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

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 RIII, and C3H/HeJ mice. In addition, cultures of MMTV-producing cells [GR-1MMTV 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-1MMTV 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 MMTV3 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 used and mean gp52 levels of serum were also relatively low.

The present study used gp52 and p27 RIA's to quantify 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.

1 This work was supported by the Willie Mae Darwin Memorial Grant for Cancer Research (American Cancer Society Grant VC-263, USPHS Grant CA-28305-01, and USPHS Grant 1Pol CA-25842).
2 To whom requests for reprints should be addressed.
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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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 × DBA/2F1, hereafter called CDBF-J] (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 CDBF-J 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 g. Tumor weights (in mg) were estimated from the empirical relationship, OA(LW2), where L and W represent, respectively, the length and width of the tumor in mm (3).

Virus and Protein Purification. RII 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.

Idodination 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 70% 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 (11, 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 kaliliein 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 pmol; Amersham/Searle Corp.) at a concentration of 10 μCi/ml. Labeled virus (~2.0 x 105 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 125I-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 125I 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 RII, C3H/HeJ, and CDBF-F1 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 RII tumors was in excess of 2.0 g and the majority of CDBF-F1 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...
all plasma samples; however, p27 could be detected in only a portion of the tumor-bearing mice of each strain. The failure to detect p27 in plasma was clearly not a function of tumor size, since mice with large tumors were also negative for p27. The nucleoid antigen was not detected by p27 RIA in 13 of 27 tumor-bearing CDBF, mice (mean gp52 level, 253 ng/ml), 6 of 10 tumor-bearing C3H/HeJ mice (mean gp52 level, 354 ng/ml), or 3 of 28 tumor-bearing RIII mice (mean gp52 level, 37 ng/ml). These results demonstrate that elevated gp52 levels of plasma from tumor-bearing animals were detected with greater frequency than elevated p27 levels.

The antigen levels of plasma samples from p27-positive animals were analyzed to quantitatively compare the abundance of gp52 and p27. Since paired antigen determinations were performed, antigen levels can be compared for individuals and groups of tumor-bearing animals. The results for CDBF, tumor-bearing mice are illustrated in Chart 1 as a semilogarithmic scatter plot. These results are also representative of those obtained with RIII and C3H/HeJ mice. Although differing CDBF, tumor burdens resulted in a range of antigen levels for both proteins, plasma concentrations of p27 were lower than concentrations of gp52, not only as a group but also for each individual tested.

Paired RIA data permitted the ratio of gp52 to p27 to be determined and evaluated for individual mice. Ratios of viral antigen concentrations obtained for individual CDBF, mice are illustrated in Chart 2 as a series of histograms. The range of gp52:p27 ratios detected (4 to 63) demonstrated a higher concentration of gp52; however, the heterogeneity of these ratios suggests that a simple relationship between the concentration of these 2 MMTV antigens does not exist. The heterogeneity of the gp52:p27 ratio was also noted in RIII and C3H/HeJ mice and could not be explained by differences in tumor size.

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 CDBF, RIII, 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. The difference in means is statistically significant for CDBF, mice (p < 0.001) and RIII 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 qualitative 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.

**Chart 3.** Accumulation of non-virion-associated gp52 in culture fluids of GR-MMTV cells. Soluble antigen fractions (virus-free supernatants) were obtained from culture medium as indicated in "Materials and Methods" at various times from 0 to 120 hr after medium change. Increasing volumes of each soluble antigen fraction were used as blocking antigen in a gp52 assay. The percentage of total [3H]-gp52 precipitated is illustrated for triplicate volumes of sample; point, mean; bars, S.D. The blocking curves obtained for each soluble antigen fraction are identified on the right by the time of collection (hr after medium change).
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></td>
<td>Concentration of MMTV gp52a (ng/10^8 cells)</td>
<td>B:A</td>
</tr>
<tr>
<td>GR-MMTV</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>MMTC(C3H)Fel I</td>
<td>15.1</td>
<td>9-24</td>
</tr>
<tr>
<td></td>
<td>7.1</td>
<td>6.8-7.3</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. Extracellular antigen fractions (virion associated and non-virion associated) were quantitated in the same RIA (either gp52 or p27).

Two cell lines were used. The GR-MMTV cell line is derived from GR mouse mammary tumor cells whereas the MMTC(C3H)Fel I line is a feline embryonic kidney cell infected with C3H MMTV.

The mean and range for determinations from 4 separate experiments are presented for both cell lines. The difference between mean concentrations of gp52 in virion-associated and non-virion-associated antigen fractions has been evaluated for significance in a 2-tailed Student’s t test. The difference in means is statistically significant for GR-MMTV cells (p < 0.01) and MMTC(C3H)Fel I cells (p < 0.01). The ranges reflect different levels of antigen in one experiment versus another. The assay variability was low and is indicated in "Materials and Methods."

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

<table>
<thead>
<tr>
<th>Time of incubation (hr)</th>
<th>Culture A</th>
<th>Culture B</th>
<th>Conditioned medium</th>
<th>DMEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80.4</td>
<td>87.8</td>
<td>93.5</td>
<td>91.6</td>
</tr>
<tr>
<td>4</td>
<td>93.0</td>
<td>93.4</td>
<td>93.8</td>
<td>83.4</td>
</tr>
<tr>
<td>8</td>
<td>91.5</td>
<td>93.1</td>
<td>94.2</td>
<td>87.6</td>
</tr>
<tr>
<td>12</td>
<td>94.5</td>
<td>92.4</td>
<td>92.1</td>
<td>90.8</td>
</tr>
<tr>
<td>24</td>
<td>85.9</td>
<td>95.3</td>
<td>96.4</td>
<td>90.1</td>
</tr>
</tbody>
</table>

Purified 3H-labeled C3H MMTV (2 x 10^9 cpm) has been added to medium in each of 4 flasks. Two flasks (Cultures A and B) contain monolayer cultures of Mm5mt/cl cells while the remaining flasks contained only medium (standard DMEM plus additives or conditioned medium). Medium samples (2.0 ml) were removed at each of the specific times and were fractionated by centrifugation as indicated in "Materials and Methods" into high-speed viral pellets and virus-free supernatants. Fractions were trichloroacetic acid precipitated, and the percent recovery of C3H MMTV was quantitated in the same RIA (either gp52 or p27). The GR-MMTV cell line is derived from GR mouse mammary tumor cells whereas the MMTC(C3H)Fel I line is a feline embryonic kidney cell infected with C3H MMTV.

These results strongly suggest that virion degradation is not a principal pathway for the release of MMTV antigens into the circulation. Even in p27-positive animals, very little similarity in the gp52:p27 ratio was noted when individuals were compared. These differences in antigen concentration ratios may not only reflect differences in the release of these 2 antigens but may also reflect secondary differences in immune response, blood clearance rates, or the presence of serum lytic activity (19).

The comparative measurement of gp52 and p10 in tumor extracts and mouse sera (2) resulted in ratios of gp52 to p10 ranging from 1.8 to 5.1; however, the average ratio of gp52 to p27 in the present study of CD8F, mice was 27. In both studies, gp52 was more abundant, but the comparatively low ratio of gp52 to p10 may indicate that p10, like gp52, may serve as a more useful marker than p27. Although lower concentrations of p27 were detected, further investigation of the physical state of p27 determinants in plasma is warranted to determine if any alterations in the p27 of plasma might render it more difficult to detect by RIA.

The extension of plasma antigen studies to an in vitro cell culture model has permitted a quantitative assessment of viral antigen release and accumulation in the absence of secondary in vivo considerations. Although the mechanisms of in vivo and in vitro viral antigen release may in part differ, the cell culture model has permitted viral antigen release to be studied in the absence of serum antibodies and potential viral lytic activity (11). Results of immune precipitation analyses have argued for the presence of gp52 in virus-free culture supernatants (11); however, the present study has not only detected non-virion-associated gp52 but has also demonstrated that virus-free gp52 continuously accumulates in GR-MMTV cultures and is approximately 12-fold more abundant than virion-associated gp52 in 24-hr medium harvests.

The high levels of non-virion-associated gp52 cannot be attributed to virus breakdown during the fractionation of particulate and soluble antigens since this procedure with 3H-labeled virus achieved recoveries 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.

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REFERENCES


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