A Tumor Antigen in Tissue Cultures Derived from Patients with Hodgkin's Disease

(pulse labeling/density-gradient sedimentation/agar-gel diffusion/immunoelectrophoresis)

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ABSTRACT Pellets obtained from supernatant fluids of monolayer cultures of cells from patients with Hodgkin’s disease were used in isopycnic density sedimentation. Material in a peak of specific gravity 1.15-1.21 g/ml from two Hodgkin’s disease cultures was used to immunize rabbits, and the antisera obtained in this manner were reacted by agar-gel diffusion and immunoelectrophoresis with antigens from the purified peaks and the unfractionated pellets of centrifuged culture medium from all the cultures. The antisera did not react with material from 9 of 10 lines derived from spleens of patients with Hodgkin’s disease, 2 of 8 cell lines from histologically negative spleens from patients with Hodgkin’s disease and with 3 of 6 lymphoma cell lines not diagnosed as Hodgkin’s disease. The antisera did not react with 12 cell cultures prepared from normal adult and fetal spleen and thymus. The antigen from cultures of patients with Hodgkin’s disease was not found in material sedimenting at lower specific gravities; it resisted Tween-ether solubilization, and migrated as a single band by immunoelectrophoresis. The antigen was not found in disrupted, noncultured tumor cells from patients with Hodgkin’s disease, and an antiserum against noncultured, minced tumor tissue did not react with the Hodgkin’s disease tissue-culture material. No immunological relationship was found between the tissue-culture antigen and Epstein-Barr, RD-114, or Rauscher murine leukemia viruses. The Hodgkin’s disease antigen may be a tumor-related antigen or a component of an oncogenic virus.

Recent epidemiological (1), immunological (2, 3), biochemical (5, 6), and tissue culture (7, 8) investigations suggest a viral etiology for Hodgkin’s disease (HD). By sucrose density-gradient labeling procedures, evidence has been obtained for a virus-like particle in HD tissue cultures (5). Intracellular and intracytoplasmic Herpes-like particles have been demonstrated by electron microscopy of long-term tissue cultures derived from HD lymph nodes (7, 8), and molecular hybridization experiments suggest that HD and other human lymphomas contain RNA homologous to that of Rauscher leukemia virus (RLV) (6). Furthermore, a tumor-associated neoantigen has been demonstrated with an antiserum against tumor tissue (9, 20).

We have cultured tumor tissue from patients with HD and, with the aid of isopycnic sucrose-density sedimentation, have obtained a fraction used to immunize rabbits. This report describes the preparation, absorption, and agar gel immunoprecipitin and immunoelectrophoretic reactivity of these antisera.

Abbreviations: HD, Hodgkin’s disease; FCS, fetal-calf serum; FeLV, Feline leukemia virus; RLV, Rauscher (murine) leukemia virus; AMV, avian myeloblastosis virus.

MATERIALS AND METHODS

Preparation of cell cultures

Monolayer cell cultures were derived from operative specimens from untreated patients during HD-staging laparotomies and incident or traumatic splenectomies, and from diagnostic lymph-node biopsies of patients with non-HD lymphomas. Cell cultures of normal human-fetal thymus and spleen were obtained from the Naval Biomedical Research Laboratories, Oakland, Calif. The 36 different cultures used in these experiments are divided into four groups (Table 1): (i) 10 splenic HD cell lines, (ii) 8 cell lines of histopathologically negative spleens removed from HD patients, (iii) 6 cell lines of non-HD lymphoma lymph nodes (3 well-differentiated lymphocytic lymphomas, 2 reticulum cell sarcomas, and 1 lymphoblastic lymphoma), and (iv) 8 cell lines of normal adult spleen and 2 each of fetal spleen and thymus. All cultures were serially passed at weekly intervals for a minimum of 2 and a maximum of 14 months with repeated checks for mycoplasma contamination.

106 Sucrose-gradient sedimentations were performed on 34 different HD and on 14 normal spleen-culture lines. [3H]U was used as a precursor 80 times and [1H]dT 26 times. 21 Different culture lines subjected to [3H]U labeling and density sedimentation form the basis for the present study.

Density gradient labeling

Six flasks containing actively proliferating cells from each cell line were labeled, each was incubated with 0.5 mCi of [3H]U for 18 hr (10). The medium was then removed and clarified twice at 7500 × g for 15 min. The supernatant was centrifuged at 78,000 × g for 2 hr. The pellet was suspended in 0.4 ml STE buffer [0.15 M NaCl-10 mM Tris·HCl-1 mM EDTA (pH 7.25)], layered on a 15-60% (w/w) linear sucrose gradient, and centrifuged at 190,000 × g for 18 hr in a Spinco SW41 rotor at 4°. 0.25-ml Fractions were collected with an Isco model 640 density-gradient fractionator, with monitoring at A250. Alternate fractions were precipitated with 5% trichloroacetic acid, collected on Millipore filters, and counted in 10 ml of scintillation fluid (0.3% PPO and 0.03% POPOP in toluene) in a Unilux II (Nuclear-Chicago). At low specific gravities, 1.08-1.10 g/ml, radioactivity peaks occurred inconstantly; these peaks could be minimized or eliminated by careful washing and draining of the pellet before sedimentation. Peaks occurring from 1.15-1.21 g/ml incorporated radioactivity from [3H]U or from [1H]dT. This “high density” sedimentation peak was used for immunization and as antigen for the immunodiffusion experiments.

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Table 1. Agar-gel immunodiffusion against pellets from culture media†

<table>
<thead>
<tr>
<th>Type of antigen preparation*</th>
<th>Anti-HD antisera 1 and 2†</th>
<th>High-density peak absorbed anti-HD antisera§</th>
<th>Antiserum to normal spleen high-density peak</th>
<th>Antiserum against noncultured tumor tissue</th>
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<tbody>
<tr>
<td>I. HD, pathologically positive spleens</td>
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<tr>
<td>(a) crude pellet</td>
<td>9/10</td>
<td>0/9</td>
<td>0/7</td>
<td>0/7</td>
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<tr>
<td>(b) high density sedimentation peak</td>
<td>4/5</td>
<td>0/4</td>
<td>0/4</td>
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<tr>
<td>(c) low density sedimentation peak</td>
<td>0/4</td>
<td>—</td>
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<td>II. Pathologically negative spleens from HD patients¶</td>
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<tr>
<td>(a) crude pellet</td>
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<td>0/2</td>
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<tr>
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<tr>
<td>(c) low density sedimentation peak</td>
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<tr>
<td>III. Normal adult spleen and thymus and spleen</td>
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<tr>
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<td>0/5</td>
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<td>IV. Non-HD lymphoma lymph nodes</td>
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<tr>
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<td>0/3</td>
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<tr>
<td>(c) low density sedimentation peak</td>
<td>0/1</td>
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* Antigens consist of three types of centrifuged, pooled tissue culture medium: (a) crude pellets prepared by clarification of 1 liter of cell-free medium at 7500 X g for 20 min, discarding pellet, centrifugation of supernatant fraction at 75,000 X g for 2 hr at 4°, and suspension of pellet in 0.5 ml of STE buffer; (b) samples of the high-density [3H]U sedimentation-gradient peak; and (c) samples of the low-density sedimentation gradient peak from cell lines with positively reacting high-density-peak samples. Extraction of the crude pellet samples with Tween-ether (13) did not alter the number or configuration of precipitin bands formed in agar gel.

† Results expressed as no. positive per no. tested. A positive reaction is a single, discrete precipitin band by gel diffusion against absorbed antiserum.

‡ The two separate anti-HD antisera 1 and 2 are included together because of identical reactivity in all instances.

§ Anti-HD antisera further absorbed with material from a high-density sedimentation gradient isolate from an HD cell line different from that used to prepare the antiserum.

¶ The two positively reacting samples in group II are the FQ and EL cell lines.

Antiserum

Three different types of antisera were prepared:

(i) Anti-HD Antiser. Pellets obtained by centrifugation of 1200 ml of nonradioactive supernatant fluids from two different HD monolayer cultures were fractionated by isopycnic density sedimentation. Peaks of specific gravity 1.15-1.21 g/ml were collected, diluted in 2 ml of STE buffer, and centrifuged at 190,000 X g for 2 hr. The pellet was resuspended in 0.5 ml of STE buffer, passed through a 45-gm Millipore membrane filter, emulsified in 0.5 ml of complete Freund's adjuvant, and injected into rabbit footpads. High density sedimentation-gradient isolates, which were prepared in a similar manner, were administered as single intravenous boosters 1 month later. 2 Weeks thereafter immune sera were collected, and the globulin fraction obtained by salting out with equal parts of 40% ammonium sulfate, followed by dialysis with water and normal saline, (11) and restoration to the original serum volume with phosphate-buffered saline (pH 7.4).

Anti-HD antisera 1 and 2 were obtained by absorbing this globulin fraction at 37° for successive 30-min periods in the following manner: three times with an equal volume of washed, packed, type-A-positive human erythrocytes; three times with an equal volume of a suspension of washed, viable, noncultured, normal adult spleen cells; and three times with 0.5 volumes of 100% fetal-calf serum polymerized with ethylene glycol (12). "High-density peak absorbed antiserum" was prepared by further absorption of 0.5 ml of anti-HD antiserum for 1 hr at 37° with a suspended pellet obtained by centrifugation of the high-density sedimentation-gradient isolate from an HD cell line different from that used to prepare the immunizing antigen for the antiserum.

The remaining two antisera were prepared as controls.

(ii) Antiserum to Normal-Spleen High-Density Peak. The pellet obtained by centrifugation of 1200 ml of cell-free supernatant from a normal spleen monolayer culture was fractionated by isopycnic sucrose gradient sedimentation. A low amplitude A260 peak of specific gravity 1.13-1.16 g/ml was pelleted, resuspended in STE buffer, filtered through a Millipore membrane, emulsified in complete Freund's adjuvant, and injected into rabbit footpads. A similarly fractionated pellet of centrifuged medium was administered intravenously 1 month later. The globulin fraction of immune serum collected 2 weeks thereafter was absorbed as described for anti-HD antisera.

(iii) Antiserum Against Tumor Tissue. 10⁴ viable cells from an HD lymph node in 0.5 ml of complete Freund's adjuvant were injected into rabbit footpads; 2 weeks later 10⁴ cells from the same source that were stored at -80° in 10% ethylene glycol were injected intravenously. Absorption of the globulin fraction of the immune serum collected 10 days later was as described for anti-HD antisera, except that absorption with fetal-calf serum was omitted.

Double immunodiffusion in 2% agar gel was done at 20° in standard Ouchterlony plates (Hyland Laboratories, Los Angeles, Calif.). Immunoelectrophoreses were performed in 1.5% barbital-buffered agar gel (pH 8.0) for 2 hr at 150 V.
erythrocytes and from a peak samples. Antiserum (absorbed with normal spleen cells and type A erythrocytes and FCS). Precipitin reaction is confined to the pellet from HD culture medium and high-density [H]U sedimentation peak samples.

Fig. 2. Agar-gel immunodiffusion of pellets from cell culture medium of five different HD cell cultures reacted with anti-HD antiserum. Wells 1, 3, and 4: high-density [H]U sedimentation peaks from 3 different HD cell lines; wells 2 and 5: crude pellets from medium of 2 other HD cell lines; well 6: anti-HD-2 antiserum (absorbed with normal spleen cells and FCS). A single precipitin of identity is seen.

Fig. 3. Agar-gel immunodiffusion of crude pellet from medium from a single HD cell culture reacted with different antisera. Well 1: unabsorbed rabbit anti-HD globulin; well 2: anti-HD-1 antiserum absorbed with normal spleen cells and FCS; wells 3 and 5: anti-HD-1, high-density peak absorbed antiserum; well 4: antibody against noncultured tumor tissue (9); well 6: HD crude pellet from medium. Simultaneous testing of crude and absorbed anti-HD antiserum demonstrates elimination of nonspecific precipitin band and persistence of tumor-related component (wells 1 and 5). Further absorption of anti-HD antiserum with material from a HD high-density sedimentation gradient peak eliminates reactivity against tumor-related component (well 3 and 5). An antibody against noncultured tumor tissue fails to react with the pellet from HD culture medium (well 4).

Feline leukemia virus (FeLV) and Rauscher murine leukemia virus (RLV) (10^11 virus particles per ml), purified by sucrose gradients, were obtained from a tissue culture source prepared by the John L. Smith Memorial for Cancer Research, Pfizer Inc., Maywood, N.J., and Electo-Nucleonics Laboratories, Bethesda, Md., respectively. These viruses were extracted with Tween–ether (13). Antisera reactive with FeLV, RLV, and avian myeloblastosis viruses (AMV) disrupted by Tween–ether were supplied by Dr. David Allen, Thorndike Memorial Laboratories, Boston City Hospital. Antiseras reactive by gel diffusion with disrupted RD-114 virus and with purified gs (group specific) protein of murine leukemia virus (21, 26) were provided by Dr. Raymond V. Gilden, Flow Laboratories, Rockville, Md. Two sera from patients with Burkitt’s tumor were provided by Dr. Werner Henle; these sera contained antibodies to the capsid antigen of Epstein–Barr virus in titers greater than 1:160, as detected by immunofluorescence. Herpes simplex viruses 1 and 2 (titer 2 × 10^6 per ml) were provided by Dr. Fred Rapp, Hershey Medical Center.

Ferritin purified from normal spleen and a monospecific antiserum to ferritin were provided by Dr. Eliot Alpert, Massachusetts General Hospital. Ferritin from HD spleens was obtained from Dr. Charles Bieber, Stanford Medical Center, Palo Alto, Calif.

RESULTS

Anti-HD antisera (absorbed with A-positive erythrocytes, normal human spleen cells, and FCS) were nonreactive when tested against: (a) tissue culture medium and 100% FCS by agar-gel diffusion; (b) suspensions of viable, noncultured peripheral blood lymphocytes and normal spleen cells, purified by a Ficoll–Hypaque gradient, by indirect immunofluorescence; (c) normal human serum by agar gel diffusion; (d) purified ferritin from normal and HD spleens; and (e) pellets from media from 12 cultures of adult and fetal spleen and thymus (Table 1). Hence, immunoreactivity against pellets from centrifuged media from normal and fetal lymphoid cell cultures, and from noncultured splenic and peripheral blood lymphocytes, was eliminated by absorption of the antiseras (Fig. 1).

Anti-HD antisera were then reacted by agar gel diffusion with antigens from 10 HD cell cultures (Table 1). Four different types of antigen preparations from centrifuged culture medium were tested. Crude pellets from medium from 9 out of 10 HD culture lines were positive. Prior extraction of the crude pellets with Tween–ether had no effect on the configuration of the precipitin lines. Four out of five samples of the high-density peaks sampled reacted positively. Simultaneous testing of crude pellets and high-density [H]U peaks yielded a single precipitin line of identity with absorbed anti-HD antisera (Fig. 2). The low-density peak from four out of four HD cell lines tested did not react by agar diffusion with either of the two “anti-HD” antisera. Positively-reacting antisera from HD media were tested against “high-density” peak” absorbed antiserum. Twenty-one out of 21 previously positively-reacting antisera failed to react with anti-HD antiserum thereby absorbed. Hence, immunoprecipitin activity is eliminated by absorption of the antibody with a high-density sedimentation peak from a HD cell line different from that used in preparation of the antibody (Table 1; Fig. 2).

Two out of eight cell lines derived from histologically negative spleens removed from HD patients reacted by gel diffusion with anti-HD antisera (FQ and EL cell lines) the remaining six were negative. When tested along with cultures of pathologically positive HD spleens, the FQ and EL lines reacted with a single precipitin band of identity, which was absent when the same lines were tested with high density peak absorbed antiserum. Media pellet fractions from three out of six cell lines derived from non-HD lymphomas reacted with anti-HD antisera forming lines of identity with HD samples.

Pellets from centrifuged media from 15 different culture lines were tested by gel diffusion with an antiserum to normal spleen high-density peak. After absorption of this antiserum (with normal spleen cells, human erythrocytes, and FCS) no reaction was observed in seven HD, five normal adult spleen, and three non-HD lymphoma culture lines. An antiserum against minced, noncultured HD tumor tissue failed to react by gel diffusion with the same 15 lymphoma and normal spleen cell cultures (Fig. 3). The antiserum to tumor tissue formed a single precipitin line in agar gel when tested with disrupted HD tumor cells obtained by rapid freeze–thawing of a splenic tumor nodule and reacting the supernatant. Anti-HD antisera did not react by gel diffusion with this preparation of noncultured HD tissue.
By agar-gel immunoelectrophoresis (Fig. 4) two distinct precipitin lines were found in pellets from centrifuged medium of both normal spleen and HD cultures reacted with nonabsorbed anti-HD globulin. A third precipitin band, which migrated toward the cathode, was not found in normal spleen cultures. This precipitin line persisted in pellets from centrifuged HD culture medium after absorption of the antiserum and is found specifically in the HD high-density sedimentation peak.

Crude pellets from medium from HD cell lines used as antigens in the present experiments were tested by agar diffusion with antisera to animal oncogenic viruses. No reaction occurred with antisera against the gs (group specific) antigen of FeLV, RLV, or AMV. No reaction occurred by agar gel diffusion between anti-HD antisera and crude or Tween-ether-extracted AMV, RLV, FeLV, or avian myeloblastosis virus. Antiserum to disrupted RD-141 virus (26) failed to react with crude or Tween-ether-extracted pellets from HD culture medium, and anti-HD antisera did not react by gel diffusion with disrupted RD-141 virus. Antisera to capsid antigen of Epstein-Barr virus of Burkitt’s tumor did not react by gel diffusion with pellets of culture medium from HD cultures nor with cells from HD monolayers by indirect immunofluorescence. Anti-HD antisera did not react by the fluorescent antibody technique with cells from a Burkitt’s tumor suspension culture containing Epstein-Barr virus (HR-1 cell line). Anti-HD antisera were nonreactive by gel diffusion with either extracts of cells containing mycoplasma or pellets from culture medium from human embryo fibroblast lines known to be contaminated with mycoplasma.

**DISCUSSION**

In the present experiments a macromolecular moiety with specific gravity 1.15–1.21 g/ml was obtained by isopycnic density sedimentation of centrifuged supernatant fluids from cell cultures from patients with Hodgkin’s disease, and was used to immunize rabbits. After removing non-specific reactivity by absorption with culture medium components and normal cellular antigens, the antisera did not react with pellets from centrifuged culture medium from 12 monolayer cultures derived from normal (adult and fetal) spleen and thymus. However, these absorbed antisera reacted strongly by gel diffusion, yielding a single precipitin line of immunological identity, with 9 of 10 monolayer cultures from patients with Hodgkin’s disease and three of six non-Hodgkin’s lymphoma cultures. Of eight cell lines derived from histologically negative spleens from patients with Hodgkin’s disease, only two reacted with the antisera: the first (FQ cell line) reacted only after a dramatic spontaneous alteration in growth characteristics after repeated tissue culture passage; the second was derived from a markedly enlarged spleen with atypical hyperplasia (EL cell line).

Several lines of evidence suggest that the anti-HD antisera react with a component confined to the high-density peak of the sedimentation gradient, the component used for immunization. Thus, there are no detectable precipitin lines with the low-density-peak material (Table 1). Furthermore, reactivity of the antisera is eliminated by absorption with the high-density-peak material (Fig. 3). Finally, simultaneous testing of crude pellets from media of cells from patients with Hodgkin’s disease and high-density peaks yields a single precipitin line of identity on gel diffusion, and similar precipitin lines on immunoelectrophoresis.

In two of eight normal cultures labeled with [3H]U and sedimented, high-density peaks were observed indicating that a high-density peak, by itself, is not specific for Hodgkin’s disease. However, such normal spleen peaks were of low amplitude and did not react with anti-HD antisera. Moreover, when an antiserum was prepared against the high-density peak from a normal spleen culture and was absorbed with tissue culture and normal cellular components, it did not react by gel diffusion with pellets prepared from Hodgkin’s disease or normal spleen culture media (Table 1).

While our data are consistent with the presence of an antigen specific for Hodgkin’s disease, there are alternate explanations. Thus, it is likely that the density-gradient fraction used as immunogen for the preparation of anti-HD antisera contains particulate cell fragments bearing histocompatibility antigens (14–16). However, it seems unlikely that the antisera are directed solely against histocompatibility antigens present in different amounts in lymphoma and normal cultures because, by fluorescence microscopy, the absorbed anti-HD antisera did not react with several different suspensions of viable, human peripheral blood lymphocytes (16). An alternative possibility is that the gel diffusion data reflect a quantitative difference in the amount of certain antigens shared by lymphoma and normal spleen. Thus, the difference in immunoreactivity between Hodgkin’s disease tissue and normal spleen could represent the variation in cell populations in the various tissue cultures. Until the question can be resolved by more sensitive methods, we cannot exclude the possibility of a quantitative difference in cell populations rather than a tumor-specific antigen.

Order et al. (9, 20, 27) have used heterologous antisera against Hodgkin’s disease tissue to demonstrate two electrophoretically separable antigens in 18 of 19 Hodgkin’s disease and 5 of 18 control spleens. Their antigens, which are not derived from tissue culture material, were designated tumor-associated rather than tumor-specific, since they were observed
in a minority of control spleens. We have prepared an antisem against Hodgkin's disease tumor tissue. After absorption with normal spleen cells, our antisem against noncultured Hodgkin's disease tissue did not react by gel diffusion with the pellets we obtained from the medium of our Hodgkin's disease cultures (Fig. 3). Anti-HD antisera derived from cultured Hodgkin's disease material did not react with fresh tumor tissue. Hence, it is unlikely that the two antisera are detecting the same antigen. Propagation in vitro may favor proliferation of the malignant cells of Hodgkin's disease, a disorder characterized morphologically by a large admixture of inflammatory with relatively few neoplastic cells. Furthermore, isopycnic density sedimentation of culture fluids may concentrate and purify the tumor antigens in Hodgkin's disease: antisera against this purified material may detect antigens not detected by antisera against minced tumor tissue.

A peak of [3H]U banding in the sedimentation gradient at 1.15-1.17 g/ml has been cited as evidence for virus-related particulate RNA in culture fluids from non-producer lines of animal (17, 18) and human (10) neoplasms. Thus, the density gradient of centrifuged media from cell cultures derived from patients with Hodgkin's disease is comparable to that of known oncogenic viruses. In addition to the sedimentation banding characteristics, other evidence suggests that the Hodgkin's disease tissue-culture antigen may be a virus component. Thus, the antigen has not thus far been detected in cultures of fetal thymus or spleen, suggesting that a fetal antigen is not involved (25), and the antigen retains its immunological reactivity after extraction with Tween-ether (23). Furthermore, a phase of in vitro proliferation in tissue culture is necessary to demonstrate the antigen by our techniques, consistent with the expression in tissue culture of an oncogenic virus present as a virogene (19, 22, 24) within the tumor cells. Thus far our anti-HD antisera do not react with either intact or disrupted noncultured Hodgkin's disease tumor cells.

We must point out, however, that radioactivity originating in [3H]U in our experience is modified during incubation in tissue culture, and can be converted to the DNA precursor TTP. It is, in addition, converted to low-molecular-weight compounds which are separable by two dimensional chromatography, but which have not been otherwise identified. [3H]dT, when added to the tissue culture medium, labels approximately the same peak (specific gravity 1.15-1.21) as does [3H]U, in a sucrose-density gradient. We have recently separated the protein, DNA, and RNA fractions from a [3H]dT-labeled peak, and have found essentially all of the radioactivity in DNA. Thus a putative virus in this sucrose density peak could be a DNA-containing one. The macromolecular location of the [H] originating in [3H]U is not so clearly defined.

The Hodgkin's disease tumor antigen does not appear immunologically related to several known oncogenic viruses. No crossreactivity has been observed between our Hodgkin's disease culture antigen and either Epstein-Barr or RD-114 virus. Although two recent reports relate Rauscher leukemia virus to Hodgkin's disease (4, 6), we could demonstrate no reaction between the anti-HD antisera and intact or disrupted Rauscher virus. Finally, an antibody to the group-specific antigen of murine leukemia virus does not react by gel diffusion with the antigen obtained from Hodgkin's disease cultures. It remains possible that our Hodgkin's disease antigen is a non-viral tumor antigen which is masked in normal cells (28) or even that it reflects a quantitative difference in cellular constitution of normal and lymphoma cultures.

We are grateful to Dr. Werner Henle for providing antisera to Epstein-Barr virus and cell cultures of Burkitt's tumor, Dr. Raymond Gilden for samples of RD-114 virus, Drs. Eliot Alpert and Charles Bieber for human spleen ferritin and Dr. Fred Rapp for Herpes Simplex 1 and 2 viral concentrates. We thank Dr. Albert H. Coons for critical comments, Drs. Leonard J. Rosenthal and Robert B. Colvin for helpful suggestions, and Mrs. Maryann Magner and Miss Sandra Svihovec for technical assistance. Supported by Training Grants 1-T01-GM02212-02 and T-T01-CA0518-16, by National Cancer Institute Contract NIH-NCI-E-71-2174, and AEC Contract AT(30-1)-2943. This is Publication No. 1454 of the Cancer Commission of Harvard University.