the exclusion of trypan blue dye. Symbols: ×, EMC-infected cells; ○, uninfected cells; ●, viability of infected cells; and, △ viability of uninfected cells.*

antiserum to "block" H-2 antigenic sites on the cell surface (2). Antiserum was added at a titer that would give 100% lysis of L cells if added in the presence of complement. The treated cells were incubated for 1 hr at room temperature with repeated mixing and were washed three times with cold Earle's solution, and viability was checked with trypan blue dye. The cells were infected with VSV, and the course of infection was followed by measuring the incorporation of ^{14}C-uridine into trichloroacetic acid-insoluble material (Fig. 6A). The rate of VSV RNA synthesis, determined by the slope of the curves, in cells pretreated with anti-H-2^k antiserum was equal to that in cells pretreated with normal mouse serum or with MEM, and the maximal amount of virus-specific RNA made by 11 hr is essentially the same in all three cultures. At 24 hr PI, the VSV produced in the cul-

TABLE 2. *H-2 activity of EMC-infected L cells*

Determinations	H-2 Units/ml	H-2 Units/ mg of protein	H-2 Units/ cell[a]
EMC virus-infected L cell..	5,263	1,012	1,754
Control.....	11,765	1,153	2,139

[a] Concentration: × 10^6.

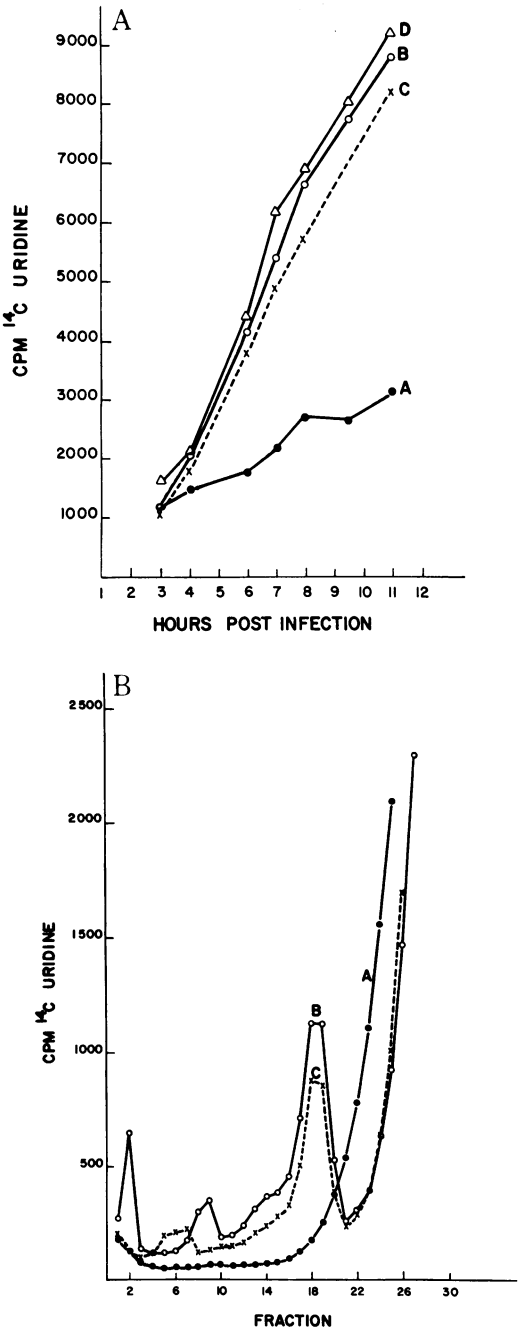

FIG. 6. *A, Incorporation of ^{14}C-uridine in VSV-infected cells pretreated with anti-H-2^k antiserum. L cells, at a concentration of 4 × 10^7 cells/0.5 ml of serum-free MEM, were treated with 15 μliters of anti-H-2^k antiserum for 1 hr at room temperature. Cells were then washed three times with cold Earle's solution, diluted to 10^7 cells/ml, and infected with VSV in the presence of actinomycin D. At 2 hr PI, 0.1 μCi of ^{14}C-uridine per ml (50 mCi/mmole) (New England Nuclear) was added, and at various times PI 1-ml samples were removed and assayed for trichloroacetic acid-insoluble material as described. B, Sedimentation velocity centrifugation of VSV. At 22 hr PI, cells were pelleted for 15 min at 700 g and discarded. The virus-containing supernatant fraction was centrifuged in a Spinco SW27 rotor for 2 hr at 18,000 rev/min. The pellet was then suspended in 1 ml of ET buffer and centrifuged in an SW27 rotor for 40 min at 18,000 rev/min. Fractions were collected and analyzed for radioactivity. The direction of centrifugation in this figure is to the left. Symbols: ●, uninfected cells; (○) infected cells pretreated with MEM; (×), infected cells pretreated with anti-H-2^k antiserum; and (△), infected cells pretreated with normal mouse serum.*

tures was purified. The amount of whole virions obtained from pretreated antiserum and control cells was similar (Fig. 6B). Thus, the presence of H-2^k antibodies on the surface of L cells does not affect entry, RNA synthesis, assembly, or exit of VSV.

Interaction of uninfected L-cell plasma membranes with radioactive VSV proteins. It is possible that during maturation of VSV insertion of viral envelope proteins into the host cell surface causes conformational changes or a masking of antigenic sites that would render these sites inaccessible to the immune assay. Therefore, an in vitro study was done to test whether VSV membrane protein associated in vitro with uninfected L-cell plasma membrane ghosts would cover up antigenic sites. Membranes, isolated from uninfected L cells, were pelleted and suspended at a concentration of 4 × 10^6 membranes/ml in 2 ml of the radiolabeled "demembraned cytoplasmic fraction" from VSV-infected cells described above. Control membranes were suspended in 2 ml of 10 mM Tris-hydrochloride buffer, *p*H 8.0. The membranes were allowed to stand on ice for 1 hr, were pelleted at 6,000 × *g* for 5 min, resuspended in 2 ml Tris-hydrochloride buffer, and centrifuged for 30 min at 6,000 × *g* over 30% (w/w) sucrose. The pellet containing the associated membranes was suspended in 0.25 ml of 10 mM phosphate buffer (*p*H 7.4) containing 2% SDS and 1% 2-mercaptoethanol and was subjected to 7.5% polyacrylamide -SDS electrophoresis (Fig. 7). The percentage of radioactive counts which attached to uninfected membranes for each of the four main virus protein peaks (II–V) (19) was calculated from the counts in each peak in the 2 ml of "demembraned cytoplasmic fraction" added to the membranes. Approximately 35 times more viral membrane protein, peak V, attached to membranes compared to the nucleocapsid protein, peak III, as previously shown for HeLa cells

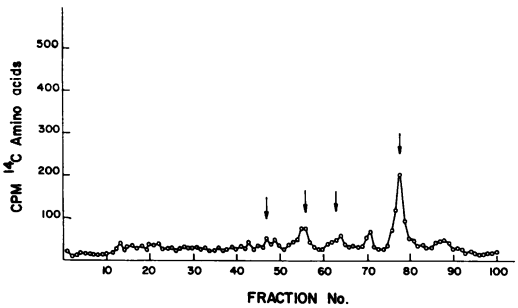

FIG. 7. *Association of VSV structural proteins with uninfected L-cell plasma membranes. Uninfected L-cell plasma membranes were associated with radiolabeled VSV proteins, solubilized in 2% SDS and 1% 2-mercaptoethanol, mixed with a ^3H amino acid VSV-infected cell marker, and subjected to electrophoresis on a 20-cm 7.5% polyacrylamide-SDS gel. The anode in this figure is to the right, and the arrows indicate the marker positions of the four major VSV polypeptides II, III, IV, V (from left to right).*

(4). These uninfected L-cell membranes with attached protein V show the same H-2 activity as control, nonassociated membranes (Fig. 8). Thus, in vitro association of VSV proteins with membranes does not cover up H-2 antigenic sites accessible to the assay.

H-2 assay of VSV grown in HeLa and L cells. H-2 antigenic activity has been demonstrated in preparations of VSV purified from L cells (T. T. Hecht, *unpublished observation*). To test whether this activity is due to cellular contamination, the following mixing experiment was performed. HeLa and L cells were infected with VSV, as described, without actinomycin D. At 24 hr PI, cells were removed by centrifugation. The virus-containing supernatant fluid from each cell type was then divided in half and mixed with either L cells or HeLa cells which were disrupted by a tight-fitting Dounce homogenizer. These mixtures were incubated at 0 C for 1 hr and then centrifuged for 15 min at 700 × *g* to remove cells and debris. VSV was then purified from each of the supernatant fluids by PEG 6000 precipitation as described. Viral pellets were suspended in ET buffer, and assayed for plaques and H-2 antigenic activity. Specific activity of H-2 antigen measured either by H-2 units/mg of protein or H-2 units/PFU in VSV made in L cells and mixed with L-cell homogenates is six to eight times greater than that of virus made in L cells but mixed with HeLa cell homogenates. However, there is no detectable H-2 activity in VSV made in HeLa cells and mixed with homogenates from either L or HeLa cells (Table 3).

TABLE 3. *H-2 activity in VSV preparations mixed with homogenates of HeLa and L cells*

VSV made in:	Mixed with homogenates from:	H-2 Units/mg of protein	H-2 Units/PFU (× 10^9)
L cells.....	L cells	4,006	962
L cells.....	HeLa cells	667	120
HeLa cells....	L cells	<30	<0.3
HeLa cells....	HeLa cells	<30	<0.3

DISCUSSION

During the process of maturation of enveloped viruses, the host cell membrane undergoes modifications which include the insertion of viral membrane protein into the host plasma membrane at a site which eventually "buds out," enveloping the nucleocapsid core which has associated with that site (5, 9, 18, 25, 30). It is not yet clear whether there are specific relationships of molecules in the host cell membrane which dictate where virus maturation will occur (16). Previous electron microscope studies have focused on the composition of the "bud" site and have shown antigenic differences at that site compared with that of the host membrane as a whole (5, 14). In this report, we have looked at changes in a specific cell membrane constituent, the histocompatibility antigen, H-2^k, on the surface of L-cell membranes after infection with VSV. The data show that infection with VSV produces a decrease in the H-2 antigenic activity on the L-cell surface such that by 22 hr PI there is greater than a 70% loss of H-2 activity. Concomitantly, L cells radiolabeled with glucosamine, which is a major component of H-2 antigen, show a greater loss of prelabel in VSV-infected cells than in uninfected controls. This makes it clear that infection with VSV causes perturbations in the host cell membrane.

The possibility that H-2 antigenic activity decreases as a result of viral inhibition of host cell protein synthesis, and thus the degradation or turnover of H-2 antigen without replacement, is ruled out since both cycloheximide-treated and EMC virus-infected cells show the same H-2 activity as control cells (Fig. 4, Table 2). Therefore, H-2 antigenic activity is stable in the plasma membrane in the absence of protein synthesis for at least 18 hr, and it seems likely that it is the process of virus maturation at cell membranes that results in the loss of H-2 activity.

We attempted to rule out the possibility that H-2 antigenic sites and virus entry or exit sites, or both, on the L-cell surface are identical. From the results obtained by incubating L cells with anti-H-2^k antiserum prior to infection, we ascertained that the presence of specific antibodies on the sur-

face of the membrane attached to H-2 antigen does not affect the normal course of VSV infection (Fig. 6A and B). This would imply that sites of entry or maturation, or both, of VSV are not identical with H-2 antigen sites. However, we cannot yet dismiss this possibility since the number of antibody molecules in our antiserum may be small compared with the number of antigenic sites on the cell membrane, and so a decrease in virus production may have been undetectable.

Another possibility is that the loss of H-2 antigenic activity is due to a masking of antigenic sites or configurational changes in the cell surface which render the antigen inaccessible to the immune assay. We have shown that H-2 antigenic activity on uninfected L-cell plasma membrane ghosts is still detectable following association of these membranes with viral structural proteins (Fig. 8). At this time we cannot rule out an apparent decrease in H-2 activity due to masking by VSV maturation, since it is not known whether the in vitro association of VSV proteins with uninfected L-cell membranes studied here reflects the in vivo process of insertion of viral proteins into the host membrane. At least we can conclude that in vitro association of VSV envelope proteins does not result in a loss of H-2 antigenic activity, as detected by the immune assay employed.

A third possibility is that VSV maturation causes a turnover or breakdown of H-2 antigen into the culture medium. It is not yet feasible to test this with the immune assay used, and so resolution of this problem awaits the use of a new soluble H-2 assay being developed in our laboratory.

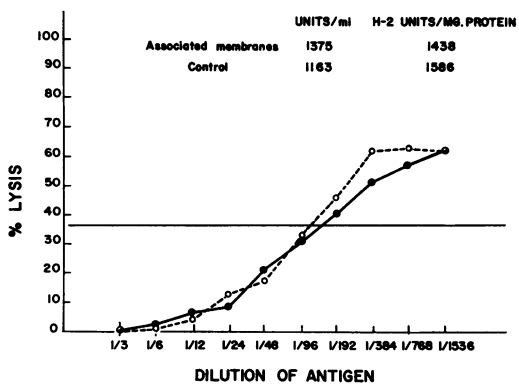

FIG. 8. *H-2 activity of L-cell plasma membranes associated with VSV structural proteins. Symbols: ●, membranes associated with VSV proteins; and ○, unassociated membranes. The horizontal line is the adjusted 50% lysis level.*

A fourth possibility is that H-2 activity decreases in cell membranes as a result of inclusion of the antigen in the viral envelope of VSV. It has never been established whether the virus budding site on the cell membrane is composed exclusively of virus-specified proteins or if the virus envelope includes a small amount of cell-specified proteins. VSV purified from L cells has been found to contain considerable H-2 antigenic activity (T. T. Hecht, *unpublished observation*). Mixing experiments in which VSV grown in L cells was mixed with L-cell and HeLa cell homogenates show that the greater H-2 activity detected in the virus preparation mixed with L-cell homogenates may be due to contamination by cellular material which associates with or purifies along with VSV during virus purification (Table 3). However, there seems to be a definite specificity involved in the association of H-2 antigen with virus since VSV grown in HeLa cells and mixed with L-cell homogenates shows no H-2 activity. If nonspecific sticking of H-2 antigen to virus occurs, one would expect VSV grown in both cell types to show equal levels of antigenic activity when mixed with L-cell homogenates. The specificity of the association may be explained by an incorporation of H-2 glycoprotein into the virus envelope upon its exit from the host cell. This incorporated antigen would act as a center around which H-2 antigen on cellular fragments in the L-cell homogenate would aggregate. HeLa cell-grown VSV, not having this incorporated glycoprotein, would not allow for H-2 antigen aggregation from L-cell homogenates. The data can also be explained by viral incorporation of L-cell-type carbohydrates in the form of other glycoproteins or glycolipids which would allow for aggregation of cellular H-2 antigen. HeLa cell-grown virus has carbohydrates that reflect that of HeLa cells (3, 17) and so would not aggregate L-cell glycoproteins. Work is now in progress to determine definitively whether VSV incorporates host glycoproteins into its viral envelope and to explore in depth the other possibilities mentioned.

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