Quantitation of Viral Antigens Released into Plasma and Culture Fluids by Murine Mammary Tumor Cells

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ABSTRACT

Radioimmunoassays capable of measuring mouse mammary tumor virus (MMTV) 52,000 M.W. envelope glycoprotein (gp52) and 27,000 M.W. protein (p27) have been used to quantitatively compare plasma concentrations of these viral antigens in mice bearing spontaneous mammary tumors. Although gp52 was detected in the plasma of all tumor-bearing mice tested, p27 was detected in only a portion of tumor-bearing animals. In P27-positive animals, gp52 was detected in higher concentrations than p27. These findings demonstrate that gp52 has preferential utility as a plasma marker for the presence of mammary tumors in MMTV-infected hybrid (BALB/c × DBA/8 F1), Paris Rill, and C3H/HeJ mice. In addition, cultures of MMTV-producing cells [GR-MMTV and MMTV(C3H)Fel I] were used as models to study the release of viral antigens in the absence of serum antibody or additional host factors. Comparisons of extracellular soluble and particulate antigen concentrations demonstrated that gp52 and p27 were present in substantially higher concentrations as soluble than virion-associated antigens. The mean ratio of non-virion-associated gp52 to virion-associated gp52 was 12.5:1 for GR-MMTV cells and 37.3:1 for MMTV(C3H)Fel I cells. The marked stability of MMTV in culture fluids suggested that virion breakdown was not responsible for the accumulation of soluble viral antigens in culture. The information obtained suggests that abundant virus-free antigens may be of greater use than virion-associated antigens as a source of viral antigen to evaluate mammary tumor status.

INTRODUCTION

The mouse mammary tumor and its associated virus MMTV\(^3\) have been used as a model to study the relationship between viral antigens in body fluids and the onset of mammary tumors. Concentrations of viral antigens in milk have correlated with the tumor incidence of mouse strains examined, and mice with higher levels of viral antigens in their milk have had a greater tendency to develop tumors (4, 7, 8, 10); however, milk antigens cannot be utilized to detect the presence of tumor in nonlactating individuals.

For this reason, gp52 was studied in plasma of tumor-bearing and tumor-free mice to determine if gp52 levels of plasma reflected the presence of mammary tumors. Results of studies utilizing gp52-specific RIA’s revealed that gp52 levels were elevated in plasma samples obtained from tumor-bearing animals (1, 15, 20). Subsequent investigation indicated a 10- to 100-fold decrease in the level of gp52 within 9 days of tumor removal, thus demonstrating that the tumor was the principal source of plasma gp52 (16). Fractionation of plasma samples by centrifugation revealed that gp52 was detected as a soluble antigen in supernatants rather than as a virion-associated antigen in viral pellets (15). Measurement of gp52 levels in plasma of tumor-bearing mice has also been used to monitor changes in tumor status during chemotherapy. This approach has provided a measure of therapeutic effect for combination 5-fluorouracil:thymidine treatment (14, 17).

Although gp52 has proven to be useful as a systemic marker for mammary disease, our knowledge of the utility of other MMTV structural proteins to serve as markers for disease is still incomplete. P10 has been detected in serum of tumor-bearing mice (2). In addition, the recent results of Osterrieth et al. (9) have suggested that the 27,000 M.W. protein (p27) is generally not found in the serum of MMTV-infected tumor-bearing Swiss albino mice; however, only a small number of a single strain of mice were studied and mean gp52 levels of serum were also relatively low.

The present study used gp52 and p27 RIA’s to quantitate these antigens in the plasma of tumor-bearing mice to determine the comparative utility of these 2 viral antigens as systemic markers for murine mammary tumors and utilized cell culture models to compare the concentrations of extracellular MMTV antigens released in the absence of potentially lytic serum factors (1, 19).

In the present study, comparison of gp52 and p27 levels in the plasma of 3 different strains of tumor-bearing mice has revealed that gp52 was detected with greater frequency and abundance than p27. In addition, the analysis of viral antigen ratios in culture fluids has demonstrated that gp52 was relatively more abundant than p27 as a soluble antigen and that both proteins were detected predominantly as soluble rather than virion-associated antigens. The marked stability of MMTV particles in culture fluids has suggested that virion degradation is an unlikely cause of the high concentrations of soluble antigens detected in virus-free supernatants.

The parallel observations that gp52 is present principally as a soluble non-virion-associated antigen in plasma of tumor-bearing animals and extracellular culture fluids of murine mammary tumor cells suggest that mammary tumor cells release an abundance of soluble viral antigen and therefore provide a useful model system for studying factors that influence the release and accumulation of soluble viral antigens.

\(^{3}\) The abbreviations used are: MMTV, mouse mammary tumor virus; gp52, 52,000 M.W. envelope glycoprotein of mouse mammary tumor virus; RIA, radioimmunoassay; p10, 10,000 M.W. protein of mouse mammary tumor virus; p27, 27,000 M.W. protein of mouse mammary tumor virus; DMEM, Dulbecco’s modified Eagle’s minimal essential medium-high glucose.

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\(^{2}\) To whom requests for reprints should be addressed.

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The present study used gp52 and p27 RIA’s to quantitate these antigens in the plasma of tumor-bearing mice to determine the comparative utility of these 2 viral antigens as systemic markers for murine mammary tumors and utilized cell culture models to compare the concentrations of extracellular MMTV antigens released in the absence of potentially lytic serum factors (1, 19).

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The parallel observations that gp52 is present principally as a soluble non-virion-associated antigen in plasma of tumor-bearing animals and extracellular culture fluids of murine mammary tumor cells suggest that mammary tumor cells release an abundance of soluble viral antigen and therefore provide a useful model system for studying factors that influence the release and accumulation of soluble viral antigens.
MATERIALS AND METHODS

Cells. The GR-MMTV cell line, which was derived from a murine mammary adenocarcinoma of a GR mouse, was acquired from Dr. David K. Howard, Meloy Laboratories (12). A feline cell line, designated MMTV(C3H)Fel I, was obtained from the same source. MMTV(C3H)Fel I is a C3H MMTV-infected feline embryonic kidney cell line (6). The Mm5mt/c1 C3H mammary tumor cell line was obtained from Dr. Fine (5). Cells were maintained on DMEM containing 10% heat-inactivated fetal calf serum, insulin (250 IU/liter), tyloxol (60 μg/ml), penicillin (100 units/ml), streptomycin (100 μg/ml), and dexamethasone (2.5 μg/ml).

Mouse Strains. For the analysis of MMTV gp52 and p27 plasma concentrations, spontaneous tumor-bearing and tumor-free mice of the following strains were used: C3H/HeJ (The Jackson Laboratory, Bar Harbor, Maine), Paris RIII (Columbia University, Institute of Cancer Research), and a MMTV-infected hybrid [BALB/c x DBA/8F1 (hereafter called DBSF1) (Catholic Medical Center Woodhaven, New York, N. Y.). Mice with only a single spontaneous breast tumor were selected for study.

Bleeding Procedure. Mice were bled (approximately 500 μl) from the retroorbital venous plexus, and the blood was collected in heparin-treated tubes. Plasma was separated from blood by centrifugation (1500 x g for 10 min) and rapidly frozen in a dry ice: methanol bath. Plasma samples were then stored at -70° prior to use in RIA. Mammary tumor size for RIII mice at the time of bleeding ranged from 0.44 to 10.4 g. A majority of tumors was greater than 2.0 g. Tumor size for C3H/HeJ and DBSF1 mice, respectively, ranged from 0.58 to 5.03 g and 0.18 to 8.11 g. A majority of tumors in these strains was greater than 10.0 g. Tumor weights (in mg) were estimated from the empirical relationship, 0.44(L^2W^2), where L and W represent, respectively, the length and width of the tumor in mm (3).

Virus and Protein Purification. RIII MMTV and structural proteins (gp52 and p27) were purified as described previously from milk of Paris RIII mice (13). RIII milk plasma was obtained from the office of Program Resources and Logistics, National Cancer Institute, NIH.

Iodination of MMTV gp52 and p27. Purified MMTV gp52 and p27 were iodinated with 125I-labeled Bolton-Hunter reagent. The iodination and chromatography procedures have been described previously (13); however, for p27, the procedure was modified by substituting Sephadex G-75 to separate iodinated protein from low-molecular-weight reaction products. Iodinated MMTV gp52 was greater than 90% immune precipitable with anti-gp52 serum and iodinated MMTV p27 was greater than 50% immune precipitable with anti-p27 serum.

RIA for MMTV gp52 and p27. Purified iodinated MMTV gp52 was used in a blocking RIA in which delayed addition of the labeled antigen was used to maximize the sensitivity of the measurement. The RIA procedure, its reproducibility, application, and lack of interference from proteases have been reported (15, 16). The RIA buffer was composed of 0.1 M potassium phosphate buffer, pH 7.4, containing 0.1% bovine serum albumin, 0.001 M EDTA, and 500 kallikrein inhibitor units of Trasylol aprotinin (Mobay Chemical Corp.) per ml.

Cross-reactivity testing with gp52, p27, and Rauscher murine leukemia virus antigen from JLSV9 cells has demonstrated that each assay was specific for its homologous protein. Repeated assays were performed on a plasma sample in both gp52 and p27 RIA’s, and the mean value (± S.D.) for 10 replicate determinations was 74.1 ± 6.9 ng/ml for gp52 assay and 31.5 ± 2.18 ng/ml for p27 assay. In each assay, a standard deviation less than 10% of the mean value was detected. Samples were stored at -70° to avoid proteolytic degradation prior to assay and assayed in the presence of Trasylol aprotinin (a protease inhibitor) to prevent degradation during assay. The RIA’s for gp52 and p27 similarly demonstrated a sensitivity of <10 ng/ml.

Separation of Soluble and Particulate Viral Antigen Fractions. Culture medium was fractionated by ultracentrifugation into a particulate antigen (virion-associated) fraction and a soluble antigen (non-virion-associated) fraction for subsequent RIA measurement. Medium was first clarified by centrifugation at 2000 x g for 20 min at 4°. The supernatant obtained was then fractionated by one of the following procedures: (a) velocity centrifugation at 100,000 x g for 90 min in an SW27 rotor; or (b) velocity centrifugation at 100,000 x g for 90 min in an SW56 rotor. In each case, the supernatant obtained following ultracentrifugation is termed the soluble antigen fraction whereas the resulting pellet is termed the particulate antigen fraction.

Collection of Extracellular Viral Antigens. To compare extracellular viral antigen levels in particulate and soluble antigen fractions, either GR-MMTV or MMTV(C3H)Fel I cells were grown in T-75 flasks until cells reached 70% confluency. At this stage of growth, fresh medium containing 10% heat-inactivated fetal calf serum and 1.2 x 10^-5 M dexamethasone was used to replace culture medium and cells were grown for 24 hr at 37°. Culture medium was then pooled from 3 parallel cultures to reduce flask-to-flask variation, clarified by low-speed centrifugation to remove debris, and centrifuged at 150,000 x g for 90 min to obtain a viral pellet and a supernatant fraction. Prior to RIA measurement, viral pellets were resuspended in 0.1 M Tris-HCl, pH 7.4, and solubilized with 0.1% Triton X-100 and 0.1 M KCl for 30 min at 37°. Viral antigen concentrations in pellets and soluble antigen fractions were standardized to cell number and have been reported as ng/10^9 cells.

Determination of Virion Stability in Culture. Virus (C3H MMTV and GR MMTV) was labeled in cell culture with [4,5-3H]leucine (52 Ci/mmol; Amersham/Searle Corp.) at a concentration of 10 μCi/ml. Labeled virus (2.0 x 10^6 cpm) was added to each of 4 T-75 flasks containing 22 ml of medium. Two of the flasks contained growing cell cultures [70% confluent monolayers of the appropriate mouse strain (C3H MMTV for Mm5mt/c1 cells and GR MMTV for GR-MMTV cells)]; one flask contained only conditioned medium (obtained from confluent monolayers after 48 hr of growth in culture); and the remaining flask contained only routine growth medium (supplemented DMEM). Flasks were incubated at 37°, and 2.0-ml samples were removed from flasks at 1, 4, 8, 12, and 24 hr after addition of 3H-labeled virions. Intact virus was recovered from each sample by centrifugation (113,000 x g for 1 hr in an SW56 rotor). The viral pellets and supernatants were precipitated with 10% trichloroacetic acid, filtered onto Whatman No. 3MM filters, washed with 95% ethanol, and counted in a scintillation counter. The distribution of 3H cpm in pellet and supernatant fractions provided a measure of the degree of virion degradation during each time interval.

RESULTS

Quantitation of gp52 and p27 in the Plasma of Tumor-bearing Mice. Previous studies have demonstrated the utility of gp52 as a systemic marker in mice bearing spontaneous mammary tumors (15, 16). The purpose of the present study is to quantitate both gp52 and p27 in plasma samples, thus comparing the relative frequency of detection and abundance of these viral antigens in tumor-bearing mice.

Plasma samples from RIII, C3H/HeJ, and DBSF1 mammary tumor-bearing mice were obtained for comparative measurement in gp52 and p27 RIA’s, and small numbers of tumor-free animals were used as controls. Viral antigen levels were compared in mice bearing a range of different tumor burdens. The majority of RIII tumors was in excess of 2.0 g and the majority of DBSF1 and C3H/HeJ tumors was in excess of 1.0 g. Volumes of plasma (10 to 200 μl) from the same bleeding of each normal and tumor-bearing mouse were assayed in the presence of trasyol, a protease inhibitor, for gp52 and p27 as described previously (13).

The gp52 levels of plasma from tumor-free controls were low (<10 ng/ml) and p27 was not detected. The results obtained with tumor-bearing mice indicated that gp52 was detected in...
Release of MMTV Antigens

Table 1

<table>
<thead>
<tr>
<th>Mouse strain</th>
<th>No. of mice tested</th>
<th>Concentration of gp52 (ng/ml)</th>
<th>Concentration of p27 (ng/ml)</th>
<th>gp52:p27</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD8F</td>
<td>14</td>
<td>446±150-840</td>
<td>24 ± 6-54</td>
<td>27±4-63</td>
</tr>
<tr>
<td>Rill</td>
<td>25</td>
<td>169±7-740</td>
<td>14 ± 1-73</td>
<td>22±1-61</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>4</td>
<td>170±39-424</td>
<td>11 ± 8-12</td>
<td>19±3-55</td>
</tr>
</tbody>
</table>

Notes:
- *Mice of each strain had a spontaneous mammary tumor. The size ranges of these tumors and bleeding procedures used to obtain plasma samples are given in "Materials and Methods."
- Plasma samples from the same animal have been assayed for gp52 and p27 by separate RIA's.
- Ranges presented for viral antigen concentrations reflect striking differences in the tumor burden of individual animals. Ranges presented for antigen ratios reflect the heterogeneity of these ratios in individual tumor-bearing animals.
- The variability of individual determinations for each assay was low and has been given in "Materials and Methods."
- The difference between mean concentrations of gp52 and p27 has been evaluated for significance in a 2-tailed Student's t test. The difference in means is statistically significant for CD8F, mice (p < 0.001) and Rill mice (p < 0.01). Although C3H/HeJ mice demonstrate the same trend, the number of p27-positive mice was too small to demonstrate a statistical difference (p > 0.1).

Chart 1. Comparison of gp52 and p27 concentrations in plasma samples of CD8F, mouse bearing mammary tumors. The concentrations of gp52 and p27 have been determined for each plasma sample by parallel determinations in their respective RIA's. The ordinate (antigen concentration) is on a log scale: ○, concentration of p27; ●, concentration of gp52.

Chart 2. Comparison of gp52 and p27 concentrations in plasma samples of mammary tumor-bearing mice. The ratio of gp52 to p27 concentration is illustrated as a series of histograms for individual tumor-bearing mice.

Although a range of viral antigen concentrations was detected within each group of mice bearing different-sized tumors, mean antigen concentrations for each group of p27-positive animals have been determined and are presented in Table 1, thus permitting group comparisons of antigen concentrations and ratios of these concentrations. This comparison indicates that gp52 is present at higher concentrations than p27 in CD8F, Rill, and C3H/HeJ mice. The difference between concentration means for gp52 and p27 has been evaluated for significance in a 2-tailed Student's t test and is statistically significant for CD8F, mice (p < 0.001) and Rill mice (p < 0.01). The ratio of gp52 to p27 in the plasma of CD8F, tumor-bearing mice (27:1) does not reflect the molar ratio of these proteins in the virion (0.91:1) (18). Ratios for Rill mice (22:1) and C3H/HeJ mice (19:1) further indicate a greater abundance of the envelope glycoprotein. Results with each mouse strain studied indicated that gp52 was detected in plasma of tumor-bearing animals with greater frequency and abundance than was possible for p27.

Accumulation of Non-Virion-associated gp52 in Extracellular Culture Fluids. Analysis of gp52 levels in plasma samples of tumor-bearing mice revealed that gp52 was detected predominantly as a soluble rather than a virion-associated antigen (15). To further our understanding of MMTV antigen release from murine mammary tumor cells, GR-MMTV cells have been utilized as a model system to study the release of non-virion-associated gp52.

As a first step in our study of viral antigen release in culture, the accumulation of non-virion-associated gp52 was examined as a function of time by gp52 blocking RIA. Culture medium was harvested from growing GR-MMTV cells cultures at various times from 1 to 120 hr after medium change. At each time point, the concentration of gp52 was determined in a parallel determination in the respective RIA. The results are illustrated in Chart 3, thus permitting group comparisons of antigen concentrations and ratios of these concentrations. This comparison indicates that gp52 is present at higher concentrations than p27 in CD8F, Rill, and C3H/HeJ mice. The difference between concentration means for gp52 and p27 has been evaluated for significance in a 2-tailed Student's t test and is statistically significant for CD8F, mice (p < 0.001) and Rill mice (p < 0.01). Although C3H/HeJ mice demonstrate the same trend, the number of p27-positive mice was too small to demonstrate a statistical difference (p > 0.1).
point, medium was clarified by low-speed centrifugation, centrifuged as indicated in "Materials and Methods" to obtain a virus-free non-virion-associated antigen fraction, and quick frozen for subsequent RIA analysis. Virus-free medium samples (volumes of 4 to 160 μl) from each time point were analyzed by blocking assay.

The blocking curves obtained for time intervals from 0 to 120 hr after medium change are presented in Chart 3. All samples (except the zero time point) produced a reduction in gp52 precipitation with the addition of increasing sample volumes, thereby demonstrating the presence of gp52 determinants in each virus-free supernatant. Further analysis of these blocking curves indicates that both the initial and maximum inhibition achieved increase with time after medium change. The results clearly demonstrate continued gp52 release and accumulation as non-virion-associated antigen for the entire time interval studied (0 to 120 hr). The extent of blocking detected indicated that a 24-hr interval would provide a sufficient amount of gp52 accumulation in culture to quantitatively compare gp52 levels in virion-associated and non-virion-associated antigen fractions.

Quantitation of Virion-associated and Non-virion-associated Extracellular Viral Antigens. Murine mammary tumor cells of GR mice (GR-MMTV cells) and cat kidney cells infected with C3H MMTV (MMTV(C3H)Fel I cells) have been used as an in vitro model to determine if gp52 and p27 antigens, released by MMTV-producing cells, are detected predominantly as virion-associated or non-virion-associated antigens. The ability to measure both gp52 and p27 by RIA has provided a direct quantitative comparison of the amount of each antigen detected in viral pellets (virion-associated antigen fractions) and virus-free supernatants (non-virion-associated antigen fractions).

Medium samples were obtained from growing dexamethasone-treated GR-MMTV and MMTV(C3H)Fel I cells, and samples were separated into soluble and particulate antigen fractions as described in "Materials and Methods." Pooled medium samples from 3 cell cultures were used to reduce variation among cultures, and 4 separate experiments were performed for each cell line. Viral pellets were solubilized (0.1% Triton X-100 and 0.1 M KCl) for 30 min at 37° prior to assay, and both antigen fractions were assayed simultaneously for comparison. Relatively mild solubilization conditions were used to permit direct assay of samples without assay interference, a possible consequence of high concentrations of salt or detergent. In each experiment, the concentration of gp52 detected in virion-associated and non-virion-associated fractions has been normalized to cell number, and the data obtained from 4 experiments with each cell line are presented in Table 2. The values obtained for both GR-MMTV cells and MMTV(C3H)Fel I cells demonstrate that gp52 is substantially more abundant as a soluble non-virion-associated antigen than as a virion-associated particulate. The difference between gp52 concentration means in these 2 antigen fractions is statistically significant for each cell line (p < 0.01), and markedly higher levels of soluble gp52 were detected in each experiment. The ratios of soluble to particulate antigen for GR-MMTV and MMTV(C3H)Fel I cells indicate that soluble gp52 is, respectively, 12.5- and 37.3-fold more abundant than virion-associated gp52. Although this comparison was conducted with dexamethasone-treated cells, soluble gp52 was also substantially more abundant in untreated cultures.*

In addition to gp52 quantitation, extracellular soluble and particulate antigen fractions were compared by p27 assay. Again, 24-hr medium harvests of growing GR-MMTV cells were obtained. The results of p27 assay for 3 separate experiments demonstrated a higher concentration of soluble compared to particulate antigen, and the ratio of soluble to particulate p27 was 2.3 ± 0.5. This ratio is substantially smaller than that obtained for gp52 (2.3 versus 12.5) and demonstrates that gp52 is relatively more abundant as a soluble antigen.

Stability of MMTV in Medium of Mammary Cell Cultures. The possibility that non-virion-associated gp52 might arise through a process of virion breakdown has been investigated using [3H]leucine-labeled MMTV purified by equilibrium gradient centrifugation. The procedures used to determine virion stability are detailed in "Materials and Methods." Labeled virus (either C3H MMTV or GR MMTV) was added to flasks containing each of the following: (a) medium and mammary cell cultures producing the same strain of MMTV; (b) conditioned medium alone, and (c) DMEM with additives routinely used for cell growth. Medium samples were removed at different time points and separated into soluble and particulate antigen fractions, and the recovery of trichloroacetic acid-precipitable [3H]leucine-labeled MMTV was determined for the particulate antigen fraction. The recovery of C3H MMTV from Mm5mt/c1 cell cultures and medium controls is presented for each time point in Table 3. These results indicated that MMTV recovery as a particulate antigen ranged from 83 to 96% and the recovery of MMTV did not decrease with increasing time of incubation for medium samples from cell cultures or controls. The high recoveries of MMTV obtained after 24 hr in culture medium and the failure to see a decline in MMTV recovery with increasing time of incubation demonstrate that the incubated MMTV was relatively stable in culture fluids. The recovery of [3H]leucine-labeled GR MMTV from GR-MMTV cultures was performed in an identical manner, and GR MMTV recoveries ranged from 91 to 96% in culture fluids and medium controls.

* B. A. Smith and E. M. Ritzi, unpublished observations.
Release of MMTV Antigens

Table 2
Distribution of gp52 in extracellular medium fractions

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Virion-associated antigen fraction (A)</th>
<th>Non-virion-associated antigen fraction (B)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GR-MMTV</td>
<td>Mean 15.1e + 9.2−14.1</td>
<td>Mean 12.5 + 9.2−14.1</td>
</tr>
<tr>
<td>MMTV/C3H Fel I</td>
<td>Mean 7.1 + 6.8−7.3</td>
<td>Mean 30.9 + 22.7−40.4</td>
</tr>
</tbody>
</table>

In each experiment, a 24-hr medium collection was fractionated by centrifugation (as indicated in "Materials and Methods") into a virion-associated antigen fraction (viral pellet) and a non-virion-associated antigen fraction (virus-free supernatant). Viral pellets were solubilized with 0.1% Triton X-100 and 0.1 M KCl prior to assay.

Table 3
Stability of C3H virus in extracellular culture fluids of Mm5mt/cl cells

<table>
<thead>
<tr>
<th>Time of incubation (hr)</th>
<th>% recovery of C3H MMTV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture A</td>
</tr>
<tr>
<td>1</td>
<td>90.4</td>
</tr>
<tr>
<td>4</td>
<td>93.0</td>
</tr>
<tr>
<td>8</td>
<td>91.5</td>
</tr>
<tr>
<td>12</td>
<td>94.5</td>
</tr>
<tr>
<td>24</td>
<td>95.9</td>
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</table>

stable and soluble antigens since this procedure with 3H-labeled virus achieved recovery in excess of 90%. This finding suggests that the majority of virus-free gp52 detected in culture and plasma samples of tumor-bearing animals does not arise as a result of virion degradation and favors alternative pathways of antigen release such as cell surface shedding. Although experiments with purified 3H-labeled virus demonstrate that...
breakdown was minimal, the possibility that a second population of less stable virions are continually released and rapidly degraded is not eliminated by the present experiments.

Quantitation of p27 in culture fluids revealed a lower ratio of soluble to particulate antigen than detected for gp52 (2:1 versus 12:1). Since virion solubilization conditions (0.1 M KCl and 0.1% Triton X-100) were relatively mild to avoid interference in assay, p27 levels obtained in assay represent minimum estimates of virion-associated p27; despite this fact, ratios demonstrated that gp52 was more abundant than p27 as a soluble antigen.

The results obtained herein argue that non-virion-associated viral antigens are present in higher concentrations and, therefore, offer greater practical utility than virion-associated antigens as markers for mammary disease. The use of mammary cell cultures to study viral antigen release should permit a more complete understanding of the factors that regulate the release and accumulation of non-virion-associated antigens. Such studies of soluble MMTV antigens can be extended to include mammary tumor cells that are cloned virus nonproducers. The amount of soluble viral antigen released by nonproducer cells should help to determine if virus production by tumor cells significantly contributes to the concentrations of soluble antigens released in culture and detected in plasma of tumor-bearing animals.

ACKNOWLEDGMENTS

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