Virus-Specific Antigens in Hamster Cells Transformed by Rous Sarcoma Virus

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Received for publication 24 July 1969

Virus-specific antigens were studied in hamster cells transformed by Rous sarcoma virus (RSV). Antigens were localized in the cytoplasm, as demonstrated by fluorescent antibody staining of fixed cells as well as by complement fixation (CF) following subcellular fractionation. Cytoplasmic extracts were analyzed by velocity and isopycnic centrifugation. CF antigens were found in a soluble form and in association with membranes and polyribosomes. Isolated plasma membranes had no CF antigen. Both soluble and particulate fractions with CF activity contained the same antigenic determinants by Ouchterlony analysis. These antigenic determinants were identical to those released by ether treatment of RSV.

Neoplastic transformation of mammalian cells by Rous sarcoma virus (RSV) frequently results in a cell population in which virus particles are undetectable. These cells do, however, harbor the RSV genome since they contain virus-specific antigens (5, 22), and they yield RSV upon fusion with chicken cells (20, 37, 39, 41). Such transformed cell systems offer an opportunity to study the virus-cell equilibrium under conditions in which the viral growth cycle is not completed, yet virus-specific molecules continue to be synthesized. A systematic study of RSV antigens in hamster cells was undertaken. Two cell lines were used: a tumor cell line carrying the Bryan strain of RSV (37), and a cloned line of BHK cells transformed by the Schmidt-Ruppin strain of RSV. This paper will report that in these cells RSV-specific antigens are exclusively cytoplasmic and exist in part in association with membranes and polyribosomes.

MATERIALS AND METHODS

Cells. H-RSV(BH) cells, a line of hamster cells transformed *in vivo* by the Bryan high-titer strain of RSV (37), were obtained from Robert Huebner. BHK-21 cells (clone 13), obtained from Michael Stoker, were transformed by the Schmidt-Ruppin strain of RSV by the procedure of Macpherson (27). A twice-cloned transformant, designated BHK-RSV(SR), was obtained, grown to a population of 10^8 , and used for experiments within the next 10 passages. H-RSV(BH) cells were grown as monolayers in Eagle's minimum essential medium (MEM) (16) with 5% tryptose phosphate and 5% agamma calf serum. BHK-RSV(SR) monolayers were grown in MEM with 10% fetal calf serum.

Viruses. The Schmidt-Ruppin strain of RSV was

obtained from Donald Armstrong. Stocks were clarified homogenates of wing-web tumors in 2-week-old chicks (COFAL-negative, SPAFAS, Norwich, Conn.). Titers averaged 2×10^6 focus-forming units/ml (35). Avian myeloblastosis virus (AMV) was purified from infected myeloblast culture fluid generously supplied by Alfred Prince. The virus was purified by repeated banding in sucrose gradients (Fig. 3 of reference 33).

Antigens. AMV viral antigens were prepared by extracting purified AMV with ether and Tween 40 (17). The aqueous layer was dialyzed and centrifuged for 30 min at $100,000 \times g$, and the supernatant fluid was used as a source of antigen for inoculation into rabbits. Viral antigens for Ouchterlony analysis were prepared from Bryan high-titer RSV (National Institutes of Health lot no. TV33) and AMV by ether treatment (2). Cell-derived antigens were concentrated with lyphogel (Gelman Instrument Co., Ann Arbor, Mich.) before use in Ouchterlony tests.

Sera. COFAL serum was obtained from Syrian hamsters (Lakeview Hamster Colony, Newfield, N.J.) bearing Schmidt-Ruppin Rous sarcomas (36). Some animals were injected as newborns with Schmidt-Ruppin virus; others were injected as weanlings with a transplantable Schmidt-Ruppin Rous sarcoma obtained from Donald Armstrong at the 10th *in vivo* passage. Rabbit antiserum against antigens from purified AMV was prepared in male albino rabbits (Lab Rabs, Inc., Westfield, Pa.).

Immunological techniques. For complement fixation tests, a micro-procedure was employed (38) with 2 units of complement and 4 units of COFAL serum. Rabbit anti-AMV was used in place of COFAL serum where noted.

Fluorescent antibody staining was carried out by the indirect method, essentially following the procedure of Kelloff and Vogt (23). Coverslips were fixed by three 1-min immersions in ice-cold acetone, or by immersion in acetone at -20°C for 1 hr after air dry-

ing. The first layer consisted of COFAL or normal hamster serum; the second layer was fluorescein-conjugated goat anti-hamster gamma globulin (Hyland Laboratories, Costa Mesa, Calif.) which had been absorbed with a washed $1,000 \times g$ pellet of frozen-thawed H-RSV(BH) cells, as well as with an acetone powder of mouse brain (kindly provided by Roller Bailey).

Immunoprecipitation was carried out by the Ouchterlony procedure, using 0.5% Noble Agar (Difco) with 1% NaCl and 7.5% glycine. Plates were developed for several days at 4 C.

Preparation of cell extracts. Confluent monolayers containing about 1.5×10^6 cells were washed with warm phosphate-buffered saline (PBS) (15) deficient in Mg and Ca, removed from the glass with 0.0625% trypsin and 0.0125% Versene in the same buffer, and poured into calf serum. Subsequent steps were performed in the cold. The cells were washed with PBS and then with reticulocyte standard buffer (RSB; 31) [0.01 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride, pH 7.4, 0.01 M KCl, and 0.0015 M $MgCl_2$] containing 1 mM dithiothreitol (RSB + DTT). The packed cells were resuspended with an equal volume of RSB + DTT and after 10 min were broken in a Dounce homogenizer with 25 strokes. The extract was centrifuged for 4 min at $1,000 \times g$, and the pellet containing nuclei with cytoplasmic tabs was washed with 2 volumes of RSB + DTT; the combined supernatant fluids constituted cytoplasmic extract no. 1 (CE 1). The washed pellet was treated twice with RSB containing sodium deoxycholate (DOC) and Tween 40 (Amend Drug Company, New York, N.Y.), by the method of Penman (31), to obtain nuclei free of cytoplasmic contamination, and the combined supernatant fluids were designated cytoplasmic extract no. 2 (CE 2). The washed nuclei were solubilized with high-salt buffer and deoxyribonuclease as described by Penman (31).

CE 1 was centrifuged at $40,000 \times g$ for 5 min in a Sorvall angle rotor (tube diameter, 1.5 cm). The pellet (P-40) containing mitochondria and large membrane structures was solubilized in RSB + DTT containing DOC and Tween as above. The supernatant fluid (S-40) was used for subsequent gradient centrifugations.

A preparation of membrane-bound antigens which would normally be extracted in CE 2 was obtained by adapting the procedure of Maggio et al. (28). The pellet containing nuclei from which CE 1 was initially separated was resuspended in RSB and centrifuged through an interface with 2.2 M sucrose. Material concentrating at the interface was collected, diluted with RSB, homogenized, and then centrifuged for 5 min at $40,000 \times g$. The supernatant fluid was analyzed by isopycnic centrifugation in the usual fashion.

Gradient centrifugation. All centrifugations were carried out at 4 C. For velocity centrifugation, linear gradients of 15 to 30% (w/v) sucrose in RSB were employed. For isopycnic centrifugation, discontinuous gradients were constructed with 8 ml each of 2.5, 2.0, 1.5, and 1.0 M sucrose in RSB + DTT, with D_2O as the solvent. On top of the 1.0 M sucrose was layered 23 ml of S-40. When the Spinco SW 41 rotor was used

in place of the SW 25.2, the layers of sucrose were 1.6 ml each, and 1.6 ml of 0.5 M sucrose was included before layering 4.5 ml of S-40.

Isolation of plasma membranes. An extract of H-RSV(BH) cells was prepared by the method of Bosmann et al. (10) as far as the centrifugation of the cell extract at $4,000 \times g$. To separate plasma membranes from endoplasmic reticulum (ER), the following modification was necessary. The $4,000 \times g$ supernatant fluid obtained by their procedure was adjusted to 1 M sucrose with 2.5 M sucrose in D_2O and then was layered over 8 ml of 2.5 M sucrose in D_2O . On top was layered 8 ml of 0.5 M sucrose and 1 ml of 0.05 M Tris, pH 7.1. Centrifugation was carried out for 20 hr at 24,000 rev/min, at 4 C in the Spinco SW 25.2 rotor. The banded membranes were diluted and collected by sedimentation (10). Plasma membranes and ER were distinguished by enzymatic and chemical tests (10).

Chemical analysis of membrane fractions. Membrane fractions were fractionated into phospholipid, ribonucleic acid (RNA), and protein by the procedure of Omura et al. (29). Phospholipid was determined as total phosphate by the method of Ames and Dubin (1), and total phospholipid was estimated by the relation: phospholipid = $25 \times$ phospholipid phosphorus (18). RNA was assayed in the hot trichloroacetic acid extracts by the orcinol test (4), and deoxyribonucleic acid was determined on portions of the same extracts by the Burton modification of the diphenylamine test (11). Protein was measured by the procedure of Lowry et al. (26).

RESULTS

Examination of RSV-transformed hamster cells for production of RSV. H-RSV(BH) cells were investigated for virus production by four different procedures. (i) No infectious virus, detectable by focus formation in chick embryo cells (35), was released into the growth medium. (ii) Pellets ($20,000 \times g$ for 2 hr) from the growth medium contained no avian leukosis group-specific antigen. (iii) Long-term in vivo radioactive labeling of tissue culture cells (33) revealed no virus particles containing labeled RSV RNA in the culture medium. (iv) No particles resembling RSV were seen in thin sections of cells examined in the electron microscope (E. de Harven, *personal communication*). These results confirm earlier reports (37, 40).

Cytoplasmic location of antigens by fluorescent antibody staining. In chick embryo cells, in which the complete growth cycle of RSV occurs, several authors have reported RSV-specific antigens detectable by fluorescent antibody staining (23, 30, 42). In one study, nuclear as well as cytoplasmic staining was reported (30). It was of interest to determine what the staining pattern would be in transformed hamster cells in which the growth cycle of RSV is incomplete.

Results for acetone-fixed H-RSV(BH) cells are given in Fig. 1. The entire cell population was

FIG. 1. Fluorescent antibody staining of *H-RSV(BH)* cells: (A, B, and C) with COFAL serum in the indirect test; (D) with normal hamster serum. The use of Evans blue as a counterstain permits discrimination of nuclei from cytoplasm. The diffuse counterstaining has a red-brown color; the punctate immunofluorescence is green. (Original Kodachrome transparencies were transcribed onto black and white film.) $\times 270$

found to be specifically stained in this fashion. Two features stand out. First, the specific fluorescence is exclusively cytoplasmic (Fig. 1 A, B, C). Second, the specific fluorescence is concentrated in discrete, punctate loci. These loci were totally absent from cells stained with normal hamster serum (Fig. 1 D). Viable *H-RSV(BH)* cells did not stain. As in the case of RSV-infected chick cells (23), staining with COFAL serum required prior fixation of the cells.

Cytoplasmic location of antigens from cell fractionation studies. The intracellular location of RSV-specific antigens was also explored by means of a second procedure, subcellular fractionation, with a different mode of antigen detection, complement fixation. The hamster sera used for complement fixation were specific for RSV-related antigens: they did not fix complement with extracts of Syrian hamster cells transformed by Moloney sarcoma virus.

H-RSV(BH) cells were broken in a Dounce homogenizer and fractionated as described in Materials and Methods. This procedure permits recovery of the total cytoplasm in two parts: the low-speed supernatant (CE 1) after sedimentation of nuclei and large membranous structures, and the remaining cytoplasm (CE 2) in a detergent solution which permits a clean separation of this fraction from intact nuclei (21, 31). In Table 1 are reported the results of a typical fractionation. More than 99% of the complement-fixing (CF) antigens was recovered in the cytoplasmic fractions CE 1 and CE 2. Of the antigens in CE 1, less than 5% sedimented at $40,000 \times g$ in 5 min; thus, mitochondria carried little, if any, antigen. More than 95% of the antigens remained in the supernatant fluid, and further analyses were carried out on this fraction.

Analysis of antigens in velocity gradients. The $40,000 \times g$ supernatant from CE 1 (S-40) was layered on a gradient of 15 to 30% sucrose and centrifuged for 3 hr. The pattern of CF activity obtained is shown in Fig. 2. (All complement fixation titers were controlled by establishing absence of anticomplementarity at antigen concentrations at least twice as high as those used in the tests, i.e. a titer of 4 means that the antigen was not anticomplementary at 1:1 and 1:2.)

Antigenic activity was found in the pellet and throughout the gradient, sedimenting with *S*-values similar to those of polyribosomes and monoribosomes, as well as in the region of soluble proteins. The CF titer of the pellet fraction as depicted is a minimum, since this fraction con-

TABLE 1. Distribution of complement-fixing (CF) antigen in *H-RSV(BH)* cells

Subcellular fraction	Total CF units ^a	Protein	Ribo-nucleic acid	Deoxy-ribo-nucleic acid
		mg	mg	mg
Cytoplasmic extract 1				
S-40 ^b	1,275	210	19.0	0.02
P-40 ^c	105	20	2.2	0.08
Cytoplasmic extract 2....	1,225	137	12.0	0.30
Nuclei.....	<12.5 ^d	43	3.4	13.0

^a One CF unit equals the amount of antigen in 1 ml with a CF titer of 1.

^b Supernatant fluid from 5-min centrifugation at $40,000 \times g$.

^c Pellet from a 5-min centrifugation at $40,000 \times g$, washed once and resuspended.

^d No CF activity detectable in assay of 25 μ liters.

FIG. 2. Velocity centrifugation of an extract from H-RSV(BH) cells on a sucrose gradient. An S-40 preparation (5 ml concentrated from 20 ml with lyphogel) was layered onto 15 to 30% sucrose and centrifuged for 3 hr at 24,000 rev/min in an SW 25.2 rotor. Fractions of 2.5 ml were collected. All CF titers were controlled by establishing absence of anticomplementarity at antigen concentrations at least twice as high as those used in tests (see text).

tained aggregated membranous material. These results raised two questions. Did the heterogeneous size distribution of antigenic activity reflect a true heterogeneity of antigenic specificities? If not, to what structures were antigens attached, thus acquiring new sedimentation characteristics?

Analysis of antigens in isopycnic gradients. To answer these questions, isopycnic centrifugation was used, permitting larger amounts of antigen to be processed. An S-40 from H-RSV(BH) cells was layered on to a stepwise gradient of 1.0 to 2.5 M sucrose in D₂O, and after centrifugation for 19 hr complement fixation tests were performed on the gradient fractions. The results are depicted in Fig. 3. There were four main regions of CF activity. Region 1 consisted of material which had moved down through part of the broad layer of extract applied to the top of the gradient. CF antigens from fractions 16 and 17 in this region had S-values of less than 12 when rerun on velocity gradients. Antigenic activity was most abundant in region 2, which is just within the sucrose gradient. Located in this region was a distinct, flocculent band of membrane material (mean density, 1.16 in 25 experiments). After dilution of the fraction containing this band, about 50% of the CF antigen could be pelleted by centrifugation at $40,000 \times g$ for 20 min, indicating an association of part of the antigen in region 2 with the banded material. Regions 3 and 4 had complement fixation maxima associated

with less prominent bands of aggregated material, and the positions of these bands varied within the density range 1.28 to 1.38, moving slightly toward the higher value when centrifugation was prolonged (see Fig. 5).

Ouchterlony analysis of antigens from isopycnic gradients. Materials from regions 1, 3, and 4 of an isopycnic gradient were pooled separately and concentrated for Ouchterlony tests. The results are shown in Fig. 4. All three gradient-derived samples contain the same antigenic determinants. Furthermore, the two lines of identity between ether-treated RSV and AMV and the hamster cell antigens show that the latter are internal structural proteins of RSV. This is true because it is well established that RSV and AMV form precipitation bands in agar with hamster COFAL serum (2, 5, 34) only if disrupted by ether or other treatments.

In extension of these findings, complement fixation assays of density gradients with rabbit anti-

FIG. 3. Isopycnic centrifugation of an S-40 from H-RSV(BH) cells. The cell extract was layered over a stepwise sucrose-D₂O gradient (see Materials and Methods) equivalent in volume to fractions 1-13. The remainder of the volume collected (23 ml) represents the volume of S-40 extract. Centrifugation was carried out for 19 hr at 20,500 rev/min in an SW 25.2 rotor. Fractions of 2.5 ml were collected. All CF titers were controlled with respect to anticomplementarity (see legend to Fig. 2).

AMV serum produced a pattern of CF activity similar to that obtained (Fig. 3) with hamster COFAL serum. This result provides additional evidence that the cell-derived antigens detected by complement fixation tests are the group-specific antigens of avian leukosis viruses, for only these antigens would be expected to fix complement with a hyperimmune serum prepared against Tween-ether-disrupted AMV.

Further analysis of viral antigens from isopycnic gradients. Representative RNA/protein and phospholipid/protein determinations for materials from regions 2, 3, and 4 are shown in Table 2. These values suggested the presence of smooth

ER in region 2, rough ER in region 3, and polyribosomes in region 4 (12, 13). Electron microscopic examination of thin sections from pellets of these fractions was carried out in collaboration with Etienne de Harven. The results confirmed the foregoing interpretations for materials in regions 2 and 4. Pelleted material from region 2 contained only smooth membranes and no ribosomes, whereas material from region 4 contained very rare membranes and large numbers of free polyribosomes. The pellets from region 3 contained ribosomes and membranes, often closely adjacent, but not in the configuration of typical rough ER. A final interpretation of the membrane structures in region 3 requires further study.

To learn more about the relationship of RSV antigens to membranes, the effect of DOC was studied. Release of CF antigens from rapidly sedimenting, dense materials could be effected by pretreatment of the S-40 with 0.7% DOC. In isopycnic centrifugation of such a DOC-treated S-40 fraction from H-RSV(BH) cells, almost all of the antigen sedimented slowly in the pre-sucrose part of the tube (Fig. 5B). A small but significant amount of antigen was left in the denser regions of the gradient.

This residual DOC-resistant antigen was subjected to further analysis. The results indicated that this antigenic activity was attached directly to polyribosomes. Starting with an S-40 from 1.5×10^9 cells, structures with a density of more than 1.3 were collected by centrifugation through 2 M sucrose by the method of Wettstein et al. (43). The pelleted material was diluted sixfold in RSB, homogenized, treated with 0.7% DOC, and after low-speed centrifugation to remove aggregates, was layered on a gradient of 15 to 30% sucrose. Centrifugation was carried out long enough to separate monoribosomes from the region of soluble antigens released by DOC. A portion of the same DOC-treated sample was exposed to 1 μ g of pancreatic ribonuclease per ml for 10 min at 0°C before gradient centrifugation, a treatment diagnostic for polyribosomes (8, 25, 32).

The results are presented in Fig. 6. As predicted from the experiment depicted in Fig. 5, 85% of the CF activity in each gradient is present in a form smaller than 74S due to the treatment with DOC. From a comparison of Fig. 6A and 6B, it is clear that the very gentle ribonuclease treatment employed has effected a striking change in the sedimentation pattern of the residual 15% of CF activity: a fourfold reduction in the titer of the pellet and a compensating increase in the titer corresponding to 74S ribosomes and their 115S dimers. Parallel changes can be seen in the optical density profiles due to the brief

FIG. 4. Ouchterlony analysis of antigens from H-RSV(BH) cells and from virions. RSV and AMV refer to ether-treated virus. Antigens 1, 3, and 4 refer to materials from regions of an isopycnic gradient (see Fig. 3). The center well contains hamster "COFAL" serum (courtesy of L. J. Old). Antigen preparations 3 and 4 were dialyzed, treated with 0.5% DOC, and centrifuged at $150,000 \times g$ before concentration. After similar treatment, material from region 2 gelled upon concentration and could not be analyzed.

TABLE 2. Characterization of fractions from isopycnic gradient centrifugation of an extract from H-RSV(BH) cells

Gradient fraction ^b	Density (g/cm ³)	CF units per milligram of protein ^a	Milligram of ribonucleic acid per milligram of protein	Milligram of phospholipid per milligram of protein
4	1.36	24	0.95	0.1
9	1.28	34	0.76	0.5
12	1.16	4	0.023 ^c	0.1 ^c

^a See Table 1.

^b Fractions are from an isopycnic gradient similar to that shown in Fig. 3.

^c These values are minimized by the presence of soluble proteins; half of the protein in this fraction does not sediment in 2 hr at $150,000 \times g$.

FIG. 5. Isopycnic centrifugation of extracts from H-RSV(BH) cells on a sucrose- D_2O gradient. (A) An S-40 preparation centrifuged for 40 hr at 30,000 rev/min in an SW 41 rotor. (B) An S-40 treated with 0.7% DOC and centrifuged as described for Fig. 5A. In each case, 4.5 ml of extract was layered on an 8-ml gradient. Fractions of 0.5 ml were collected. All CF titers were controlled with respect to anticomplementarity (see legend to Fig. 2).

ribonuclease treatment: a loss of polyribosomes, especially from the pellet, and an increase in 74S ribosomes and their dimers. Two experimental details require mention. The 20% increase in total CF activity and optical density in Fig. 6B as compared to Fig. 6A, as well as the minimal titer of the pellet in Fig. 6A, is explained by the presence of aggregated material in the resuspended pellet fraction of Fig. 6A. The small residual CF activity and optical density in the large polyribosome region of Fig. 6B can be traced to the very large amount of ribosomes (more than 10 mg in 1 ml) layered on each gradient to obtain detectable CF activity on ribosomes: not all polyribosomes were broken down by ribonuclease.

The conclusion drawn from the experiments presented in Fig. 5 and 6 was that the bulk of the CF antigen found in the dense regions of sucrose gradients was solubilized by DOC, and hence membrane-bound, whereas a smaller amount of antigen was associated directly with polyribosomes.

It seemed likely that CF antigen appeared in CE 2 also by virtue of an original association with membrane structures. If so, the structures involved might be similar to ones found in CE 1, only complexed in larger aggregates. Since CE 2 already contained DOC and Tween 40, a different procedure was required to study the membrane structures in question without the use of detergents. Membranes normally extracted in CE 2 were separated from nuclei by centrifugation to a density interface (see Materials and Methods) and subjected to isopycnic analysis. As predicted, CF activity was found in regions 2, 3, and 4 and in a pattern similar to that in Fig. 3.

Absence of viral antigens from plasma membranes. In cells in which RSV grows to maturity, virus is formed by budding from the external (plasma) membrane (42). Thus, the question could be posed: do viral core proteins form an association with the plasma membrane in H-RSV(BH) cells in which the viral replicative process is blocked prior to emergence of mature virus? To answer this question, it was necessary to separate plasma membranes from ER membranes. This was accomplished by a modification of the isopycnic centrifugation procedure of Bosmann, et al. (see Materials and Methods). Plasma membranes and ER were found at the top and bottom, respectively, of the 1 M sucrose

FIG. 6. Velocity gradient centrifugation of polyribosomes from H-RSV(BH) cells. The position of 74S monoribosomes is in fraction 8, and that of 115S ribosome dimers in fraction 6. (A) Polyribosomes centrifuged for 90 min at 40,000 rev/min in an SW 41 rotor. (B) Polyribosomes treated for 10 min at 0°C with 1 µg of pancreatic ribonuclease per ml and then centrifuged. In each case, 1 ml of the DOC-treated preparation was layered on a gradient of 11 ml of 15 to 30% sucrose in RSB. All CF titers were controlled with respect to anticomplementarity (see legend to Fig. 2).

layer, as indicated by measurements of certain parameters which serve to distinguish the two membrane fractions (10, 24; C. G. Gahmberg et al., *unpublished data*). In Table 3, isolated plasma membranes are compared with ER with respect to these parameters as well as CF activity. The complement fixation data indicate that there is little CF antigen associated with plasma membrane compared to the amount associated with ER.

Investigations with hamster cells transformed by Schmidt-Ruppin virus. Most of the fluorescent antibody and complement fixation analyses described above were repeated with BHK-RSV(SR) cells with results similar to those obtained with H-RSV(BH) cells.

DISCUSSION

A number of authors have presented evidence that the group-specific antigen of the avian sarcoma and leukosis viruses is in reality a complex of antigens (2, 3, 6, 7, 14, 22, 34). An analogous complexity can be found in the antigens of the murine sarcoma and leukemia group (19). The avian group-specific antigens all appear to be internal components of the virions (2, 6, 34). This is consistent with the fact that hamster COFAL sera do not contain neutralizing (strain-specific) antibodies (23).

The work reported here permits three principal conclusions to be drawn concerning RSV-specific antigens in virus-free hamster cells. (i) The antigens are located exclusively in the cytoplasm. (ii) The antigens are group specific; that is, they are immunologically identical with core proteins released from RSV-related viruses by treatment with ether. (iii) The same antigens occur in a soluble form and in association with membranes and polyribosomes within cells.

TABLE 3. *Comparison of plasma membrane and endoplasmic reticulum fractions of H-RSV(BH) cells*

Determination	Plasma membrane	Endoplasmic reticulum
Total CF units ^a	<4.0 ^b	290
CF units per mg of protein.....	<1.2 ^b	11
Ratio of moles cholesterol to moles phospholipid...	0.82	0.15
5' Nucleotidase (units/mg of protein).....	1.8	0.27
Adenosine triphosphatase (units/mg of protein)....	3.0	0.63

^a See Table 1.

^b No CF activity detectable in assay of 25 μ liters.

The evidence for the first conclusion is based on two highly sensitive methods of detection: complement fixation with subcellular fractions and fluorescent antibody staining. Both methods reveal no detectable antigen in nuclei.

In regard to the second conclusion, derived from Ouchterlony analysis, it is striking that transformed hamster cells appear to contain at least two group-specific antigenic components. This result confirms and extends the findings of Bauer and Janda (5) and of Armstrong (2), who found one precipitating component in Rous-transformed hamster cells.

The evidence for the third conclusion is derived from CF tests used in conjunction with isopycnic centrifugation, chemical analyses, and electron microscopic examination. Solubilization of the membrane-attached antigens by DOC fits the concept that the integrity of membrane structure is necessary for antigen to appear in a "particulate" form.

ACKNOWLEDGMENTS

I thank Caryl Lambek and Leslie Tilzer for skillful technical assistance. I am indebted to Peter J. Gomatos and to other members of the Virology Division for many helpful discussions.

This investigation was supported by Public Health Service grant CA 08758 from the National Cancer Institute, and by The John A. Hartford Foundation, Inc.

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