Isolation and Development of a Reticuloendotheliosis Virus-Transformed Lymphoblastoid Cell Line from Chicken Spleen Cells†

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The establishment and characterization of a reticuloendotheliosis virus (strain T)-transformed lymphoblastoid cell line, designated TV-1, was reported. These cells, isolated from the spleen of a moribund chick infected with reticuloendotheliosis virus, were maintained in suspension culture for over 74 weeks at a stable generation time of 15 h. The cells were found to carry a female karyotype. Both TV-1 cells and cell-free TV-1 culture supernatant produced lesions and mortality patterns in chicks which were identical to those caused by reticuloendotheliosis virus (strain T) infection. Examination of TV-1 cells by electron microscope revealed the presence of C-type virions budding from the plasma membrane. Cytotoxicity assays and fluorescent antibody tests indicated the presence of B-cell determinants on the TV-1 cell surface membrane.

The T-strain of a reticuloendotheliosis virus (REV-T) was first described by Sevoian et al. (12) as an avian leukosis virus with high lethality and a short incubation period when injected into various genetic lines of chickens. REV-T was then originally cultured from spontaneous lymphomas in adult turkeys (11) and was later studied in Japanese quail and chickens (17). REV-T was then characterized as antigenically distinct from other known avian leukemia viruses (13), although they share a C-type morphology. REV-T is now considered a prototype of the reticuloendotheliosis group of viruses (9) and is the only virus of this group proven capable of inducing a neoplastic disease (2). Although REV-T-transformed cells were shown to be present in livers and spleens of REV-T-infected chickens (1), attempts to culture these cells in vitro were unsuccessful until Franklin et al. (2) maintained a culture of the bone marrow cells of an REV-infected chick.

We now report the in vitro establishment and characterization of a REV-T-transformed lymphoblastoid cell line from the spleen of a moribund SPAFA (SPAFAS, Inc., Norwich, Conn.) chick infected with REV-T.

MATERIALS AND METHODS

Cell line establishment. REV-T-infected cells were obtained from the dissociation of a splenic lymphoma in a Ten-Broek grinder. The splenic cells were washed twice with phosphate-buffered isotonic saline (PBS) (pH 7.2) before being layered on Ficoll-Paque (Pharmacia Fine Chemicals, Piscataway, N.J.) for separation of the buffy coat. The separated cells were then washed three times in PBS and suspended in culture medium RPMI 1640, supplemented as previously described (3), to a cell density of 5 x 10⁶ to 1 x 10⁶ cells per ml. The cells were incubated in 50-ml volumes in glass bottles at 37 and 41°C in a 5% CO₂ in-air atmosphere. Cultures were transferred to fresh glass bottles after 2 days of incubation. Thereafter, the top 5% of the culture medium was aspirated and replaced with fresh medium every 2 to 3 days or when a pH change was evident.

Growth studies. REV-T-transformed cells were suspended in fresh RPMI 1640 medium at a density of 5 x 10⁵ cells per ml. To determine cell replication and viability, assays were performed daily by treating 0.5 ml of cell suspension with trypan blue and counting the viable cell population on a hemacytometer.

Karyotyping. Air-dried chromosome preparations of REV-T-transformed cells were made by a modification of the technique of Goh (4). Cells (3 x 10⁵) were subjected to colchicine arrest (10⁻⁶ M/ml for 4 h), to a hypotonic solution (3:1, distilled water-RPMI 1640) treatment for 20 min, and then to three 10-min treatments in freshly prepared fixative (3:1, methanol-acetic acid). The final cell suspensions in fixative were dropped onto glass slides previously cleaned with acetone and wetted with distilled water. When the slides were completely air-dried, C banding patterns were induced by the alkaline-SSC method of Stefos and Arrighi (14).

Electron microscopy. Lymphoblastoid cell pellets obtained from 30-ml samples of REV-T culture (10,000 rpm, 10 min) were suspended in 3 ml of 1% glutaraldehyde (0.2 M sodium cacodylate, pH 7.3) for 2 h,
washed in 0.05 M sodium cacodylate (pH 7.3), and postfix in 1% osmium tetroxide (0.2 M sodium cacodylate, pH 7.3) for 2 h. After the suspended cells were washed with 0.05 M sodium cacodylate, centrifuged pellets were enrobed in 1 ml of 2% Noble agar, dehydrated in graded ethanols, and embedded in Epon 812. Silver sections were cut with a diamond knife on an LKB Ultratome III ultramicrotome and mounted in 400-mesh copper grids. The sections were stained for 15 min with 5% (wt/vol) uranyl acetate, followed by 15 min with 0.4% (wt/vol) lead citrate. The stained sections were immediately examined with a Zeiss EM 9S-2 electron microscope at an accelerating voltage of 60 kV.

### Biological assay of cells and cell-free supernatant from REV-T-transformed cell line

Titrations of REV-T-transformed cells and cell-free culture supernatant were assayed in day-old chicks. Ten-fold serial dilutions in PBS were prepared from REV-T-transformed cells (10⁶ per ml) or from the culture supernatant, which had been filtered through a 0.22 μm BioRad filter (BioRad Laboratories, Richmond, Calif.). Five birds were inoculated intraperitoneally with 1 ml of each dilution and maintained for 4 weeks in Horsfall isolation units. Chicks inoculated with culture supernatant dilutions were challenged 4 weeks postinoculation with 0.5 ml of liver and spleen homogenates from a moribund chick infected with REV-T.

Five birds per trial were maintained in separate units as uninoculated controls. All dead birds were necropsied and examined for gross lesions. Birds that died within 5 days postinoculation or had no gross lesions of the liver or spleen upon necropsy were considered nonspecific for T-virus. The 50% lethal dose (LD₅₀) and 50% protective dose were calculated by the method of Reed and Muench (10).

### Antisera production

B-cell and T-cell antisera were produced in New Zealand white rabbits by giving rabbits 3 to 10 inoculations of bursal or thymic cell suspensions (5 × 10⁶ to 10 × 10⁶ cells per ml) from 3- to 4-week-old chickens at 6- to 10-day intervals. Pooled anti-T or anti-B sera from weekly bleedings were inactivated at 56°C for 30 min and absorbed twice with chicken liver and up to six times with either thymic or bursal cell homogenates. In addition, portions of T-cell and B-cell antisera were reabsorbed with either thymic or bursal cell homogenates, respectively, to insure specificity in fluorescent antibody and cytotoxicity assays.

REV-T was isolated from the plasma of freshly drawn heart blood (in 0.02 M ethylenediaminetra-acetic acid) from moribund REV-T-infected chicks. The plasma was centrifuged twice at 2,000 × g for 15 min to ensure complete separation from residual cells, then centrifuged at 80,000 × g for 1 h in a Beckman model L Ultracentrifuge (Beckman type SW50L rotor). The virus pellets were suspended in 1 ml of Freund complete adjuvant and inoculated into New Zealand white rabbits. The rabbits were boosted twice with REV-T that had been obtained as described above and suspended in 1 ml of PBS. Serum was pooled from weekly bleedings, inactivated at 56°C for 30 min, and extensively absorbed with chicken thymus, bursa, spleen, and liver tissue.

Antimacrophage serum was prepared and kindly provided by Keith Haffer of this department.

Marek's disease-associated tumor-specific antigen antisera was produced in chickens inoculated with JMV-1 (7), a nonproductive Marek's disease herpes-virus-transformed T-cell line, and Marek's disease virus (MDV) antisera was obtained from chickens which were naturally infected with MDV by contact exposure. Both antisera were prepared and kindly provided by David C. Munch of this department.

### Fluorescent antibody staining

REV-T-transformed cells were stained with T-cell, B-cell, REV-T, and macrophage antisera by a modification of the indirect technique of Möller (6) using fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin G (Miles Labs, Inc., Kankakee, Ill.). In addition, REV-T-transformed cells were stained directly with FITC-protein A (Pharmacia Fine Chemicals, Piscataway, N.J.), diluted 1:2, to specifically stain for immunoglobulin G. Thymic and bursal cells and JM-1 cells, from a productive Marek's disease herpes-virus-transformed T-cell line (JM strain) (7) were included in all trials to distinguish nonspecific from specific staining reactions.

REV-T-transformed cells were also examined by immunofluorescent staining for possible contamination with both MDV and avian leukosis virus.

A 15-ml sample of 5 × 10⁶ viable REV-T-transformed cells per liter was treated for 24 h with 12.5 μg of iododeoxyuridine (Sigma Chemical Co., St. Louis, Mo.) to induce MDV antigens. Iododeoxyuridine-treated and nontreated cells were acetone fixed overnight at −20°C and then stained for the presence of MDV. Non-acetone-fixed cells were stained with Marek's disease-associated tumor-specific antigen antisera. All staining was performed by the indirect technique of Möller (6) using FITC-conjugated rabbit anti-chicken immunoglobulin G (Nutritional Biochemicals Corp., Cleveland, Ohio). Controls included JMV-1, JM-1, and purified spleen cells from non-JM-exposed chicks kept in Horsfall isolation units.

To detect the presence of avian leukosis virus, REV-T-transformed cells, Rous sarcoma tumor cells from infected chicks, and chicken embryo fibroblast (CEF) cultures from SPF RA chicks were stained indirectly with Rous sarcoma (Schmidt-Ruppin strain) hamster tumor antisera (1:20; Microbiological Associates, Bethesda, Md.) and then by FITC-conjugated anti-hamster immunoglobulin G (Rb) (1:16; Miles-Yeda, Ltd., Miles Labs, Inc., Elkhart, Ind.) by the indirect technique of Möller (6).

Stained preparations were immediately examined on a Zeiss RA fluorescent microscope. All specimens were examined alternately in conventional and ultraviolet light. Photographs were taken with Kodak Tri-X Pan film with exposure time varying between 4 and 16 min.

### CEF cultures and media

Growth medium 199 was supplemented with 5% tryptose phosphate broth, 8% bovine fetal calf serum, 100 U of penicillin, 25 μg of amphotericin B (Fungizone) per ml, and 100 μg of streptomycin per ml. Maintenance medium 199 was the same as above except that bovine fetal calf serum was reduced to 1%.

CEF cultures were prepared from 9- to 11-day-old embryos. Aseptically harvested embryos were forced
through a 10-ml syringe and trypsinized in 0.25% trypsin in PBS for 8 min at 25°C. Cell suspensions were then filtered through two layers of cheesecloth and centrifuged for 10 min at 1,000 × g. The resulting cell pellet was washed twice by centrifugation in maintenance medium and finally suspended in growth medium at a concentration of 5 × 10^5 cells per ml. Samples of 5 ml of this suspension were put into plastic tissue culture plates (60 by 15 mm). Cultures were incubated at 37°C in a 5% CO₂-in-air atmosphere. Monolayers were confluent in 24 h.

A purified REV-T pellet was isolated from TV-1 culture supernatant as previously described above. The virus pellet was suspended in 7 ml of maintenance medium and overlaid on the 24-h CEF cultures for 2 h. The cultures were then washed four times with PBS, given 5 ml of maintenance medium, reincubated as above, and observed for the development of cytopathic effects for 8 to 10 days.

Trypan blue cytotoxicity assay. Optimal dilutions of guinea pig complement (C') and T- and B-cell antisera were determined by trypan blue cytotoxicity tests. C' and T- and B-cell antisera were titrated against REV-T-transformed cells and JM-1 cells from suspension cultures and against thymic and bursal cells from 3- to 4-week-old chickens.

All cells and antisera were diluted with RPMI 1640 growth medium supplemented with 10% fetal bovine serum (Flow Labs, Rockville, Md.). Suspensions of thymic and bursal cells were prepared by dissociating the respective tissue in a Ten-Broek grinder and washing four times in PBS by centrifugation at 1,500 × g for 5 min. REV-T-transformed cells and JM-1 cells from suspension cultures were washed twice in PBS as described above. All cell densities were standardized to an optimal dilution of 2 × 10^5 to 3 × 10^5 cells per ml of RPMI 1640.

C' was obtained from a 2-month-old female English guinea pig and was stored at −20°C in aliquots sufficient for each trial. All C' dilutions were made with Glucose-GVB * buffer (Cordis Labs., Miami, Fla.).

For the assay, 100-μl samples of cells suspended in antiserum dilution were placed in wells of Costar Cluster Plates (Costar, Cambridge, Mass.), and 100 μl of C' dilution was added to each well. Control wells contained either 100 μl of cells suspended in antiserum dilution plus 100 μl of RPMI 1640, 100 μl of cells suspended in RPMI 1640 plus 100 μl of C' dilution, or 200 μl of cells suspended in RPMI 1640 only (no treatment). Samples were run in triplicate. The cluster plates were incubated at 37°C for 2 h. The plate was then placed on ice, and 50 μl of 1% trypan blue in PBS was added to each well. The cells were counted on a hemacytometer. The cytotoxicity index was calculated as: [mean percent dead in test − mean percent dead in no-treatment controls]/(mean percent live in no-treatment control) × 100.

RESULTS

Cell line establishment, growth rate, and karyotyping. Cultures of REV-T-transformed cells began to multiply within 2 to 4 weeks. Cultures incubated at 37°C grew more slowly than cultures grown at 41°C, and cultures from the lower temperature died within 6 weeks. Splenic cultures grown at 41°C have been maintained in suspension culture for more than 74 weeks.

Cell counts conducted over several months using trypan blue exclusion tests determined that these cells had a generation time of from 12 to 15 h. Three weeks after cell line initiation, their culture doubled in cell population every 12 h. Thirty-four weeks after initiation, the doubling rate had slowed to approximately 15 h, and it stabilized thereafter. Although cultures would reach a viable cell density of 5 × 10^6 to 6 × 10^6 cells per ml, the optimal density for growth was 5 × 10^5 to 1 × 10^6 cells per ml (Table 1). Passages were made every 48 to 72 h by inoculating 2.0 ml of suspension culture into 16 ml of fresh RPMI 1640.

Karyotype analysis of these cells indicated the presence of a female chromosome (ZW) (Fig. 1). This REV-T-transformed lymphoblastoid cell line has been designated TV-1.

Microscopy. In suspension culture, TV-1 cells grew as single cells or in large aggregates which temporarily dissociated with gentle agitation. When stained with hematoxylin and eosin, the cells appeared pleomorphic and possessed a prominent nucleus. Mitotic figures were frequently observed (Fig. 2).

When examined by an electron microscope, the lymphoblastoid cells appeared irregular in shape and frequently exhibited cytoplasmic processes. The cytoplasm was basophilic and contained numerous mitochondria, abundant polyribosomes, and a well-developed endoplasmic reticulum. A pronounced nucleolus and peripherally distributed chromatins were easily distinguishable within the large nucleus (Fig. 3).

<table>
<thead>
<tr>
<th>Age of culture (weeks)</th>
<th>Viable cells after hours in culture:</th>
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<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>5 × 10^5</td>
</tr>
<tr>
<td>35</td>
<td>5 × 10^5</td>
</tr>
<tr>
<td>66</td>
<td>5 × 10^5</td>
</tr>
</tbody>
</table>

* Numbers represent viable cells only, as determined by trypan blue exclusion. Numbers are an average of the count from at least four cell cultures. ND, Not done.
Fig. 1. Chromosome spread of TV-1 lymphoblast expressing a female (ZW) karyotype.

Fig. 2. TV-1 lymphoblasts stained with hematoxylin and eosin. Note pleomorphism and cell in mitosis.

Fig. 3. An electron micrograph of a REV-T-infected lymphoblastoid cell from chicken spleen. Note the pronounced nucleolus (Nu) in the nucleus (N), spherical mitochondria (M), rough endoplasmic reticulum (RER), and irregular plasma membrane (PM). Virus particles (VP) are evident in the proximity of the plasma membrane.
Similar to the findings of Kang et al. (5), C-type REV-T budding virions were observed in the region of the plasma membrane. However, no budding was evident within smooth-walled, intracytoplasmic vesicles. During release from the cell, the virus particles were seen in various stages of maturation. Intermediate and inner rings were observed in the nucleoid region of virions still associated with the plasma membrane, whereas an outer envelope and an intermediate layer surrounding a dense core were characteristic of REV-T particles located extracellularly (Fig. 4).

Assay for infectivity. TV-1 cells and their culture supernatant produced lesions typical of REV-T infection (8) when inoculated into various lines of 1-day- to 1-week-old chickens. Table 2 indicates the results of titrating TV-1 cells and TV-1 cell-free culture supernatant in 1-day-old chicks. The LD<sub>50</sub> remained constant at 10<sup>4.337</sup> from 9 weeks after culture initiation to 10<sup>4.228</sup> after 24 weeks of cell line growth. After 31 weeks of growth, the LD<sub>50</sub> had dropped to 10<sup>-2.124</sup>.

The amount of infectious virus released into the culture supernatant was low as evidenced by the LD<sub>50</sub> of 10<sup>0.341</sup>. All birds that survived the initial dose were challenged with REV-T-infected liver and spleen tissue homogenate to determine whether immunity to T-virus was present. A 50% protective dose was calculated to be 10<sup>2.345</sup>.

All dead birds were necropsied and typically had mottled livers. In addition, splenomegaly and hepatomegaly were frequently found. Control birds had no lesions of T-virus infection.

Fluorescent antibody staining. The findings of fluorescent antibody staining for cell membrane and intracellular antigens are summarized in Table 3. Positive staining of TV-1 cells resulted with B-cell antiserum (Fig. 5). The presence of immunoglobulin on TV-1 cell surfaces was subsequently confirmed by positive staining with FITC-protein A. When T-cell and B-cell antisera were absorbed with T- and B-cells, respectively, no significant membrane staining of any cell population was evident.

The absence of MDV and ALV contaminants was verified by the lack of fluorescent staining of TV-1 cells, including those that had been treated with iododeoxyuridine, when stained with anti-MDV, anti-Rous sarcoma virus, or anti-Marek's disease-associated tumor-specific antigen serum, in the presence of appropriate controls. Furthermore, CEF cultures that were infected with virus obtained from TV-1 cell culture supernatant produced no cytopathic effects characteristic of Marek's disease herpesvirus. Although in 25% of the CEF culture trials foci of dead cells, as described by Temin and Kassner (16), were found within 48 h after CEF infection,
Table 2. Titration of TV-1 cells and TV-1 cell-free culture supernatant in chicks inoculated at 1 day of age

<table>
<thead>
<tr>
<th>Determination</th>
<th>Weeks after culture initiation</th>
<th>Dilutions</th>
<th>Mortality*</th>
<th>Control</th>
<th>LD$_{50}$/ml</th>
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<tr>
<td></td>
<td></td>
<td>10$^9$</td>
<td>10$^{-1}$</td>
<td>10$^{-2}$</td>
<td>10$^{-3}$</td>
</tr>
<tr>
<td>TV-1 cells</td>
<td>9</td>
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<td>3/5</td>
<td>5/5</td>
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<tr>
<td></td>
<td>24</td>
<td>5/5</td>
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<td></td>
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<td>5/5</td>
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<tr>
<td>TV-1 culture supernatant</td>
<td>31</td>
<td>4/5</td>
<td>2/5</td>
<td>1/5</td>
<td>0/5</td>
</tr>
</tbody>
</table>

*Numbers represent specific mortality/total inoculated.

Table 3. Cell membrane and intracellular antigens of TV-1 cell line indicated by fluorescent antibody staining

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>TV-1</th>
<th>JM-1</th>
<th>JMV-1</th>
<th>RSV* tumor cells</th>
<th>T-cells</th>
<th>B-cells</th>
<th>Macrophages</th>
</tr>
</thead>
<tbody>
<tr>
<td>T-cell</td>
<td>±</td>
<td>+++</td>
<td>ND</td>
<td>ND</td>
<td>+++++</td>
<td>±</td>
<td>-</td>
</tr>
<tr>
<td>B-cell</td>
<td>+</td>
<td>±</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>REV-T</td>
<td>++</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>Macrophage</td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>FITC-protein A</td>
<td>+</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>+</td>
<td>ND</td>
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<td>T absorbed with</td>
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<td>ND</td>
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<td>T-cells</td>
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<td>B absorbed with</td>
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<td>B-cells</td>
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<td>ND</td>
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<td>-</td>
<td>-</td>
<td>ND</td>
</tr>
<tr>
<td>MATSA*</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>MDV</td>
<td></td>
<td></td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>RSV</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*Intensity of fluorescence was graded from + to +++. ±, Insignificant fluorescence; -, no fluorescence. ND, Not done.

no consistent pattern of cytopathic effects was observed.

Cytotoxicity assay. Lysis of TV-1 cells by either T-cell or B-cell antiserum was performed to confirm the results of fluorescent antibody staining. The cytotoxic indexes presented in Table 4 are averages of three to six trial values. Indexes measured for T, B, TV-1, and JM-1 cells against T-cell antiserum were 100, 12.75, 4.09, and 31.90, respectively. Indexes measured for the same cells against B-cell antiserum were 6.46, 99.1, 55.98, and 10.58, respectively. B-cell antiserum was cytotoxic for most TV-1 cells that remained disassociated from a clump during a given trial.

DISCUSSION

We report the establishment and characterization of an REV-T-transformed lymphoblastoid cell line designated TV-1. The cell line has a stabilized generation time of 15 h when cell densities are maintained between 5 x 10$^4$ and 1 x 10$^6$ cells per ml, and it has been growing in suspension culture for at least 74 weeks.

We observed the cells to be pleomorphic and
Table 4. Cell membrane antigens of TV-1 cell line indicated by cytotoxicity assay

<table>
<thead>
<tr>
<th>Antiserum</th>
<th>Cytotoxicity index on cell population:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>TV-1</td>
</tr>
<tr>
<td>T-cell</td>
<td>8.18</td>
</tr>
<tr>
<td>B-cell</td>
<td>55.98</td>
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</table>

* Cytotoxicity index = [(mean percent dead in test – mean percent live in no-treatment control)/(mean percent live in no-treatment control)] × 100. Index values are an average of three to six trials.

to grow primarily in large aggregates. Ultrastructurally, the presence of abundant ribosomes, free and attached, suggested active protein synthesis related to cell proliferation and to the synthesis of protein for export. The REV-T budding was found to be associated with cytoplasmic processes of the plasma membrane but was not observed within intracytoplasmic vesicles.

The amount of virus released from the cell was found to be low. The LD₅₀ values obtained for both cell-associated and cell-free virus were lower than values obtained by Sevoian et al. (12) in mortality studies of 1-day-old chicks tested with cellular and cell-free preparations of liver homogenate from moribund REV-T-infected chicks. However, the mortality patterns and histopathological lesions resulting from TV-1 cell and cell-free culture supernatant inoculation of 1-day-old chicks were indistinguishable from those obtained by inoculation of REV-T-infected tissue homogenate. In both instances, chicks showed no sign of illness until a few hours before death, and most deaths occurred 6 to 10 days postinoculation. Upon necropsy, greatly enlarged and mottled livers and spleens were consistently found.

The demonstration of a female karyotype for TV-1 cells was remarkable in the light of the observations of Stephens et al. (15) and Hahn et al. (3), who found that the JMV lymphoblastic leukemic cell line was always of female karyotype. It has been speculated that the discrepancies in transplantability studies with JMV may be indicative of an initial proliferation of transplanted lymphocytes, followed by a late proliferation of host lymphocytes (D. C. Munch, Ph.D. dissertation, University of Massachusetts, Amherst, 1978). Insofar as both TV-1 cell-free supernatant and TV-1 cells produced typical reticuloendotheliosis in chicks, it appeared unlikely that TV-1 was acting as a cell transplant. However, the difference in chick lethality caused by REV-T-infected tissue homogenate versus TV-1 cells and their culture supernatant, combined with the female karyotype of the TV-1 cell line, suggested that the function of REV-T was altered in vitro and that transplantability studies with the TV-1 cell line would be informative.

Positive staining of TV-1 cells with B-cell antiserum and with FITC-protein A by fluorescent antibody technique showed the presence of cell surface immunoglobulin indicative of B-cells. Lysis of TV-1 cells by B-cell antiserum in the cytotoxicity assay also indicated the presence of B-cell determinants on membrane surfaces. The identification of TV-1 as a B-cell was further supported by ultrastructure studies which showed the presence of abundant endoplasmic reticulum within the cells.

It is anticipated that the establishment of the TV-1 cell line and its characterization as a female, B-cell line will facilitate further research related to the mechanisms of viral cell transformation and tumor cell immunity.

Literature Cited

