RADIOIMMUNOASSAY OF MAMMALIAN TYPE C VIRAL PROTEINS

III. DETECTION OF VIRAL ANTIGEN IN NORMAL MURINE CELLS AND TISSUES

BY WADE P. PARKS, DAVID M. LIVINGSTON, GEORGE J. TODARO, RAOUl E. BENVENISTE, AND EDWARD M. SCOLNICK

(From the Viral Carcinogenesis Branch and the Viral Leukemia and Lymphoma Branch, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20014)

(Received for publication 26 October 1972)

Induction of infectious type C viruses by physical and chemical means from avian and murine cells (1-4) strongly supports the view that most, if not all, mouse and chicken cells contain at least one normally repressed copy of a complete type C viral genome (5). The state of this endogenous viral information is presently unclear. The recent development of a radioimmunoassay for the major internal viral protein (6-8), the group-specific (gs) antigen of murine type C viruses (9-12), provides a method for the detection of small quantities of this protein in murine cells below the level of sensitivity of complement fixation or gel diffusion assays (9, 11, 13).

Using this technique, we have been able to measure an immunological reactivity that is similar to the virion gs protein in both virus-producing and virus-free mouse cell lines and also in natural nontumored murine tissues. The findings reported here provide additional evidence for the widespread prevalence of type C viral genes in a variety of mouse cells and strongly suggest a low level of synthesis of at least one virion protein both in apparently virus-negative tissues in the animal and in virus-free cultured cells.

Materials and Methods

Cells.--The cell lines and strains listed in Table I were employed for the present studies. Cells were grown and maintained with Dulbecco's modification of Eagle's medium supplemented with either 10% calf serum or 10% fetal calf serum (Colorado Serum Co., Denver, Colo.).

Virus.--The Rauscher strain of murine leukemia virus (MuLV) was obtained from Electro-Nucleonics, Bethesda, Md., as twice sucrose density gradient purified material concentrated 1,000-2,000-fold relative to the starting material.

Antigens and Antisera.--The preparation of murine gs antigen [MVP3(gs)], the major polypeptide (30,000 daltons), from disrupted MuLV by Sephadex G-100 gel chromatography and electrophoresis in ampholytes has been described (5), as has the preparation and specificity testing of rabbit anti-MVP3(gs) (6). Cell extracts were prepared by several methods designed to solubilize maximally the gs reactivity. Generally, cell monolayers were washed one to two times with phosphate-buffered saline, pH 7.2, and after removal with a rubber policeman, pre-

1 Abbreviations used in this paper: CF, complement fixation; gs, group specific; MuLV, murine leukemia virus; MVP3(gs), murine group-specific antigen; NRK, normal rat kidney.
pared as 20% (vol/vol) extracts. Subsequent treatments included two cycles of freezing at
-70°C and rapid thawing, treatment with 1% Triton X-100 and 0.7% deoxycholate in 2 M
KCl, followed by removal of the Triton with ether and dialysis to remove the salt and deoxy-
cholate. These procedures improved the yield of murine cellular gs reactivity by 40-60% over
repeated freeze-thawings comparable to the results previously reported by Fleissner (21) for
the extraction of avian gs antigen(s) from hamster cells.

Affinity Chromatographic Procedures.—The
method of preparation and coupling to Sepharose
of the goat IgG was that described previously for rabbit IgG (22). IgG was coupled to Sepha-
rose 4B using a ratio of Sepharose to protein of 30/1 (wt/wt); cell extracts were applied to the
column in 2-3 ml of buffer containing: 0.05 M Tris-HCl, pH 7.8; 0.30 M KCl; 2% Triton
X-100; and 10 mg/ml of bovine serum albumin (buffer A). After 5-10 min to allow for equil-
ibration, the column was washed with 10 ml of buffer A; followed by 10 ml of 0.05 M Tris-
HCl, pH 7.8, and 0.30 M KCl (buffer B); and eluted with 15-20 ml of 1.0 M NH4OH and
0.30 M KCl (solution C). Fractions of each wash (1.0 ml) were collected and were then dialyzed
overnight against 0.05 M Tris-HCl, pH 7.8. Concentration of pooled positive fractions to a
volume of 0.5-1.0 ml by ultrafiltration was performed in an Amicon concentration device
(Amicon Corp., Lexington, Mass.) using a PM-10 filter. Recovery of antigenic reactivity
through these procedures averaged 50% of the reactivity in the starting material in repeated
experiments. Regeneration of columns was as described (22).

Assays.—Protein
determinations were performed by the Lowry method using bovine serum
albumin (Pentex Biochemical, Kankakee, Ill.) as a standard (23). The radioimmunoprecipita-
tion assay for the murine intraspecies group-specific antigen and the preparation of 125I-labeled
MVP3(gs) were as described (6) with the following minor modifications. All dilutions were
made in 0.01% (vol/vol) Triton X-100 with 1% normal rabbit serum (6.8 mg/ml) as carrier
in 0.01 M potassium phosphate, pH 7.8. This concentration of rabbit serum was determined
by prior titrations to provide the optimal amount of carrier IgG for maximal precipitation of
marker [125I]rabbit IgG.

Data Analysis.—Dose-response curves generated by radioimmunoprecipitation inhibition
assays were normalized to 100% to facilitate intrassay comparability and linearized by logit
transformation (24). Regression analysis of linearized dose-response curves was by the un-
weighted least squares method and subsequent comparison of slopes and intercepts was done
using the standard Student’s t test. Computational analysis and data plotting were greatly
facilitated by a Honeywell 1340 computer programmed for radioimmunoassay by Steve
Watson, Meloy Laboratories, Springfield, Va.

Nucleic Acid Hybridizations.—The procedures employed for the synthesis of [3H]thymidine
DNA product from KiMuLV(Ki-MSV) virus preparations grown in normal rat kidney (NRK)
cells (17) and hybridization methods using S-1 nuclease are as described (25, 26). Over 70% of
the [3H]thymidine DNA product hybridized to 70S RNA from purified MuLV virions (26).

RESULTS

Measurement of Antigen in Murine Tissue Culture Cells.—By using the
radioimmunoassay, uninfected murine tissue culture cells were found to contain
measurable levels of antigen that cross-reacted with MuLV gs. Estimates of the
concentrations noted in several representative murine, rat, and human cell
systems with and without known exogenous virus infections are shown in Fig. 1.
The expressed values can be compared with one another; however, absolute
quantitation assumes that the antigen detected in cells is immunologically
comparable to purified MVP3(gs) since the antigen levels are determined by
reference to the 10% intercept on a linearized standard dose-response curve of
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Fig. 1. Dose-response curve of crude antigen preparations. 20% (vol/vol) cell extracts of NRK (O-O) (21.5 mg/ml), BALB/c 3T3 clone A31 (■-■) (6.2 mg/ml), NIH 3T3 (○-○) (9.0 mg/ml), and disrupted R-MuLV (▲-▲) (0.00052 mg/ml) were assayed at the dilutions indicated using radioimmunoprecipitation inhibition of [125I]MVP3(gs) as described (6, 7) with rabbit anti-MVP3(gs). Maximum cpm were 8,110 and in the presence of antibody without added unlabeled antigen, 2,268.

purified MVP3(gs) protein. Two cell lines producing murine leukemia virus, KiMuLV(Ki-MSV)-NRK and HEK-HRV, produce approximately 1,000 times more viral antigen per milligram of total cell protein than cultures not releasing virus. Since the MuLV measured grows in cells of heterologous species (rat and human), the reaction is virus specific.

Levels of antigen in contact-inhibited, virus-negative monolayer cultures shown in Table I ranged from 16 to 36 ng/mg protein. Levels varied from preparation to preparation; however, repeated determinations on the same preparation did not vary by more than 30% of the mean. It was of interest that several lines of non-producer-transformed cells derived by four separate methods, SV40 transformation, murine sarcoma virus transformation, X-irradiation-induced, or “spontaneous” transformation, had comparable levels of antigenic reactivity. This indicated that transformation per se, and particularly nonproducer “sarcoma” virus infection (17), does not result in significantly (<2-fold) increased levels of gs reactivity compared with untransformed cells.

Attempts to recover infectious virus from the cell lines listed in Table I other than the producer cultures have been negative. Similarly the cells have been regularly tested and found negative for viral RNA-instructed DNA polymerase and morphological type C particles by electron microscopy.

Several specificity tests of the radioimmunoassay reaction were performed. First, in the mouse species-specific (gs-1) assay, normal rat cells and human fibroblasts were negative, suggesting the reactivity is specific for mouse cells. Secondly, mouse cells were negative in species-specific radioimmunoassays for
TABLE I
Radioimmunoassay of Type C Antigenic Reactivity in Virus and Cell Extracts

| Species | Cell line or strain | Reference | Phenotype | Average concentration of reactivity* |
|---------|---------------------|-----------|-----------|------------------------------------|
| Mouse   | BALB/c 3T3 clone A31 (14) | Normal | 19        |                                    |
|         | SVA31 clone 5 (14)       | Transformed | 29        |                                    |
|         | KA31 (14)               | Transformed | 16        |                                    |
|         | R4 (15)                | Transformed | 22        |                                    |
|         | 3T12 line 3 (14)        | Transformed | 36        |                                    |
|         | NIH Swiss 3T3 (16)      | Normal    | 26        |                                    |
| Rat     | KNRK (17)              | Transformed | <1        |                                    |
|         | KiMuLV (MSV)-NRK (17)  | Transformed (virus producing) | 5,950 |                        |
|         | NRK (18)               | Normal    | <1        |                                    |
| Human   | HEK-HRV (19)           | Normal (virus producing) | 1,620 |                                    |
|         | Woolly monkey, infected |                       |           |                                    |
|         | M413B (20)             | Normal    | <1        |                                    |
|         | M413B (20)             | Normal    | <1        |                                    |

Assays were performed with twice frozen and thawed 20% cell extracts of antigen (vol/vol) as described (5, 6). Concentrations were determined by extrapolation to standard dose-response curves at the 10% intercept on a linearized scale normalized to 100% in the absence of added antigen.

* Based on at least two separate preparations.

the 30,000 dalton “gs” proteins of feline, woolly monkey (27), and RD114 (28, 29) type C viruses. The sensitivity of each of these assays in their particular homologous systems is comparable to or exceeds that of the mouse radioimmunoassay where 1–3 ng can be detected.

Detection of Antigen in Natural Tissues.—Studies of murine gs antigens in tissues that are free of demonstrable infectious virus by complement fixation and gel diffusion (30) provide another means of studying the specificity and distribution of the reactivity measured by radioimmunoassay in normal mouse cells. The C57 Leaden/Jax (L/J) strain has a very low incidence of tumors and rarely expresses antigen detectable by complement fixation until very late in life (H. Meier and R. J. Huebner, personal communication). Similarly, studies of BALB/c mice show an intermediate level of leukemias and lymphomas and infectious virus although antigen is usually not detectable by complement fixation before 6 mo of age (31). We examined different groups of male and female C57L/J at weaning (3 wk) and, as shown in Table II, readily found antigen in lymphoid tissues, kidney, and liver, but not in muscle. Antigen levels
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TABLE II
Detection of Cross-Reacting Murine gs Antigen in Tissue Preparations

| Species or strain | Organ       | Concentration range* |
|-------------------|-------------|----------------------|
|                   | Spleen      | 20–50 ng/mg protein  |
|                   | Thymus      | 4–15                 |
|                   | Kidney      | <1–24                |
|                   | Liver       | 22–36                |
|                   | Muscle (cardiac) | <1                 |
| C57L/J            |             |                       |
| BALB/c            | Spleen      | 12–330 ng/mg protein |
|                   | Kidney      | 5–52                 |
|                   | Muscle      | <1–12                |
| Feline            | Spleen      | <1                   |
| Human             | Spleen      | <1                   |

Weanling C57L/J mice kindly supplied by Dr. Hans Meier, Jackson Laboratory, Bar Harbor, Maine, were sacrificed by cervical dislocation, tissues were harvested, and 20% extracts (vol/vol) were prepared as described (36). Weanling and young adult (<6 mo) BALB/c mice were supplied by Mr. Sam Pulley, NCI, NIH; extracts from five tumored cats and from three human lymphoma patients were kindly provided through Doctors Murray Gardner and Brian Henderson, USC, Los Angeles, Calif.

* Based on at least three separate organs in each case.

in a given tissue were variable from mouse to mouse, but the lymphoid tissues (spleen and thymus) were consistently positive.

As in the tissue culture systems, BALB/c tissues were positive. The positives varied over a wider range than C57L/J tissues and were often higher; nevertheless, the patterns of tissue reactivity were comparable with the C57L/J in that the spleen was consistently positive and muscle tissue low or negative. Cat spleens [positive for feline VP3(gs)] and human spleens were negative in the mouse assay for the mouse VP3(gs). Positive murine tissues did not react in the feline, woolly, or RD14 gs protein assays but were positive for the interspecies (gs-3) antigenic reactivity (32) by radioimmunoassay (7, 8). This organ distribution of viral antigen coincides with the distribution of murine gs antigens detected by complement fixation (30). Thus it appears that the murine radioimmunoassay can specifically measure an antigenic reaction in tissues, as well as in cell culture systems, that is specific for murine tissues and shows a similar organ distribution to that noted later in life when the tissues have levels of antigen that can be detected with less sensitive methods.

Dose-Response Curve of Murine Cellular Reactivity.—As shown in Fig. 1, the addition of increasing amounts of NIH 3T3 or BALB/c 3T3 cells to the radioimmunoprecipitation competition assay resulted in a decreased number of [125I]MVP3(gs) counts being precipitated by specific antibody, indicating the
The presence of an unlabeled antigen. The displacement is only partial with unconcentrated cell extracts since the levels of antigen in uninfected cell extracts are relatively low. Fig. 1 demonstrates that the degree of displacement of labeled antigen is complete using disrupted MuLV.

The BALB/c 3T3 and NIH 3T3 mouse cell preparations, although differing slightly in their relative amounts of antigen, show a similar partial displacement with increasing concentrations. NRK does not displace labeled antigen at up to eight times greater concentrations of total cellular protein. Mixing experiments indicated that the NRK extracts do not contain an inhibitor that would interfere with detection of limiting amounts of MuLV gs antigen.

Antibody Consumption by Extracts of Normal Mouse Cells.—The radioimmunoprecipitation inhibition assay is a competition assay between the test material and the highly purified [125I]MVP3(gs) protein with limiting amounts of antibody. If the reaction noted with uninfected mouse cells in the mouse competition assay is specific for the MuLV virion protein, MVP3(gs), then tissue extracts should adsorb antibody prepared against purified MVP3(gs) protein. A representative antibody consumption assay with NIH 3T3 cell extracts and monospecific rabbit anti-MVP3(gs) sera is shown in Fig. 2. NIH 3T3 extracts specifically remove limiting concentrations of rabbit antibody against MVP3(gs) antigen. In the same experiment, quantitative studies with purified MVP3(gs) protein showed the antibody consumption assay easily detected 0.5–1.0 ng of antigen and was comparable to the sensitivity of the radioimmunoprecipitation inhibition assay (1–3 ng). Several of the murine cell lines described in Table I were tested in the antibody consumption test with results that were similar to those noted in the standard competition assays. NRK cells and woolly monkey type C virus-infected cells did not remove detectable antibody, and in parallel studies woolly type C virus antibody was not adsorbed by the murine cell extracts (NIH 3T3) or purified MVP3(gs) (data not shown). As a control for that system, woolly type C virus-infected cells removed antibody to the woolly gs protein with an efficiency comparable to that noted in the homologous system.

These results indicate that “normal” mouse cells specifically adsorb antibody to the MuLV group-specific protein and suggest that these cells, though containing no demonstrable infectious virus, are producing a cross-reacting antigen.

Immunoaffinity Chromatography of MVP3(gs) and Cross-Reacting Antigen.—To partially purify the cross-reacting antigen in NIH 3T3 mouse cells, an affinity chromatographic procedure similar to that described for the viral reverse transcriptase was employed (22). The antibody (IgG) coupled to Sepharose in this case was from a goat serum (IS-8) prepared against disrupted feline leukemia virus; by radioimmunoassay the serum contained a high antibody titer (1:32,000) to the interspecies reactivity of mammalian type C viruses (gs-3) (32). Since species-specific reactivity (gs-1) and mammalian interspecies reactivity (gs-1) are contained on the same polypeptide (33), such
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Fig. 2. Antibody consumption. A 1:5000 dilution of rabbit anti-MVP3(gs) (124 μg/ml) was mixed with equal volumes of extracts of NRK cells (○—○) (21.5 mg/ml), NIH 3T3 (●—●) (9.0 mg/ml), disrupted MuLV (Rauscher) (□—□) (0.00052 mg/ml), and NRK productively infected with KIMuLV(MSV) (■—■) (8.6 mg/ml). After 3 h of incubation at 37°C the mixtures were centrifuged at 100 g in an International PR-6 centrifuge (International Equipment Co., Needham Heights, Mass.) at 4°C. The supernatant fluid was removed and titrated for its ability to precipitate [125I]MVP3(gs) in a double antibody assay method (6) compared with untreated antibody (△—△).

A column was useful in purifying the reactivity [presumably mouse VP3(gs), i.e. gs-3] from the NIH 3T3 cells. To test the specificity of the immunoadsorbent for the mouse gs-1 protein, 125I-labeled murine VP3(gs) was applied either to the antibody column or to a column coupled to nonimmune IgG. The results are shown in Fig. 3. The labeled antigen binds to and can be eluted with 1 M NH₄OH from the IS-8-containing column but not to the column containing control IgG. Thus the immunoadsorbent can be used to bind gs antigen.

Immunological Comparison of Antigenic Reactivity in NIH 3T3 Cells with
FIG. 3. Immunoaffinity chromatography of ([125I]MVP3(gs). 0.13 ml of [125I]MVP3(gs) (5,900 cpm) was applied to a 1 ml anti-gs-3 IgG-Sepharose and a 1 ml nonimmune IgG-Sepharose column after having been equilibrated with buffer A. Column operations are as described in Materials and Methods. The first 5 fractions represent the buffer A wash, the next 3 the buffer B wash, and the last 10, the solution C eluate. Each fraction's volume was 1 ml. 1 ml of each fraction was counted in 10 ml Redi-Solv VI (Beckman Instruments, Inc., Fullerton, Calif.).

Known MuLV VP3(gs).—By employing concentrated material partially purified by adsorption to and elution from the anti-gs-3 column, it was possible to obtain a complete dose response curve of the NIH cellular antigen and purified MuLV VP3(gs) that subsequently had been similarly treated by adsorption to and elution from the same column. As shown by the linearized plots in Fig. 4, the slopes of the dose-response curves are parallel, indicating that the antigens are highly related immunologically. Without a precise estimate of the amount of NIH antigen, it however cannot be concluded the antigens are identical.

The slope of known MVP3(gs) was not affected by the treatments employed to prepare the NIH 3T3 cellular reactivity since the treated and untreated materials had the same slope and intercept. These data, combined with the antibody consumption data, support the conclusion that the murine cellular antigen is highly related to the virion VP3(gs) antigen. In preliminary studies using guanidine-HCl chromatography, the major cross-reactivity of reactive NIH antigen has approximately the same molecular weight as the virion antigen (30,000 daltons).

Detection of Viral-Specific RNA in Normal Mouse Cells.—An independent
approach to demonstrate the expression of subinfectious viral information in murine cells is molecular hybridization (34). By employing \([3^H]DNA\) synthesized from the viral RNA with endogenous viral RNA-instructed DNA polymerase, it is possible to detect significant amounts of virus-specific RNA by hybridization (Table III). Increasing concentrations of NIH 3T3 cellular RNA.

![Dose-response curves of NIH 3T3 antigen and MVP3(gs). NIH 3T3 clone A antigenic reactivity from approx. 2 × 10⁶ cells was purified by adsorption to and elution from an anti-gs-3 immunoadsorbent. Assays were performed as described (6, 7) and in the legend to Fig. 1. Maximum cpm were 4,150 and in the presence of antibody without added unlabeled antigen, 1,810. The percentage precipitation at each antigen concentration was determined in duplicate and assayed at the same time as purified MVP3(gs). Linearization and linear regression analyses were as described in Materials and Methods. The slope of the purified MVP3(gs) dose-response curve (not graphed) was 1.475 with a variance of 0.0001. The slope of the MVP3(gs) eluted from the immunoadsorbent (▲—▲) was 1.494 with a variance of 0.005. The slope of the NIH 3T3 cell antigen (●—●) was 1.456 with a variance of 0.006. The slope of the MVP3(gs) and the NIH 3T3 cell antigen appeared to be identical to the standard, \(T[10] = 0.258\) and \(T[10] = 0.426\), respectively.

| Total cellular RNA added | Zero time % | Hybridized % | Difference % |
|--------------------------|-------------|--------------|--------------|
| 100                      | 134         | 167          | 33           | 3            |
| 310                      | 172         | 248          | 76           | 6            |
| 530                      | 184         | 410          | 226          | 19           |

Total cell RNA was extracted using sodium dodecyl sulfate and phenol. Reaction mixtures contained 1,204 \([3^H]DNA\) TCA-precipitable cpm. The details of the RNA preparation, the \([3^H]DNA\) product, and the analysis of hybridization by S-1 nuclease are described elsewhere (26).
resulted in larger amounts (up to 19%) of labeled DNA being resistant to an enzyme (S-1 nuclease) that is specific for single-stranded nucleic acids. NRK cell RNA that were employed for growing the virus bound less than 2% of the labeled DNA at comparable levels of RNA. Other specificity tests and results in other cells are described in greater detail elsewhere (26).

Tsuchida et al. (34) have reported the presence of viral-specific RNA in an unusual virus-negative line of NIH Swiss murine cells (NIH-X), which has levels of MuLV gs antigen that are high enough to be detected by complement-fixation (CF) assay. Since the NIH 3T3 cell line we are studying has 10-50-fold less gs antigen than would be required for detection by CF, the detection of viral-specific RNA by hybridization strongly supports the view that viral genes are being expressed at very low levels in a subinfectious form in these cells.

DISCUSSION

The observation of an antigen in murine cells that is structurally and immunologically related to the type C viral group-specific (gs) antigen strongly suggests some degree of expression of at least one type C viral gene in normal murine cells. Various lines of evidence have already led to the conclusion that there is a latent type C viral genome in all rodent cells (5, 35). However, previous studies have generally implied that the genome in postembryonic cells is dormant until activated by age, chemicals, radiation, or other factors. The present findings in virus-negative strains of mice indicate at least a portion of the viral genome is expressed widely, suggesting that host cell repression of the viral genome is not complete. Whether the expression is somehow linked to the cell growth cycle is not known. Further, present data do not allow one to distinguish clearly between the possibility that the antigen being measured represents a low level of virus release or a selective expression of a particular viral gene product in the absence of virus production. The failure to isolate virus from the cells studied by the most sensitive biological assays (focus-forming assays from nonproducers, XC test, or viral polymerase-inducing ability) support the latter view.

Huebner et al. (36) have reported the presence of murine gs antigen in NIH Swiss mouse embryos by CF tests and demonstrated by gel diffusion its identity to virion gs protein. Since there is an apparent absence of either morphologically mature or infectious virus in these embryos, they postulated an association between factors controlling gs antigen expression and growth. Abelev and Elgort (37) have independently reported the presence of MuLV gs antigen in both embryonic and adult tissues from low leukemic incidence strains of mice such as C57BL/6 and BALB/c using indirect immunoautoradiography.

Unless some threshold phenomena exists, it seems likely that the presence of this particular viral structural gs protein is not in itself a "determinant" of cellular transformation since morphologically normal cells contain levels of
antigen comparable to those found in transformed tumorigenic cells in culture. Neither spontaneous transformation, transformation by DNA tumor viruses, nor radiation-induced transformation significantly increase the levels of expression of this viral gene product (Table I). Therefore, transformation per se does not necessarily increase the expression of all type C viral gene functions; however, the transforming functions may be coded for by other portions of the viral genome.

Since the increased expression of the viral gs antigen during embryogenesis qualifies it as an “embryonic” antigen, the existence of low levels of this antigen in adult mouse tissues has parallelisms with certain tumor-associated embryonic antigens (38–40). In mice, the loss of host cell repression of the synthesis of gs antigen has a strong correlation with an increased risk for the development of lymphoid tumors. Similarly, the levels of certain embryonic antigens is increased in the serum of animals or patients with certain types of tumors. If common biological mechanisms are involved in the regulation of these proteins and of transformation, questions concerning a viral etiology of tumors would involve another dimension. The existence of vertically transmitted viral genes (36) will require different approaches to establish cause and effect than those that have been previously applicable to epigenetic factors in disease.

Finally, the finding that host cell RNA from normal murine cells specifically hybridizes with murine type C viral DNA product provides an essential support for the finding of a viral protein, and vice versa. It would appear that a combination of both nucleic acid hybridization for the detection of viral-specific RNA and sensitive immunological assays for viral proteins might provide a stronger approach for detecting low levels of viral expression in other species than does either alone.

SUMMARY

A radioimmunoassay specific for a murine leukemia virus structural protein, the gs antigen, detects an antigenic reactivity in normal murine cells in culture and natural tissues. The assay was shown to measure an antigen that is highly related to the virion protein as shown by absorption tests, immunoadsorbent chromatography, and by analysis of linearized dose-response curves. These findings combined with the finding of viral-specific RNA indicate that portions of the viral genome are being expressed with a much greater frequency than previously appreciated.

The authors appreciate the excellent technical assistance of Lou Noon, Lois Saerxner, and Cyndy Watson. Dr. Robert J. Huebner kindly provided several helpful discussions. This work was partially supported by contract NIH-NCI-E-72-2006 to Meloy Laboratories, Springfield.

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