Detection of Infectious Centers in C57BL/Ka Lymphoid Cell Populations Infected in Vitro by the Radiation Leukemia Virus

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ABSTRACT

The cocultivation of nonproducer lymphoma cells derived from a radiation-induced lymphoma of the C57BL/Ka mouse with cultures of lymphoid cell populations from the thymus, spleen, and marrow of the same strain 48 hr after their infection by the C57BL/Ka leukemia viruses permits the detection of infectious centers in these cultures. A quantitative assay is described which allows the estimation in lymphoid cell subpopulations of the numbers of target cells susceptible to productive infection by the thymotropic and leukemogenic viruses of C57BL/Ka mice in vitro. This assay should greatly facilitate the identification and characterization of such target cells.

INTRODUCTION

RadLV,4 derived from radiation- and virus-induced thymic lymphomas of C57BL/Ka mice (2), and RadLV/LTC, the virus produced by lymphoma cell lines in vitro (3, 17), are both L+ in this strain of mice (2, 3, 7). They are also designated as T+ because virus expression occurs selectively in the subcapsular area of the thymus after i.v. inoculation into C57BL/Ka hosts (7, 8). RadLV and RadLV/LTC can be assayed in vivo on the basis of their thymotropic and leukemogenic activity (2, 3, 5). They can also be assayed in vitro due to their ability to act as helper viruses in the murine sarcoma virus focus assay (1, 11) and to their ability to replicate in nonproducer lymphoma cell lines of C57BL/Ka origin (19). We now present an indirect cocultivation assay which reliably leads to the detection of infectious centers in cultures of lymphoid cell populations from the thymus, spleen, and marrow of C57BL/Ka mice after infection and cultivation of such cells in vitro. From a practical standpoint, the present procedure greatly facilitates in vitro studies on the identification and characterization of the target lymphoid cell populations susceptible to productive infection by the T+L+ viruses of C57BL/Ka mice.

MATERIALS AND METHODS

Mice. Four-week-old female C57BL/Ka (Fv-1b+) inbred mice and NIH/Swiss (Fv-1+) random-bred mice were obtained from our colony. Viruses. RadLV/VL3, a T+L+ virus biologically and serologically identical to RadLV (3), was obtained from the culture fluids of the BL/VL3 cell line derived from a RadLV-induced C57BL/Ka thymic lymphoma (17). The RadLV/VL3 preparation used in these experiments had a titer of 2.5 x 104 infectious units/ml as estimated by the immunofluorescence assay in vivo (5).

BL/Ka(B) is a cloned B-fibrotropic virus isolated from C57BL/Ka fibroblast cultures (2). This virus is nonthymotropic and nonleukemogenic (2, 4). The isolate used in these experiments had a titer of 2.5 x 106 infectious units/ml as estimated by immunofluorescence (6) and reverse XC assay (26) in vitro.

Cells. The BL/RL12-NP lymphoma cell line was derived from a radiation-induced C57BL/Ka thymoma. These cells do not produce infectious viruses and are negative for the presence of cytoplasmic or membrane viral antigens, as analyzed by immunofluorescence and radioimmunocompetition assay. They are permissive for infection by both RadLV/VL3 (17) and BL/Ka(B) (18). The BL/RL12-NP cell line is maintained in Eagle’s MEM supplemented with 10% FCS, penicillin (100 units/ml), and streptomycin (100 μg/ml).

Cell Suspension and Short-Term Cultures. Thymuses and spleens were minced with scissors in PBS (Grand Island Biological Co., Grand Island, N.Y.) and maintained in culture at 37° in a 5% CO2 incubator. The tubes were shaken every 1@.

Infection Procedure and Culture Conditions for the BL/RL12-NP Cocultivation Assay. Unless otherwise stated, thymus, spleen, and marrow cell suspensions were first incubated in RPMI medium 1640 containing 5% FCS and DEAE-dextran (25 μg/ml) (30) for 10 min at 4° for 10 min. The cells were then resuspended at a concentration of 2.5 x 105/ml in RPMI Medium 1640 containing 5% FCS.

Infection Procedure and Culture Conditions for the BL/RL12-NP Cocultivation Assay. Unless otherwise stated, thymus, spleen, and marrow cell suspensions were first incubated in RPMI medium 1640 containing 5% FCS and DEAE-dextran (25 μg/ml) (30) for 15 min and centrifuged at 1000 rpm at 4° for 10 min. The cells were then resuspended at a concentration of 2.5 x 105/ml in RPMI Medium 1640 containing 5% FCS. The cells were then resuspended at a concentration of 2.5 x 105/ml in RPMI Medium 1640-5% FCS containing polybrene (4 μg/ml) (29) and mixed with 1 ml of undiluted RadLV/VL3 in a 5-ml plastic tube before incubation for 2 hr at 37° in a 5% CO2 incubator. The tubes were shaken every 15 min to prevent cell sedimentation. After infection, the cells were spun at 1000 rpm at 4° for 10 min and resuspended in RPMI Medium 1640 supplemented with 10% heat-inactivated FCS, antibiotics, 2 mm L-glutamine, 1 mm sodium pyruvate, 1% nonessential amino acids, and 50 μM 2-mercaptoethanol. The cells were plated in 35-mm dishes (Lux Scientific Corporation, Newbury Park, Calif.) and maintained in culture at 37° in a 5% CO2 incubator.

In a typical cocultivation assay, the live cells were counted...
by the trypan blue exclusion test 48 hr after infection. The cell suspensions were then spun down for 10 min at 1000 rpm in the cold and resuspended in Eagle's MEM with 10% FCS and antibiotics to obtain a final concentration of $1 \times 10^7$ cells/ml. These suspensions were then irradiated with 5000 R in a $^{137}$Cs irradiator (Mark I, Model 30; Shepherd and Assoc., Glendale, Calif.). Serial dilutions of the individual thymus, spleen, and marrow cell preparations were made in 1 ml of medium and then cocultivated, in triplicate, in 35-mm dishes with $2 \times 10^6$ BL/RL12-NP cells suspended in 1 ml of Eagle's MEM supplemented with 10% FCS and antibiotics. These cocultures, in which only the unirradiated BL/RL12-NP indicator cells survive, were passaged every 3 days at a concentration of $2 \times 10^6$ cells/35-mm dish. At 3 and 10 days after cocultivation, they were analyzed by IF for the presence of CVA (6). In some instances, the BL/RL12-NP cocultivation assay was performed in microtiter plates (Falcon Microtest II Tissue Culture Plate 3040, Lid 3041; Falcon Plastics, Los Angeles, Calif.) in which 0.1 ml of various lymphoid cell concentrations was mixed with 0.1 ml of BL/RL12-NP cells at a concentration of $2 \times 10^5$ cells/ml.

RESULTS

Detection of Infectious Centers by Cocultivation of Thymic Lymphocyte Cultures with BL/RL12-NP Lymphoma Cells. Preliminary experiments were first designed to determine whether virus production by lymphoid cells could be detected by cocultivation in vitro with BL/RL12-NP indicator cells. Cell suspensions containing from 1 to 100 IF-positive thymocytes obtained from a C57BL/Ka mouse thymus 3 weeks after intrathymic inoculation of $10^6$ infectious units of RadLV/VL3 were mixed with normal thymocytes obtained from a normal weanling C57BL/Ka mouse to yield total cell suspensions with concentrations ranging from $10^5$ to $10^7$ cells/ml of tissue culture medium. These samples were then irradiated with 5000 R and mixed in 35-mm dishes with 1 ml of a suspension containing $2 \times 10^6$ BL/RL12-NP cells. Triplicate dishes for each experimental point were then passaged 3 times and maintained in culture for a total of 10 days, at which time the BL/RL12-NP indicator cells were processed for detection of CVA by IF. The data (not shown) of these experiments indicate that this procedure permits the detection of 1 virus-positive cell in $10^2$ to $10^6$ thymocytes. A 5- to 10-fold decrease of sensitivity was observed when thymocyte populations larger than $10^5$ cells/ml were mixed with the indicator cells. This could be explained by the observation that the growth of BL/RL12-NP cells is significantly reduced when the ratio of lymphoid cells to BL/RL12-NP cells is greater than 5.

In the next experiment, thymus cell suspensions obtained from normal weanling C57BL/Ka mice were infected in vitro with $2.5 \times 10^5$ infectious units of RadLV/VL3 and kept in culture for 3 days. Cell viability decreased from 80 to 90% after 24 hr in culture to about 50% after 48 hr and less than 25% after 3 days (Chart 1A). In contrast, DNA synthesis, as measured by tritiated thymidine uptake, decreased sharply immediately after the cells were seeded in vitro (Chart 1B). These data, which are in agreement with those observed by others (12), also showed no difference between the infected and noninfected thymocyte cultures in either cell survival rate or thymidine uptake.

In the experiment shown in Table 1, various concentrations...
of thymic lymphocytes from weanling C57BL/Ka mice were seeded in 35-mm dishes in 1 ml tissue culture medium and incubated with an equal volume of a RadLV/VL3 preparation containing 2.5 x 10^7 infectious particles. Some culture dishes were seeded with virus alone. After the virus infection procedure was completed, one-half of the cell cultures for each experimental point were irradiated with 5000 R in order to prevent cell division and active virus replication. The irradiated and unirradiated cultures were then returned to the incubator and maintained in culture for 1 to 4 days. At each time interval, 3 dishes per point were irradiated with 5000 R. Then 0.1 ml of a BL/RL12-NP cell suspension containing 2 x 10^6 cells/ml was added to each culture and to the dishes containing virus alone. The BL/RL12-NP indicator cells were examined by IF 10 days later. The IF positivity observed in the indicator cells plated at the 1-day interval is attributed either to free virus present in the culture fluid or to virions passively absorbed on carrier cells, since the BL/RL12-NP cells became infected in both the irradiated and unirradiated cultures as well as in dishes which contained only virus (Table 1). In accordance with a previous report from this laboratory (25), RadLV/VL3 maintained in tissue culture medium for 2 days was no longer infectious. BL/RL12-NP indicator cells added 48 hr after infection and irradiation of the thymocyte cultures did not reveal viral antigens, whereas these cells became IF positive when added to infected nonirradiated thymus lymphocyte cultures at the 48-hr interval, which strongly suggests that the detection of virus can be attributed to the presence of infectious centers in such cultures.

We next proceeded to exclude the possibility that passive absorption of viral particles by carrier cells might be responsible for infection of the detector cells at the 48-hr interval in the cocultivation assay. Since Fv-1 restriction is not related to virus absorption or penetration but rather involves an intracellular event (15, 28), C57BL/Ka thymocytes (Fv-1'') and NIH/Swiss thymocytes (Fv-1'''') were both infected in vitro with RadLV/VL3 (which is B-tropic) and then assayed in the cocultivation assay with BL/RL12-NP detector cells. The differentiation restriction system was also used in this experiment. As demonstrated earlier in this laboratory (2, 4), normal thymocytes from weanling mice can undergo productive infection by T+ virus but not by the fibrotropic-ecotropic isolates from strain C57BL/Ka. Therefore, C57BL/Ka thymocytes were also infected with BL/Ka(B), a B-tropic fibrotropic-ecotropic virus. As in the previous experiment, indicator cells were added to culture dishes that contained only BL/RL12-NP cells. The presence of IF-positive cells in the indicator cell cultures was determined 10 days after cocultivation.

Table 1: Virus detection in thymocyte cultures at intervals after infection of various numbers of thymocytes with RadLV/VL3

<table>
<thead>
<tr>
<th>No. of thymocytes at time of infection</th>
<th>Infected +</th>
<th>Infected +</th>
<th>Infected +</th>
<th>Infected +</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 day</td>
<td>2 days</td>
<td>3 days</td>
<td>4 days</td>
</tr>
<tr>
<td></td>
<td>only R</td>
<td>only R</td>
<td>only R</td>
<td>only R</td>
</tr>
<tr>
<td>5 x 10^7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 x 10^7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>1 x 10^6</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 x 10^5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1 x 10^4</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>None (virus only)</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* The thymocyte cultures were infected with 2.5 x 10^7 infectious units of RadLV/VL3, and one-half of the cultures were then immediately irradiated with 5000 R.

† At each time interval, the infected cultures were irradiated with 5000 R and then cocultivated with 2 x 10^6 BL/RL12-NP cells. The presence of IF-positive cells in the indicator cell cultures was determined 10 days after cocultivation.
fibroblasts (26) and BL/VL3 cells in the XC cell assay (17),

though the cell is no longer able to divide.

do not prevent it from acting as an infectious center even

acting as infectious centers in the BL/RL12-NP cocultivation

per centage of CVA-positive BL/RL12-NP cells was scored by IF at

cocultivation. Representative curves obtained with infected

cells were harvested and either used for the detection of

cytoplasmic viral antigens by indirect immunofluorescence or

and thoroughly washed before the addition of the indicator cells.

Thymocytes cultures were irradiated at a dose of 5000 R immediately

after 2 hr of incubation with RadLV/VL3.

Detection of Virus Producer Target Cells after in Vitro

Infection by RadLV/VL3. Results are expressed as the percentage of IF-positive

BL/RL12-NP indicator cells 5 ( ) and 16 ( O) cell doublings after cocultivation

with various concentrations of irradiated (5000 R) thymocytes.

Table 2

Virus detection by BL/RL12-NP detector cells plated 48 hr after inoculation of

culture dishes with virus, cells, or both

<table>
<thead>
<tr>
<th>Thymocytes</th>
<th>Virus type</th>
<th>Additional treatment</th>
<th>% of IF-positive detector cells 15 cell doublings after plating</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>RadLV/VL3</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>BL/Ka(B)</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>C57BL/Ka (Fv-1+)+</td>
<td>RadLV/VL</td>
<td>None</td>
<td>90</td>
</tr>
<tr>
<td>C57BL/Ka (Fv-1+)+</td>
<td>RadLV/VL</td>
<td>Trypsin</td>
<td>90</td>
</tr>
<tr>
<td>C57BL/Ka (Fv-1+)+</td>
<td>RadLV/VL</td>
<td>Irradiation</td>
<td>0</td>
</tr>
<tr>
<td>C57BL/Ka (Fv-1+)+</td>
<td>BL/Ka(B)</td>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>NIH/Swiss (Fv-1+)+</td>
<td>RadLV/VL</td>
<td>None</td>
<td>0</td>
</tr>
</tbody>
</table>

* RadLV/VL3 (5 x 10^6 infectious units/ml) and BL/Ka(B) (1 x 10^6 infectious units/ml) were maintained at 37° in 2 ml of tissue culture medium for 2 days in 35-mm tissue culture dishes prior to the addition of these dishes of 2 x 10^6 indicator cells in 0.1 ml of medium.

Thymocytes (10^6) were incubated with the concentration of virus isolates indicated in Footnote a and maintained in culture for 2 days prior to irradiation with 5000 R and addition of BL/VL3 cells in 0.1 ml of medium.

Two-day-old cultures were treated with 0.05% trypsin and EDTA for 5 min and thoroughly washed before the addition of the indicator cells.

Thymocyte cultures were irradiated at a dose of 5000 R immediately after 2 hr of incubation with RadLV/VL3.

Chart 2. Detection of infectious centers in thymocyte cultures 2 days after infection by RadLV/VL3. Results are expressed as the percentage of IF-positive BL/RL12-NP indicator cells 5 ( ) and 16 (O) cell doublings after cocultivation with various concentrations of irradiated (5000 R) thymocytes.

Table 3

Number of infectious centers detected by the BL/RL12-NP cocultivation assay at 48 hr in cultures of RadLV/VL3-infected cell suspensions from thymus, marrow, and spleens of weanling C57BL/Ka mice

<table>
<thead>
<tr>
<th>Cell types</th>
<th>% of IF-positive cellsa</th>
<th>No. of infectious centers/cell</th>
<th>total no. of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal thymocytes</td>
<td>&lt;0.1</td>
<td>1/3 x 10^5-2 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Corticosteroid thymocytes</td>
<td>ND</td>
<td>1/3 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Normal marrow cells</td>
<td>&lt;0.1</td>
<td>1/1 x 10^5-1 x 10^6</td>
<td></td>
</tr>
<tr>
<td>Normal spleen cells</td>
<td>&lt;0.1</td>
<td>1/1 x 10^6</td>
<td></td>
</tr>
</tbody>
</table>

* Percentage of CVA-positive cells detected by IF.

0 The variations observed from one experiment to another can be attributed to variations in the number of target cells in the various cell samples at time of infection (±5%).

0 The animals were treated with 5 mg of cortisone sodium succinate (Solu-Cortef; Upjohn Co., Kalamazoo, Mich.) 2 days prior to sacrifice.

0 ND, not detectable.

cells contain similar numbers of target cells (1:1,000,000 to 1:200,000) for productive infection by RadLV/VL3, whereas only 1 cell in 3 x 10^6 corticosteroid thymocytes is susceptible to productive infection by this T+L+ virus.

The progeny virus released into the culture fluids of the IF-positive indicator cells at 15 doubling times after cocultivation with these thymus, marrow, and spleen cultures was tested in vivo (5) for thymotropic activity (5 to 10 mice/point) and was found to be highly thymotropic in all cases. Thus, the virus released by the infectious centers present in these RadLV/
VL3-infected cultures retains this biological property of the parental virus.

**DISCUSSION**

The isolation, identification, and characterization of target cells for infection and transformation by RadLV has been of great interest to laboratories studying the pathogenesis of thymic lymphomas in C57BL/Ka mice. Previous experiments have demonstrated that lymphoid cells isolated from the spleen and marrow of weanlings, the thymus of neonates, and the fetal liver of C57BL/Ka mice can be infected in vitro with RadLV and can then give rise to thymomas of donor origin when inoculated into C57BL × BALB/c F1 hosts immediately after infection (16, 21). Other in vivo experiments have shown that i.v. inoculation of RadLV into young adult C57BL/Ka mice results in virus production by thymocytes present in the subcapsular region of the thymus (8). Such studies would be greatly facilitated by the availability of an entirely in vitro assay which would allow the cultivation of C57BL lymphoid cells from various tissues, infection of these cell populations by T+L+ viral isolates from that strain, detection of the virus producer cells in such cultures, and finally the isolation and characterization of these target cells. The XC assay and the direct assay of viral antigens in lymphoid cultures by immunofluorescence have been of little use for the detection and reliable quantitation of cells which have been productively infected among the total cell population of cultivated lymphocytes. Indeed, it has been demonstrated that virus-related antigens can appear in the cytoplasm of infected thymic lymphocytes concomitantly with cell death (22) and that virus-producing cells present in RadLV-induced lymphomas in vivo do not always induce plaque formation in the rat XC cell assay in vitro (17). The BL/RL12-NP cocultivation assay 48 hr after infection of the target lymphoid cell population by a T+L+ virus is thus the first quantitative assay by which productive in vitro infection can be detected in thymus, bone marrow, and spleen cell populations maintained in short-term culture.

The data clearly show that infection of the indicator cells is initiated by virus-producing cells (infected centers) which have undergone one or 2 divisions. Infection is not attributable to free virus surviving in the culture fluids or to virus particles passively adsorbed on cell membranes 48 hr after virus inoculation. The assay can detect a single infectious center among 10² to 10⁶ lymphoid cells, provided that a ratio of infectious particles to total cell number greater than 0.1 is used in the infection procedure. It can thus be concluded that any single cell producing RadLV-like virus in the culture will be detected by the assay, as a consequence of the remarkable permissiveness of BL/RL12-NP cells for infection by RadLV (19).

In the experiments described in this report, infectivity has been examined in thymus, spleen, and bone marrow cultures under conditions in which many of the cells were rapidly dying. Cell growth parameters, which are better documented for the thymocyte cultures than for the bone marrow and spleen cultures, show that DNA synthesis occurs only during the initial 24 hr of a thymocyte culture. Since dividing thymocytes have an average doubling time of 10 to 12 hr (23), one can assume that thymocytes could have undergone a maximum of 2 cell divisions during the cultivation period. Therefore, under optimal conditions, one could expect that each target cell for productive infection by RadLV could give rise to a maximum of 4 progeny cells after 24 hr in culture (at the end of the period of cell replication), and thus to 4 infectious centers detectable by the cocultivation assay performed 48 hr after the initiation of the cultures. However, because of the high level of cell death in this culture system, it is likely that the number of infectious centers derived from each target cell ranges between 1 and 4 at the time of the assay.

It would be more convenient to use an in vitro system in which the pattern of cell proliferation could be controlled and quantitated accurately. However, to our knowledge, there is no efficient way to maintain the proliferation of immature lymphoid populations in vitro. For example, cocultivation of thymocytes with thymus reticuloepithelial cells does not significantly improve cell survival, although it induces thymocyte maturation (24). On the other hand, mitogens and T-cell growth factors rapidly modify the differentiation patterns of prothymocytes and thymocytes in vitro (9, 14, 27) and thus select for mature T-cell subpopulations, which would not necessarily retain the same susceptibility to infection by RadLV. Preliminary experiments have shown that the use of such growth factors does not increase the number of infectious centers detectable in short-term cultures of thymocytes after infection with RadLV.

At the present time, therefore, the in vitro system described in this report seems to be the only available method for estimating the frequency of target cells permissive for productive infection by RadLV in lymphoid or hematopoietic cell populations, although the kinetic limitations discussed above must be kept in mind. For example, if the true abundance of target cells in a thymocyte culture were 1/3 × 10⁶, then, considering the above-cited estimate that such target cells could give rise to from 1 to 4 progeny cells at 48 hr, the cocultivation assay might yield frequency estimates ranging from between 1 infectious center/3 × 10⁴ to 1 infectious center/1.2 × 10⁵ cells. It should be noted that the experiments actually gave somewhat lower estimates (1 infectious center/2 × 10⁵ to 1/8 × 10⁵). If similar calculations are applicable to normal bone marrow cultures, the numbers of bone marrow cells susceptible to RadLV infection in vitro would range between 1/1 × 10⁴ and 1/4 × 10⁵ cells.

It is of interest that the estimates thus obtained from these in vitro studies are in good agreement with previous in vivo observations which indicated that 1 in 2 to 4 × 10⁴ thymus and 1 in 1.5 × 10⁴ to 2 × 10⁵ bone marrow cells could serve as target cells for RadLV in C57BL/Ka mice (4, 16). In light of these small numbers of primary target cells for RadLV infection, one can now readily explain the earlier observation that it requires at least 4 days after RadLV inoculation into mice before CVA-positive cells can be detected (7, 8). Indeed, these estimates have been recently confirmed in vivo; using the BL/RL12-NP cocultivation assay, it was found that as few as 1 in 10⁴ to 1 in 10⁵ thymocytes produce detectable virus 2 days after direct intrathymic injection of RadLV, in excellent agreement with the above-cited in vitro and in vivo data. Conversely, these results are incompatible with a published claim by Haas and Kaplan.

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and Hilgers that 80% of C57BL thymocytes are productively infected by RadLV after 48 hr in culture (10). This finding, which has not been confirmed in our laboratory (19), is in addition not supported by the growth kinetics of thymic lymphocytes in culture (Ref. 13; this paper) or by studies on the in vivo replication of RadLV in C57BL mice (4, 5, 7, 8).

The numbers of infectious centers detected by the BL/RL12+ NP cocultivation assay in thymus, bone marrow, and spleen cultures are compatible with the hypothesis that the normal target cell for infection and possibly for transformation by RadLV belongs to a restricted subpopulation which might be, as suggested by previous in vivo studies, a bone marrow or spleen prothymocyte (12, 16) or a subset of the subcapsular blast cell population in the thymus (8). The present availability of the assay described in this report allows one to test this hypothesis by using techniques of enrichment for various lymphoid cell populations from marrow, spleen, or thymus before in vitro infection with RadLV/VL3. Also, it will be possible to analyze the interaction of RadLV with non-T-cell populations. These experiments, which are now in progress in our laboratory, will hopefully provide us with enriched target cell populations for RadLV infection; these populations could then be tested for neoplastic transformation either in vivo, using the bioassay described by Lieberman and Kaplan (16, 21), or in vitro by cultivation in the presence of thymus reticuloepithelial cells and/or growth factors.

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