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Viral Proteins and RNAs in BHK Cells Persistently Infected by Lymphocytic Choriomeningitis Virus

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Some Syrian hamster cell lines persistently infected with lymphocytic choriomeningitis virus (LCMV) do not produce extracellular virus particles but do contain intracytoplasmic infectious material. The proteins of these cells were labeled with [^{35}S]methionine or with [^{3}H]glucosamine and [^{3}H]mannose, and immunoprecipitates were prepared with anti-LCMV sera. A substantial amount of the LCMV nucleocapsid protein (molecular weight about 58,000) was detected, along with GP-C, the precursor of the virion glycoproteins GP-1 and GP-2. GP-1 and GP-2 themselves were not detected. A new method of transferring proteins electrophoretically from sodium dodecyl sulfate-polyacrylamide gels to diazotized paper in high yield revealed several additional LCMV proteins present specifically in the persistently infected cells, at apparent molecular weights ($\times 10^3$) of 112, 107, 103, 89, 71 (probably GP-C), 58 (nucleocapsid protein), 42 to 47 (probably GP-1), and 40 (possibly GP-2). By iodinating intact cells with I_3^-, GP-1 but not GP-2 or GP-C was revealed on the surfaces of the persistently infected cells, whereas both GP-1 and GP-C were found on the surfaces of acutely infected cells. The absence of GP-C from the plasma membrane of the persistently infected cells might be related to defective maturation of the virus in these cells. Cytoplasmic viral nucleoprotein complexes were labeled with [^{3}H]uridine in the presence or absence of actinomycin D, purified partially by sedimentation in D_2O-sucrose gradients, and adsorbed to fixed *Staphylococus aureus* cells in the presence of anti-LCMV immunoglobulin G. Several discrete species of viral RNA were released from the immune complexes with sodium dodecyl sulfate. Some were appreciably smaller than the 31S and 23S species of standard LCMV virions, indicating that defective interfering viral RNAs are probably present in the persistently infected cells. Ribosomal 28S and 18S RNAs, labeled only in the absence of actinomycin D, were coprecipitated with anti-LCMV serum but not with control serum, indicating their association with LCMV nucleoproteins in the cells.

Some lines of Syrian hamster cells persistently infected with lymphocytic choriomeningitis virus (LCMV) fail to produce extracellular standard or defective virus particles but nevertheless do contain an appreciable amount of intracellular infectious material in their cytoplasm (18). Some properties of the intracellular infectious particles and how such an infection might be propagated in the absence of extracellular particles have been described. We now describe the viral proteins and RNAs associated with these persistently infected cells.

MATERIALS AND METHODS

Cells and antisera. The Syrian hamster cell lines C13/SV28, BHK-21F, and BHK-21L have been described (18). Persistently infected cell lines are indicated by appending (LCMV). Thus, BHK-21L and BHK-21L(LCMV) indicate the uninfected and persistently infected lines, respectively. The preparation of hamster anti-LCMV serum has been described (18), rabbit anti-LCMV (WE strain) was a gift from F. Lehmann-Grube, and mouse anti-LCMV (Armstrong strain) was prepared by infecting 3-week-old Swiss mice intraperitoneally with 5.6×10^4 50% tissue culture infective doses of virus and bleeding them after 6 weeks. The titers of these sera in the immunoradiometric competition assay (18) and in a plaque reduction assay are shown in Table 1.

Labeling and immunoprecipitation of proteins. Subconfluent monolayers of cells in 25-cm^2 tissue culture flasks were labeled with L-[^{35}S]methionine (1,000 Ci/mmol, 50 μCi/ml) in 4 ml of methionine-deficient Eagle medium with 5% fetal calf serum. Glycoproteins were labeled in Eagle medium containing 1/10 the normal concentration of glucose and 10% fetal calf

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TABLE 1. Properties of the anti-LCMV sera used in these studies[a]

Species	Amt of serum for half-maximal inhibition in competition assay (μl)	Plaque reduction titer[b]
Hamster	0.01	<100
Mouse	2.0	<100
Rabbit	0.01	5,000

[a] The immunoradiometric competition assay and plaque assay were performed as described previously (18).

[b] Plaque reduction titers are expressed as the final dilution giving 50% reduction of 100 PFU of the WE strain of LCMV in conjunction with anti-hamster IgG, anti-mouse IgG, or anti-rabbit IgG diluted 1:50.

serum. Both D-[2-^{3}H]mannose and D-[1-^{3}H]glucosamine (16 and 4.1 Ci/mmol, respectively, 100 μCi/ml of each) were used. After 4 h, the cells were washed with phosphate-buffered saline (PBS) and lysed with 0.5 ml of TES buffer (20 mM Tris-hydrochloride, pH 7.4, 1 mM EDTA, 100 mM NaCl) supplemented with 0.5% Triton X-100, 0.5% 1,5-naphthalene disulfonate disodium salt, and 2 mM phenylmethylsulfonyl fluoride. The lysates were centrifuged at 10,000 × *g* for 5 min and the supernatant solutions were stored at −70°C. For immunoprecipitation, lystates were clarified after thawing and incubated overnight with 1/25 volume of antiserum at 4°C. KCl was added to a final concentration of 0.5 M, followed by a 10% suspension of protein A-Sepharose (10 μl/μl of antiserum; Pharmacia, Uppsala, Sweden) in TES buffer containing 0.1% Triton X-100. After 30 min, the protein A-Sepharose was pelleted and washed three times with TES buffer containing 0.1% Triton X-100. Some experiments were done with fixed *Staphylococcus aureus* cells (8) instead of protein A-Sepharose. The immune complexes were eluted into sample buffer for electrophoresis, boiled for 2 min, and analyzed in sodium dodecyl sulfate (SDS)-polyacrylamide gels.

Immunological detection of intracellular LCMV proteins after separation in SDS-polyacrylamide gels and transfer to diazotized paper. A confluent 10-cm plate of cells was placed on ice and washed twice with ice-cold buffered saline solution. Two milliliters of ice-cold 10% trichloroacetic acid was spread over the monolayer, and after 5 min the precipitate was scraped into a tube and centrifuged at 3,500 × *g* for 10 min at 4°C. The trichloroacetic acid was decanted and the pellet was drained for a few minutes by inverting the tube and the excess acid was blotted away. The pellet was suspended in a mixture of 380 μl of water plus 100 μl of 5× sample buffer (5% SDS, 250 mM dithiothreitol, 50 mM sodium phosphate pH 7.0, 50% glycerol, 0.03% bromphenol blue), and 20 to 30 μl of 2.5 M Tris (free base) was added to titrate the dye from yellow to blue. After brief sonication to disperse the pellet, the mixture was boiled for 3 min. A method described by Reiser and Stark (Methods Enzymol., in press) was used for transfer. Briefly, electrophoresis was carried out in the borate-sulfate system of Neville (12), avoiding amines, which inactivate diazonium groups. Transfers were carried out in 15 mM sodium phosphate buffer, pH 6.5, at 20°C by using a Transphor apparatus (Hoefer Scientific Instruments, San Francisco, Calif.) for 1 h at 2 A and 50 V. Diazotized aminophenylthioether paper (15) was used to bind the tranferred proteins covalently (Reiser and Stark, in press). For labeled standard proteins with molecular weights similar to those of the LCMV nucleocapsid proteins, the efficiency of transfer was about 50%. After inactivating excess diazonium groups, the paper was incubated first with rabbit or hamster anti-LCMV serum diluted 1/25 to 1/100 and then with ^{125}I-labeled protein A, about 10^6 cpm per sheet. The positions of labeled bands were determined by autoradiography.

Detection of cell surface LCMV antigens by immunofluorescence. About 2.5 × 10^4 cells per 16-mm well were plated in Eagle medium as modified by Dulbecco, with 10% fetal calf serum. After 30 h, some cultures were infected with 0.3 PFU of LCMV (WE strain) per cell in 0.2 ml of Dulbecco modified Eagle medium with 10% fetal calf serum for 1 h at 37°C. At 48 h after plating, the cells were washed with PBS and incubated for 30 min at 4°C with a 1:20 dilution of rabbit anti-LCMV serum in PBS (Table 1) or with murine monoclonal antibodies to LCMV (3), kindly provided by M. Buchmeier, Scripps, Institute, La Jolla, Calif. The cells were then washed three times with cold PBS, and the procedure was repeated with a 1:20 dilution of fluorescein-labeled goat anti-rabbit immunoglobulin G (IgG) (Miles-Yeda Ltd., Rehovot, Israel) or fluorescein-labeled goat antimouse IgG (Nordic, Tilburg, The Netherlands). Finally, the cells were fixed with 95:5 methanol-acetic acid (vol/vol) for 10 min at −20°C, washed with PBS, and assayed for immunofluorescence.

Surface radioiodination. Cells were plated in 35-mm wells and infected as above. At 48 h after plating, the cells were washed twice with PBS, and 1 ml of PBS containing 500 μCi of Na^{125}I was added. The reaction was started by floating a cover slip (18 by 18 mm) coated with 10 μg of Iodogen (9) on top of this fluid. After 10 min at room temperature, the cover slip was removed and tyrosine was added to 1 mM. The cells were washed twice with PBS plus 1 mM tyrosine and then lysed with 0.3 ml of TES buffer containing 0.5% Triton X-100, 0.5% disodium 2,5-naphthalenedisulfonate, and 2 mM phenylmethylsulfonyl fluoride. Viral proteins were detected by SDS-polyacrylamide gel electrophoresis of immunoprecipitates prepared with rabbit anti-LCMV serum as described above, using fixed *S. aureus* cells (8). As a control, BHK-21L cells were infected with vesicular stomatitis virus (VSV; 20 PFU per cell) or mock-infected 4 h before surface radioiodination. Immunoprecipitation was carried out with anti-VSV serum prepared as described previously (7).

Preparation of RNA from intracellular LCMV ribonucleoproteins. Hamster anti-LCMV IgG and normal hamster IgG were precipitated with ammonium sulfate, chromatographed on DEAE-cellulose and carboxymethyl cellulose (18), and stored at 4°C in the presence of 0.02% sodium azide. RNase activity was assayed by incubating the IgGs with rRNAs overnight at 4°C, followed by analysis in agarose gels. The activity found, much too high to allow isolation of intact RNAs, was reduced to an acceptable level by incubating the IgGs with the hectonite clay Macaloid (a kind gift from National Lead Industries, Baroid Division, Houston, Tex.) as described by Meredith and Erlanger (11). The Macaloid was heated to boiling

in water, dispersed at high speed in a blender, and then autoclaved. After centrifugation, the pellet was resuspended in 0.1 M Tris-hydrochoride buffer, pH 7.4, and autoclaved again. A portion corresponding to 1 mg was added to each milliliter of IgG, and the resulting colloidal suspension was kept at 4°C for 1 week before centrifugation. Oren and Levine (13) have described alternative methods for ridding IgG preparations of RNase activity.

All glassware was sterilized and all solutions were autoclaved or treated with diethylpyrocarbonate to inactivate RNase. BHK-21F(LCMV) cells were incubated overnight with [^{3}H]uridine (0.5 mCi per plate) in the presence or absence of actinomycin D (2 μg/ml, applied 45 min before the uridine). Cell-free extracts (from 10-cm plates of cells) were prepared on ice (18), and all subsequent steps were performed at 0°C. D_2O-sucrose gradients, 20 to 50% (wt/vol) in the absence of detergent and 40 to 80% (wt/vol) in the presence of 0.25% Nonidet P-40 (NP40), were run as described by van der Zeijst et al. (18) and the fractions containing most of the antigen were pooled. From each pool, 250 μl was diluted with 4.5 ml of NET buffer (30 mM NaCl, 1 mM EDTA, 10 mM Tris-hydrochloride, pH 7.4) containing 2 mg of bovine serum albumin and 0.5 mg of heparin per ml. After 2 h on ice, a small amount of flocculent precipitate was removed by centrifugation, and 250 μ of IgG was added. After 16 h at 4°C, fixed *S. aureus* cells (Enzyme Center, Boston, Mass.) washed and suspended in NET buffer plus 0.25% gelatin and 0.05% NP40 were added. One volume of a 10% suspension was used, and the mixture was incubated for 15 min on ice with occasional swirling. After centrifugation in a microfuge, the pellet was washed once with NET-gelatin-NP40, once with the same buffer plus 0.5 M NaCl, and once again with NET-gelatin-NP40. The pellets were then incubated twice with 2% SDS for 15 min at 25°C and the supernatant solutions were combined. After adding tRNA as carrier, the RNAs were extracted with phenol, precipitated

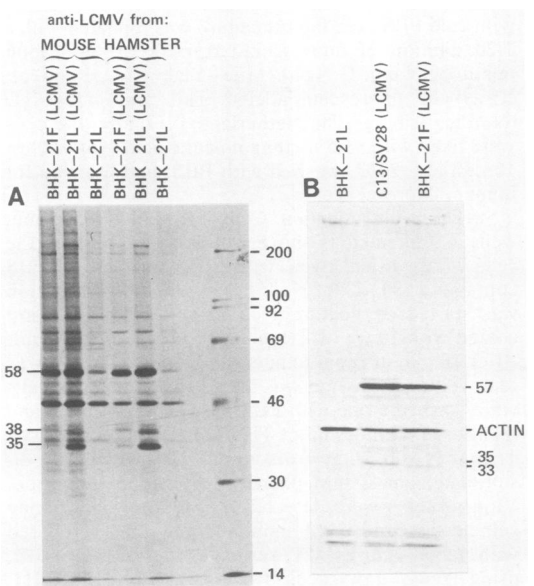

FIG. 1. LCMV proteins labeled with [^{35}S]methionine in persistently infected BHK-21 cells. Immunoprecipitates from cytoplasmic lysates, prepared with mouse or hamster anti-LCMV serum, were separated in SDS-polyacrylamide slab gels. Material from about 2×10^5 cells was analyzed in each track, and fluorography was carried out for 1 day. The positions of marker proteins are indicated by their molecular weights ($\times 10^3$). (A) Mouse or hamster anti-LCMV sera revealed three proteins in immunoprecipitates from BHK21F(LCMV) cells. The immunoprecipitates were collected with protein A-Sepharose. (B) The smaller of the two small LCMV proteins from C13/SV28(LCMV) is different from that from BHK21F(LCMV) cells, and the larger has two components. Hamster anti-LCMV serum was used, and the immunoprecipitates were collected with fixed *S. aureus* cells.

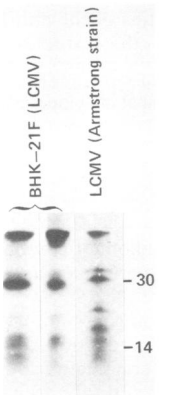

FIG. 2. Comparison by peptide mapping of the LCMV nucleoprotein from acutely infected cells with the 58,000-molecular-weight LCMV proteins from persistently infected cells. The labeled protein with a molecular weight of 58,000 obtained from BHK-21F(LCMV) cells with hamster anti-LCMV serum was cut from the dried gel shown in Fig. 1 by using the fluorograph as a template. A similar band was prepared from BHK-21L cells infected with the Armstrong strain of LCMV (0.01 PFU per cell) and labeled with [^{35}S]methionine 4 days after infection. A modification of the method of Cleveland et al. (5) was used to map peptides derived from these proteins. The gel fragments were soaked in elution buffer (0.125 M Tris-hydrochloride, pH 6.8, 0.1% SDS, 0.001% bromophenol blue, 1 mM EDTA, 1% dithiothreitol), boiled briefly, and transferred to a 6-mm-wide slot in a 3-cm-long 4.5% polyacrylamide stacking gel atop a 17% separating gel, both prepared as described by Studier (17). Elution buffer supplemented with 20% glycerol was added to the slot until the gel fragments were just covered and they were overlaid with 20 μl of elution buffer–20% glycerol containing 1 μg of *S. aureus* V8 protease (Miles Laboratories Ltd., Slough, England). Electrophoresis was carried out until the bromophenol blue marker migrated to a position just above the separating gel and the current was switched off for 30 min and electrophoresis was resumed. Fluorography was carried out for 7 days. The positions of marker proteins are indicated by their molecular weights ($\times 10^3$).

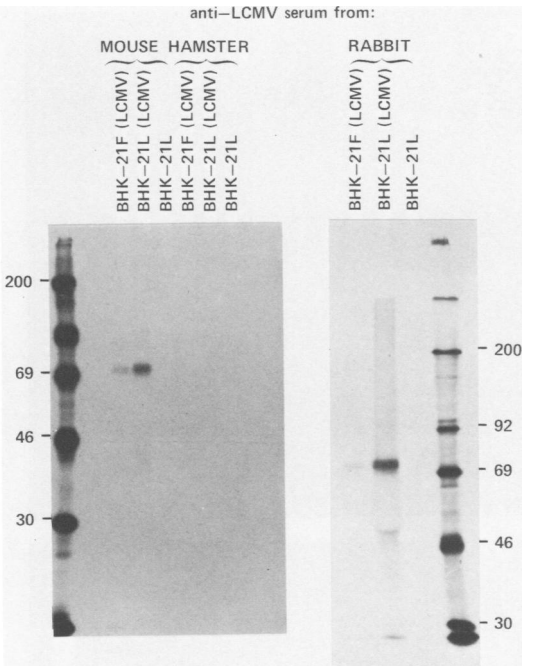

FIG. 3. LCMV proteins labeled with [^{3}H]glucosamine and [^{3}H]mannose in persistently infected BHK-21 cells. Immunoprecipitates from cytoplasmic lysates, prepared with mouse, hamster, or rabbit anti-LCMV serum, were separated in SDS-polyacrylamide slab gels. Material from 10^6 labeled cells was analyzed in each track. Fluorography was carried out for 14 days (mouse and hamster) or 4 days (rabbit). The positions of marker proteins are indicated by their molecular weights ($\times 10^3$).

with ethanol, denatured with glyoxal for 1 h at 50°C, and analyzed in agarose gels as described by Wahl et al. (19). The gels were treated with enhancing solution for fluorography, dried, and autoradiographed.

Preparation of labeled LCMV RNA. Mouse L-929 cells were infected with 0.01 PFU of the WE strain of LCMV per cell, and [^{3}H]uridine (50 μCi/ml, 29 Ci/mmol) was added 18 h after infection. The virus was pelleted from the medium 42 h after infection and was purified further in a 10 to 40% gradient of Urografin (Schering AG, Berlin, Germany). Viral RNA was extracted with phenol after treating the virus suspension with proteinase K (16), and the RNA was precipitated with ethanol.

RESULTS

Intracellular LCMV proteins. LCMV virions contain a nucleocapsid protein (NP) with an approximate molecular weight of 63,000 and two glycopeptides, GP-1 and GP-2, with approximate molecular weights of 44,000 and 35,000, respectively (2, 4). Only small amounts of GP-1 and GP-2 have been reported to be present in infected cells. Instead, GP-C, a precursor glycopeptide with a molecular weight of 74,000, is more abundant. We have investigated the LCMV proteins present in BHK-21F(LCMV) cells and BHK-21L(LCMV) cells, using uninfected BHK-21L cells as a control. When hamster or mouse anti-LCMV sera were used for immunoprecipitation of the ^{35}S-labeled proteins (Fig. 1A), a major protein with a molecular weight of 58,000 was found in both lines of persistently infected BHK-21 cells. Two smaller proteins with molecular weights of 38,000 and 35,000 were also observed. Similar results were obtained with rabbit anti-LCMV serum (data not shown). The size of the smallest protein (molecular weight about 35,000) is somewhat different in BHK-21F(LCMV) or C13/SV28(LCMV) cells (Fig. 1B). This figure also shows that the amount of the protein with a molecular weight of 58,000 from control cells was very low in some immunoprecipitations. The protein with a molecular weight of 58,000 comigrated with the virion NP of the WE strain of LCMV and with the NP (reported molecular weight, about 63,000 [2]) from cells acutely infected with the Armstrong strain of LCMV (data not shown). Comparison of the 58,000-molecular-weight protein from BHK-21F(LCMV) cells with the NP protein from cells infected with the Armstrong strain showed that they were indeed closely related (Fig. 2).

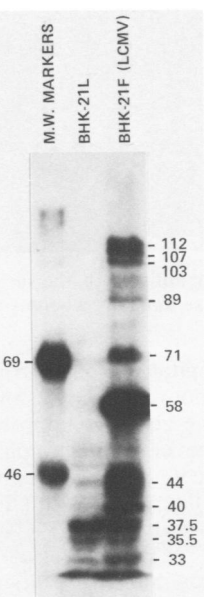

FIG. 4. LCMV antigens in BHK-21F(LCMV) cells, revealed by transfer from an SDS-polyacrylamide gel to diazotized paper. Whole cell extract from 5% of a 10-cm plate of cells was run in each slot of a gel containing 8% acrylamide and 0.08% *N,N'*-methylene-bis-acrylamide. The standards were iodinated ovalbumin and bovine serum albumin. The transfer was probed with rabbit anti-LCMV serum.

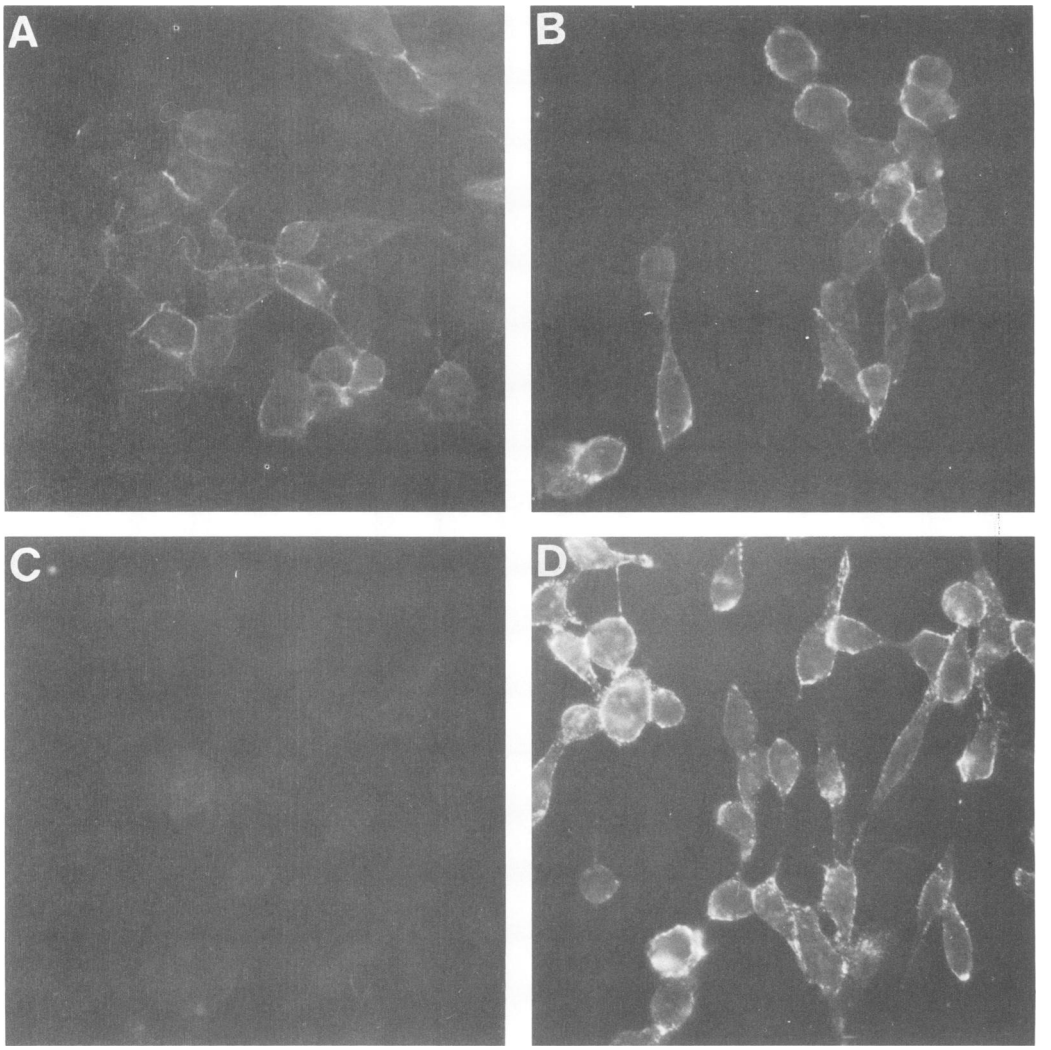

FIG. 5. LCMV antigens on the surfaces of BHK-21F(LCMV) (A) and BHK-21L(LCMV) (B) cells. BHK-21L cells (C) lack these antigens whereas they are present on acutely infected BHK-21L cells (D).

Immunoprecipitates from [^{35}S]methionine-labeled cells did not reveal GP-C, the intracellular precursor for the virion glycoproteins GP-1 and GP-2 (4). However, GP-C (molecular weight, 72,000) was detected readily in extracts of BHK-21L(LCMV) cells labeled with [^{3}H]glucosamine plus [^{3}H]mannose (Fig. 3). GP-C was also found in BHK-21F(LCMV) cells, but in somewhat smaller quantities. Similar results were obtained with rabbit and mouse anti-LCMV sera. However, GP-C was not precipitated with hamster anti-LCMV serum, consistent with the fact that this serum was raised against purified intracellular nucleoprotein antigen from persistently infected cells (18).

As shown in Fig. 4, rabbit anti-LCMV serum reveals several proteins in BHK-21F(LCMV) extracts after separation in SDS gels and transfer to paper. The NP protein (molecular weight, 58,000) is readily seen, as are bands in the positions of GP-C (molecular weight, 71,000), GP-1 (molecular weight, 42,000 to 47,000), and possibly GP-2 (molecular weight, 40,000). The viral glycoproteins were more readily revealed by the transfer method than they were in the previous experiment in which [^{35}S]methionine or ^{3}H-labeled sugars were employed. In addition, a set of high-molecular-weight proteins is present specifically in the BHK-21F(LCMV) cells. Bruns et al. (1) have found 12 proteins of the following molecular weights ($\times 10^{3}$) to be structural components of virions: 19, 25, 26, 35, 38, 44, 60, 63, 77, 85, 130, and 200. All of these proteins bind to anti-LCMV serum. In addition

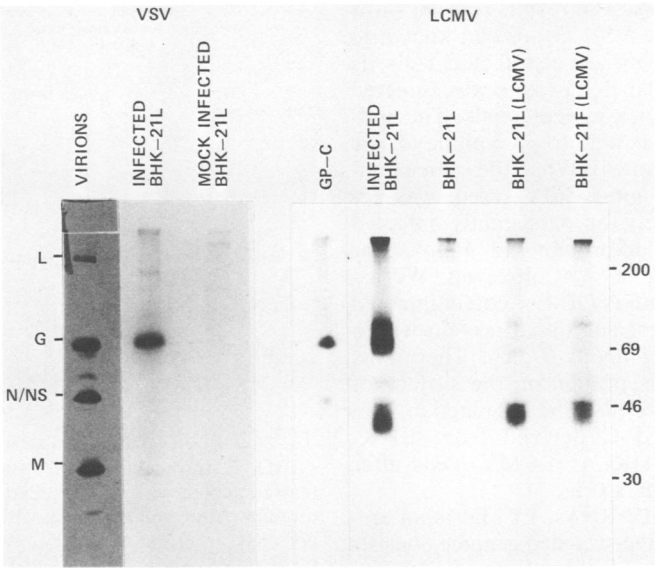

FIG. 6. Surface radioiodination of LCMV proteins on acutely and persistently infected BHK-21 cells. Immunoprecipitates were separated in a 12.5% SDS-polyacrylamide slab gel, using the material from 1.3×10^5 cells in each track. An immunoprecipitate from BHK-21L(LCMV) cells labeled with [^{3}H]glucosamine and [^{3}H]mannose was run as a marker for GP-C (Fig. 3), and surface radioiodination of VSV-infected cells was used as a control for the method. These latter two tracks were exposed for only 1 day, compared to 12 days for the LCMV tracks. VSV labeled with [^{35}S]methionine was grown in BHK-21L cells and purified by sucrose gradient centrifugation.

to the possibility that they may be virion components, the proteins of high molecular weight in Fig. 7 may correspond to the LCMV polymerase or precursors to viral structural proteins. It should be noted that the transfer efficiency is usually somewhat less for large proteins, so their relative abundance may be under-represented in the experiment of Fig. 4. The two smaller proteins (molecular weights, 35,000 and 38,000) that were revealed by immunoprecipitation (Fig. 1) are shown in Fig. 4 to be present at equivalent levels in transfers from BHK-21L and BHK-21F(LCMV) cells. This result indicates that they were probably normal cellular constituents which coprecipitated with LCMV particles in the experiments with [^{35}S]methionine. It is not clear why the rabbit anti-LCMV serum would recognize normal cellular proteins in the transfer experiment. Perhaps they were present at low levels in the preparation of virions used for immunization. Transfers exposed to normal rabbit sera show very little background (data not shown).

Hamster anti-LCMV serum prepared against intracellular nucleoproteins does not react even with the abundant NP protein in transfers from SDS gels, although it does immunoprecipitate this protein (Fig. 1). The reason for this difference is not known.

Cell surface LCMV antigens in persistently infected cells. During processing of GP-C to GP-1 and GP-2, the mannose and glucosamine labels are largely removed (4), possibly explaining why GP-1 and GP-2 were not revealed in the experiment of Fig. 3. Alternatively, GP-C might not be processed properly in the persistently infected cells, possibly blocking virus maturation. Therefore, we determined by immunofluorescence whether any viral antigens were present on the surface of persistently infected cells, using uninfected and infected BHK-21L cells as negative and positive controls. The results (Fig. 5A and B) show clearly that BHK-21F(LCMV) and BHK-21L(LCMV) cells do contain LCMV antigens on their surfaces. The immunofluorescence assay was positive with monoclonal antibody II WE-6.2 against GP-1 (3) but not with monoclonal antibody 9-7.9 against GP-2 (3), in agreement with results of Buchmeier and Oldstone (4), who found GP-1 but not GP-2 on the surface of acutely infected cells.

To determine more precisely which viral glycoproteins were present on these cells, we labeled the surface antigens on cells in an intact monolayer with ^{125}I, immunoprecipitated them, and analyzed them by SDS-polyacrylamide gel electrophoresis. To ascertain that this method labeled only surface antigens, we also applied it

to VSV-infected cells. The results (Fig. 6) show clearly that only the VSV G protein, known to be present on the surface of infected cells, is labeled and that no labeled protein was detected on the surface of mock-infected cells. The anti-VSV serum used was able to precipitate all the viral proteins (not shown). When the same method but with rabbit anti-LCMV serum was applied to cells acutely or persistently infected with LCMV, a labeled protein with a molecular weight of about 46,000 was observed. We assume that this protein is GP-1. Acutely infected cells had a prominent additional protein with a molecular weight of about 69,000. There was little or none of this protein on the surface of persistently infected cells. The iodinated protein comigrates with GP-C derived from BHK-21L(LCMV) and BHK-21F(LCMV) cells after labeling with ^{3}H-sugars (Fig. 3).

Intracellular LCMV RNAs. LCMV is an arenavirus whose negative-stranded genome consists of two single-stranded RNAs, L and S, 3.9 and 6.35 kilobases (kb) long (6). The RNAs of BHK-21F(LCMV) cells were labeled in the presence or absence of actinomycin D, and the labeled RNAs bound to LCMV proteins were precipitated with anti-LCMV IgG after partial purification of the intracellular nucleoprotein complexes. Cell-free extracts were first sedimented in D_2O-sucrose gradients (20 to 50%) in the absence of detergents (gradients not shown, but see reference 18 for an example), and the broad peaks of antigen were pooled. This material was sedimented again in D_2O-sucrose gradients (40 to 80%) in the presence of detergent (0.25% NP40), and the prominent peaks of antigen at densities 1.34 to 1.29 (no actinomycin) or 1.30 to 1.29 (actinomycin) were pooled (gradients not shown but see reference 18 for an example). Immunoprecipitations were performed under RNase-free conditions, and RNA was recovered, denatured with glyoxal, and separated by electrophoresis in agarose gels (Fig. 7). In several control experiments, normal IgG precipitated only about 5% of the ^{3}H label brought down with anti-LCMV IgG, and electrophoresis of this material revealed no discrete bands (data not shown). Similar RNA species were precipitated from the pooled peaks in gradient 1 and gradient 2, except for the high-molecular-weight RNA (probably cellular) that was removed in gradient 2. With actinomyin D, the 28S rRNA was not labeled and the 18S rRNA was labeled very little. Note that the hazy, diffuse background of cellular RNAs was also eliminated with actinomycin D. We strongly suspect that the discrete RNAs labeled in the presence of actinomycin D and specifically precipitated with IgG directed against LCMV proteins were indeed LCMV specific, but we have no direct proof. The

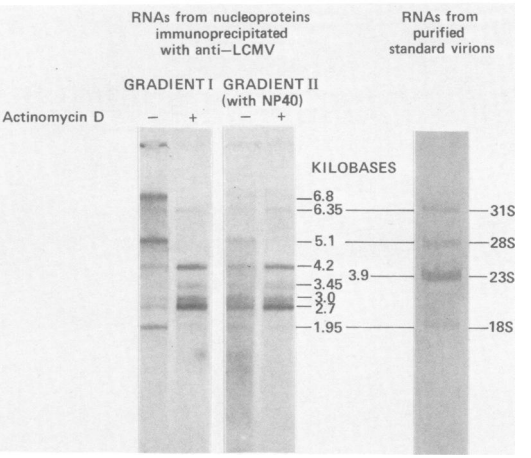

FIG. 7. Intracellular RNA species in cells persistently infected with LCMV. Partially purified nucleoproteins from cells labeled with [^{3}H]uridine in the presence or absence of actinomycin D were immunoprecipitated with hamster anti-LCMV serum. RNAs recovered from precipitates were denatured with glyoxal and separated by electrophoresis in agarose gels. See the text for more details of gradients I and II. A gel with the RNA species present in standard LCMV virions (WE strain) is also shown for comparison. The sizes of the LCMV RNAs were calculated by comparison with the known sizes of the rRNAs.

pooled, immunoprecipitated LCMV RNAs did not yield translation products in vitro in a rabbit reticulocyte system and did not bind to a column of oligodeoxthymidylate-cellulose (data not shown), indicating that they probably were not messengers and did not contain long tracts of polyadenylic acid. Only the largest of these RNAs comigrated with a standard LCMV RNA derived from ^{3}H-labeled virions (Fig. 7). The prominent band at 4.2 kb, larger than the 3.9-kb (23S) LCMV RNA from standard virions, may be a defective interfering (DI) RNA derived from the 6.35-kb (31S) standard LCMV RNA. Similarly, the prominent species at 2.7 kb may be derived from the 3.9-kb standard RNA. Dutko et al. (6) have observed that extra bands can be observed in gels of glyoxal-denatured RNA of arenaviruses if secondary structure has not been disrupted sufficiently. The conditions we used, 50°C for 1 h, should have caused complete denaturation (6), so that all the bands shown in Fig. 7 probably do represent discrete, different RNA molecules.

DISCUSSION

RNAs. Prior analyses of extracellular DI particles from cells persistently infected with LCMV (20) or of extracellular interfering particles from acutely infected cells (10) have revealed no LCMV-specific RNAs substantially smaller than

the 23S and 31S species found in standard virions. Shorter LCMV RNAs comparable to those revealed in the experiment of Fig. 7 may have been present intracellularly in the persistent infections studied previously, but may not have become incorporated into virions. The immunoprecipitation procedure employed is capable of revealing the small amount of LCMV RNAs present in the BHK(LCMV) cells, and careful attention to achieving RNase-free conditions allowed these RNAs to be seen with little degradation. This latter point can be appreciated from the integrity of the 18S and 28S ribosomal RNAs, which are coprecipitated through their association with LCMV nucleoproteins and are unlabeled in the presence of actinomycin D. There are several distinct species among the RNAs labeled in the presence of actinomycin D. Although minor bands are seen at the positions of standard LCMV RNAs, most of the precipitated RNA is smaller and is very likely to be DI RNA, which could well be the predominant type of LCMV RNA present. The DI particles released from some persistently infected cells have been reported to either contain smaller RNAs (14) or not (20). The interfering particles released during acute infections apparently lack the 23S RNA of standard virions (10).

Proteins. Most of the LCMV proteins previously observed to be present in virions (2) or in DI particles (20) are also detected in the persistently infected BHK(LCMV) cells described here, including the NP and GP-1. GP-C is also present in the BHK(LCMV) cells we studied, as are some larger LCMV proteins. Some of these proteins may correspond to the species found in purified virions by Bruns et al. (1).

Our finding that GP-C is present on the surface of acutely infected cells but not on the surface of persistently infected BHK(LCMV) cells may provide a clue about the defect in virus maturation that prevents the formation of extracellular virus particles in this case (18). If GP-C were cleaved to GP-1 and GP-2 on the cell surface in a step closely linked to virus assembly, a mutational defect in GP-C affecting cleavage could lead to the result observed. Clearly much more work needs to be done to pursue this observation in depth.

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