Human Teratocarcinomas Cultured in vitro Produce Unique Retrovirus-like Viruses

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SUMMARY

We have previously reported that among a series of human tumours investigated, only human teratocarcinoma cell lines derived from testicular tumours or pulmonary metastases of patients in Germany and the U.S.A. produced retrovirus-like particles spontaneously, albeit in low amounts. In a recent publication electron microscopical data suggested that the human teratocarcinoma-derived (HTD) particles were morphologically closely related, but not identical, to the type C retroviruses of animals. In this communication, the explantation of three human teratocarcinoma cell lines is briefly described. Evidence is presented that HTD particles (i) are synthesized only in a fraction of the epithelioid and differentiating cells; (ii) can be induced biochemically in a manner characteristic of retroviruses; (iii) either are not infectious or possess a peculiar host range; (iv) are immunologically unrelated to animal retrovirus strains; (v) possess an endogenous RNA-dependent DNA polymerase activity that can be banded at 1.16 g/ml in linear sucrose gradients. These results may be taken as suggestive evidence that HTD particles represent a novel group of unique retroviruses.

INTRODUCTION

The question of the existence of human retroviruses remained a widely debated enigma (Deinhardt, 1980; Gallo et al., 1977; Gardner et al., 1977) until the remarkable isolation of unique retroviruses from adult T-cell lymphomas, first in R. C. Gallo's laboratory (Pozie et al., 1980; Reitz et al., 1981) and subsequently by Hinuma et al. (1981).

Previously, probably the most convincing piece of evidence that retroviruses may exist in man came from electron microscopical observations that particles with retrovirus morphology could be seen budding from human placental trophoblasts (Dalton et al., 1974; Dirksen & Levy, 1977; Imamura et al., 1976; Kalter et al., 1973a; Vernon et al., 1974). However, all attempts (including our own) to grow placenta-derived particles in tissue culture have failed. Subsequently, retroviruses have also been detected in the placentas of non-human primates (Ahmed et al., 1974; Benveniste et al., 1974; Kalter et al., 1973b, 1975; Schidlovsky & Ahmed, 1973; Seman et al., 1975; Smith et al., 1977) and of other mammals (Gardner, 1971; Gross et al., 1975; Hsiung et al., 1974; Smith et al., 1975).

In a series of related experiments performed by us and other laboratories, precipitating antibodies recognizing antigens of retroviruses from monkeys and mice were detected in human sera (Kurth et al., 1977; Kurth & Mikschy, 1978). Subsequent detailed studies showed, however, that these antibodies are probably heterophil in nature as they are directed against the carbohydrate moieties of the retrovirus envelope glycoproteins and thus may not represent antibodies primarily induced by retroviruses (Barbacid et al., 1980; Snyder & Fleissner, 1980; Löwer et al., 1981a). Before the carbohydrate-specificity of the antibodies was determined, we found that teratocarcinoma (TC) patients exhibited an unusually high anti-viral antibody titre
(Kurth et al., 1980). In retrospect, this increase may reflect the recognized influence of neoplasia and chemotherapy on natural heterophil antibody levels (Hirshaut et al., 1974; Shiku et al., 1977).

The two observations, namely the presence of retrovirus-like particles in placentas and the anti-viral immune reactivity in the sera of TC patients prompted us to investigate retrovirus production particularly in human tumours that contain embryonal or placental tissues. We describe in this communication that, of the many human tumour lines studied (e.g. from choriocarcinomas, seminomas, embryonal carcinomas), only the human teratocarcinoma cell lines produce particles with retrovirus characteristics. Independently of us, Bronson et al. (1978, 1979) also published electron microscopical observations of retrovirus-like particles in human TC cultures. In addition to the electron microscopical data from D. L. Bronson’s and our laboratory (Boller et al., 1983), evidence is now presented that human teratocarcinoma-derived (HTD) particles can be distinguished from virtually all presently known animal retrovirus strains not only on morphological, but also on immunological grounds. As HTD particles can be banded at the density characteristic of retroviruses and possess endogenous RNA-dependent DNA polymerase (RDDP) activity, they may indeed represent a new group of retroviruses.

METHODS

Cells. The Tera-1 and -2 human TC cells (Fogh & Trempe, 1975) were obtained from Dr J. Fogh (Sloan-Kettering Memorial Cancer Center, Rye, N.Y., U.S.A.). Three additional TCs, the GH, HL and ER lines, were established from surgical material obtained from the Department of Urology, Tübingen University (Kurth et al., 1980; Löwer et al., 1981b). Cell culture conditions have been previously described (Kurth et al., 1980; Löwer et al., 1981b).

Induction of HTD particles. A variety of chemicals known to increase animal retrovirus synthesis in tissue culture cells were investigated for their effects on TC cells and HTD particle production. We finally settled on a protocol that involved the addition of 10 μg/ml iododeoxyuridine (IUdR), 10^{-6} M-dexamethasone (DXM) and 1% dimethyl sulphoxide (DMSO) to the medium. One day later, medium was changed and supplemented only with 10^{-6} M-DXM and 1% DMSO. In other studies, a quantitatively similar virus induction effect was achieved by adding 3 μM-5'-azacytidine for 24 h to the tissue culture medium. TC cells were subsequently monitored for another 2 weeks to follow the synthesis of HTD particles.

Determination of beta human chorionic gonadotrophin (β-HCG). The β-HCG content of culture media was measured using a commercially available radioimmunoassay (Serono/Biodata, Freiburg, F.R.G.). Background β-HCG values, e.g. in the medium of IUdR-induced human embryo lung cell cultures, were below 5 × 10^{-3} International Units/ml (mIU/ml).

Co-cultivation. The indicator cell lines used for virus rescue attempts are listed in Table 2. These cells were either obtained from the American Type Culture Collection (C2Th, TblLu, Vero, FL, RD) or kindly supplied by Dr M. Essex, Boston (EF), Dr R. C. Gallo, Bethesda (CCL 64) or Dr G. Darai, Heidelberg (TEK, HF-AVTS). Prior to co-cultivation, all indicator cell lines were extensively tested for the absence of retroviruses by means of electron microscopy and RDDP assays. For the co-cultivation, TC cells were chemically induced by IUdR, DXM and DMSO. Two to 4 days after induction, indicator cell lines were added to the flasks. HTD particles could be observed electron microscopically in early passage co-cultivations. After 3 to 4 weeks and four to ten cell transfers, the teratocarcinoma cells had usually been lost from the cultures, as the indicator cells were growing faster. These indicator cells were then used at least one more time for another co-cultivation cycle with freshly induced TC cells. Cultures were checked for HTD particle production every 4 to 6 weeks by electron microscopy and RDDP assays.

Radioimmune competition assays. Purification and iodination of the main structural proteins (p30, gp70) of simian sarcoma-associated virus (SSAV) and baboon endogenous virus (BEV) used as labelled antigens have been described (Kurth & Mikschy, 1978; Wernicke & Kurth, 1981). These proteins were kind gifts of Dr W. Schäfer, Dr H.-J. Thiel and Dr D. Wernicke (Tübingen).

The following goat antisera to a wide variety of retroviruses were employed: anti-BEV, anti-RD114 (an endogenous feline retrovirus), anti-gp70 of feline leukaemia virus (FeLV), anti-Mason–Pfizer monkey virus (MPMV), anti-squirrel monkey retrovirus (SMRV), anti-bovine leukaemia virus (BoLV), anti-p30 BEV, anti-p30 RD114, anti-p30 FeLV and anti-p30 Friend leukaemia virus (FLV). All antisera were obtained through the courtesy of Dr H.-J. Thiel and Dr D. Wernicke (Tübingen).

The two observations, namely the presence of retrovirus-like particles in placentas and the anti-viral immune reactivity in the sera of TC patients prompted us to investigate retrovirus production particularly in human tumours that contain embryonal or placental tissues. We describe in this communication that, of the many human tumour lines studied (e.g. from choriocarcinomas, seminomas, embryonal carcinomas), only the human teratocarcinoma cell lines produce particles with retrovirus characteristics. Independently of us, Bronson et al. (1978, 1979) also published electron microscopical observations of retrovirus-like particles in human TC cultures. In addition to the electron microscopical data from D. L. Bronson’s and our laboratory (Boller et al., 1983), evidence is now presented that human teratocarcinoma-derived (HTD) particles can be distinguished from virtually all presently known animal retrovirus strains not only on morphological, but also on immunological grounds. As HTD particles can be banded at the density characteristic of retroviruses and possess endogenous RNA-dependent DNA polymerase (RDDP) activity, they may indeed represent a new group of retroviruses.
concentrations of cell lysates were preincubated (1 h, 4 °C) with limited amounts of antisera previously titrated to determine the 75% precipitation range of radiolabelled antigens. After preincubation, radioactive antigen was added and the radioimmunoassay continued as described (Kurth & Mikschy, 1978).

**Purification of HTD particles.** TC cells were grown in 1585 cm² roller vessels on a modular cell production roller apparatus (Belco). Cells were treated with 3 μM-5'-azacytidine for 24 h. Two days later, the supernatant was harvested twice per day for a period of 10 days, clarified by centrifugation at 15000 g for 15 min, and stored at −70 °C. After collecting about 40 litres, the material was thawed and processed (35000 rev/min, 15 °C, 5 l/h) in a continuous flow ultracentrifuge (model RK, Electro-Nucleonics, Fairfield, N.J., U.S.A.). During centrifugation, a 5 to 50% sucrose gradient was formed by the spontaneous mixing of a 60% sucrose cushion and the flow-through material. The gradient fractions (50 ml) were diluted to 100 ml with 0.01 M-Tris–HCl pH 7–2, 5 mM-MgCl₂, and pelleted at 60000 g for 60 min. The pellets were resuspended in 0.5 ml 0.01 M-Tris–HCl pH 7–2, 0.25 M-sucrose, 5 mM-MgCl₂.

**Endogenous RNA-dependent DNA polymerase assays.** Forty μl of each sucrose gradient fraction were tested for an endogenous RDDP activity in an assay mixture (final vol. 100 μl) containing 0.05 M-Tris–HCl pH 8–2, 0.08 M-KCl, 0.01 M-MgCl₂, 2 mM-dithiothreitol, 4 mM-phosphoenolpyruvate, 10 μg/ml pyruvate kinase, 0.15% NP40, 0.1 mM-actinomycin D, 2 mM-dATP, 2 mM-dCTP, 0.015 mM-[Me-3H]dTTP (sp. act. 10 Ci/mmol), 0.015 mM-[8–3H]dGTP (sp. act. 10 Ci/mmol). The reaction was performed at 37 °C for 30 min.

**Exogenous RNA-dependent DNA polymerase assays.** Culture supernatants were freed of cells and debris by centrifugation for 15 min at 1600 g. Virus was pelleted for 90 min at 90000 g and resuspended in 0.01 M-Tris–HCl pH 7–2, 0.25 M-sucrose, 5 mM-MgCl₂. Standard assay conditions: 0.05 M-Tris–HCl pH 8–3, 0.08 M-KCl, 0.5 mM-MnCl₂, 1 mM-dithiothreitol, 0.05% NP40, 0.005 mg/ml poly(rA)·(dT)₁₀, 0.5 mM-dATP and 0.2 mM-[3H]TTP (sp. act. 2.5 Ci/ml). Incubation was for 45 min at 37 °C. The results are calculated for a 1000-fold concentration of virus in the test tube compared to the culture supernatant. As control for DNA-dependent DNA polymerase, parallel assays with poly(dA)·(dT)₁₀ as template instead of poly(rA)·(dT)₁₀ were always performed.

**RESULTS**

**Growth of explanted teratocarcinoma cells**

Primary cultures were established from 1 mm³ cubes of tumour tissue. These explants as well as early passage cultures were freed from normal human fibroblasts by prolonged culturing without transfer. Under these conditions, fibroblasts, which were originally present in large excess, finally ruptured as monolayers, dissociated from the plastic dish and died in the supernatant medium. The TC cells, which are very sensitive to alkaline pH and trypsin treatment, remained attached to the plastic and exhibited a limited capacity to differentiate in culture, as they tended to form domes and vesicles. These vesicles could be aspirated and transferred to new dishes as an additional means of removing fibroblasts (Fig. 1).

Chromosomally integrated retroviruses are often silent, i.e. their expression is suppressed by largely unknown host cell controls. Explanting tumours in culture represents a stress situation often sufficient to activate retrovirus production. In accordance with these observations, retrovirus-like particles could not be detected in tissue specimens directly embedded from surgically excised tumours. However, subsequent electron microscopical studies of TC cells cultured for at least 6 to 8 weeks revealed that HTD particles were budding predominantly from cells growing in domes and vesicles (Löwer, 1983). Cultures were kept at high cell densities to increase vesicle formation and to facilitate their transfer. These cultures exhibited constitutive, increased particle production. Even Tera-2 cells, which originally grew as epithelioid monolayer cells and which were reported to be virus-negative (Bronson et al., 1979; Kurth et al., 1980; Löwer et al., 1981 b) were found recently to be low particle producers after long-term culture, during which the cells began to form domes and vesicles.

Another approach to improve HTD particle production was based on experiments with animal cells infected by retroviruses. Treatment of cells with a combination of IUdR, DXM and DMSO or with 5'-azacytidine led to a transiently increased synthesis of HTD particles, as judged by serial thin sections of TC vesicles. In Fig. 2, representative particles from four human teratocarcinoma cell lines are illustrated. Although they resemble C-type retroviruses (Frank et al., 1978), HTD particles can be morphologically distinguished by the lack of an electron-lucent space between envelope and nucleoid and by the absence of mature, collapsed cores. HTD particle morphology is not influenced by the host cell, as BEV or SSAV replicate with unaltered morphology in superinfected TC cells (Boller et al., 1983, and unpublished observations).
Restriction of HTD particle replication

In all five human TC lines only a proportion of the cells produced HTD particles. The fraction of particle-producing cells is difficult to evaluate, but may range from approximately a few percent in the case of Tera-2 cells to up to 20% in the case of GH cells, as judged from serial thin sections. It is not yet known why a proportion of the cells always remains virus-negative. These cells can neither be induced nor can they be infected by the HTD particles that are in any case
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Fig. 2. Electron micrograph of HTD particles. (a) HTD-2 particles from Tera-2 cells; (b) HTD-3 particles from GH cells; (c) HTD-4 particles from HL cells; (d) HTD-5 particles from ER cells. For an analysis of the fine-structural composition of HTD particles, see Boller et al. (1983). Bar marker represents 100 nm.

Table 1. Effect of iododeoxyuridine, dexamethasone and dimethyl sulphoxide on HTD particle production and β-human chorionic gonadotrophin synthesis

<table>
<thead>
<tr>
<th>Tumour</th>
<th>Particle designation</th>
<th>Particle production</th>
<th>β-HCG synthesis (mIU/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Before induction</td>
<td>After induction</td>
</tr>
<tr>
<td>Tera-1</td>
<td>HTD-1</td>
<td>+ *</td>
<td>+ +</td>
</tr>
<tr>
<td>Tera-2</td>
<td>HTD-2</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>GH</td>
<td>HTD-3</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>HL</td>
<td>HTD-4</td>
<td>+</td>
<td>+ + +</td>
</tr>
<tr>
<td>ER</td>
<td>HTD-5</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>JEG (human choriocarcinoma)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>JAR (human choriocarcinoma)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HEF (human embryonic fibroblasts)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* Arbitrary judgement based on electron microscopical evaluation of approximately 100 cells/cell line. † NT, Not tested.

present in the culture. It remains to be seen whether viral interference, virus defectiveness or cellular repressor mechanisms are responsible for the limitation of virus spread.

On the other hand, HTD particle production may be restricted to a certain differentiated cell type among the heterogeneous teratocarcinoma cell population. The virus-like particles in
human placentas bud only from the trophoblastic cell layer (Kalter et al., 1973a; Vernon et al., 1974), and so a characteristic marker of this cell type, namely β-HCG, was determined in the supernatant of TC cultures. As shown in Table 1, induction leads to significant levels of β-HCG in all tested TC lines. These results demonstrate the presence of trophoblast-like cells in the heterogeneous tumour cell cultures. The data may also be taken as suggestive, albeit not conclusive, evidence that cells of the trophoblastic lineage are responsible for the production of HTD particles.

**HTD particles are not infectious for a variety of animal cells**

Despite forced dome and vesicle formation and the use of various induction regimens (which are usually toxic to the cells), HTD particle production remained low, thus hampering virological characterization. It was desirable, therefore, to find new host cells which would support increased particle production. These studies turned out to be disappointingly negative. Despite repeated co-cultivation of the slowly growing teratocarcinoma cells, which therefore eventually disappeared from the culture, with a wide variety of virus-free human and animal indicator cell lines, no signs of virus production were observed in any of the indicator cells (Table 2). Knowing that the rescue of (endogenous) retroviruses by indicator cells may require months of intimate co-cultivation (for example, see Todaro et al., 1978), induced teratocarcinoma cells were repeatedly co-cultivated with the same batch of indicator cells for periods exceeding, in some cases, more than 1 year. In contrast, BEV could easily be rescued from superinfected TC cells simply by successfully infecting normal Cf2Th cells with supernatant from superinfected TC cells (data not shown).

**Animal retrovirus antigens cannot be detected in TC cells**

Immunological relationships between individual retrovirus strains can be demonstrated in so-called competition radioimmunoassays (RIAs). In these RIAs, the precipitation of a purified and radioactively labelled virus antigen by an anti-viral antiserum is competed by virus preparations or lysates from virus-infected cells. Successful competition means that the competing virus is immunologically related to the test virus antigen. We have used purified and iodinated major internal p30 core and gp70 envelope antigens of two prototype primate retrovirus strains, namely BEV and SSAV, which are marginally related immunologically. Against these antigens, antisera were employed which were specific for a wide variety of mammalian retrovirus strains or individual polypeptides purified therefrom. Lysates from induced TC cells, lysates from TC and animal cells infected by BEV, as well as lysates from uninfected human cells were used for competition.

As exemplified in Fig. 3, lysates from induced TC cells were unable to compete for p30 BEV or p30 SSAV in any RIA, no matter which anti-viral antiserum was used. In the controls, TC cells superinfected with BEV as well as infected animal control cells were able to compete successfully (Fig. 3a).

A summary of more than 170 additional permutations of different virus antigens, anti-viral antisera and cell lysates is presented in Tables 3 and 4. Lysates from human TC cells did not compete significantly for the iodinated viral antigens. The results suggest that HTD particles are at best marginally related, if at all, to any of the presently known retrovirus strains.

**Demonstrations of endogenous RDDP activity**

Initial attempts to concentrate HTD particles by density-gradient ultracentrifugation were unsuccessful. Forced dome and vesicle formation in combination with chemical induction procedures and adaptation to growth in roller bottles finally resulted in particle yields that were demonstrable by endogenous RDDP activity (Fig. 4) and by electron microscopy (Boller et al., 1983). Particles banded at the density of 1.16 g/ml in 20 to 55% (w/v) linear sucrose gradients, which is characteristic of the density of animal retroviruses. Contamination of density gradient-purified HTD particle preparations by cellular DNA polymerases, which could mimic endogenous RDDP activity, appears highly unlikely as these polymerases should not band at 1.16 g/ml and would accept exogenous templates.
Table 2. Co-cultivation of Tera-1, Tera-2 and GH human teratocarcinoma cells with a variety of indicator cell lines for the determination of the infectivity and host range of HTD particles*

<table>
<thead>
<tr>
<th>Indicator cell line</th>
<th>Source</th>
<th>Tera-1 No. of passages†</th>
<th>Virus search by</th>
<th>Tera-2 No. of passages†</th>
<th>Virus search by</th>
<th>GH No. of passages†</th>
<th>Virus search by</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cf2Th</td>
<td>Canine thymus</td>
<td>72</td>
<td>–</td>
<td>0.9</td>
<td>60</td>
<td>–</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>FEF</td>
<td>Feline embryo fibroblasts</td>
<td>10</td>
<td>–</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td>CCL 64</td>
<td>Mink lung</td>
<td>60</td>
<td>–</td>
<td>&lt;0.2</td>
<td>58</td>
<td>–</td>
<td>1.9</td>
</tr>
<tr>
<td>TblLu</td>
<td>Bat lung</td>
<td>70</td>
<td>–</td>
<td>2.8</td>
<td>55</td>
<td>–</td>
<td>1.6</td>
</tr>
<tr>
<td>Z-5</td>
<td>Goat connective tissue</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>TEK</td>
<td>Tupaia embryo kidney</td>
<td>52</td>
<td>–</td>
<td>0.7</td>
<td>55</td>
<td>–</td>
<td>2.9</td>
</tr>
<tr>
<td>HF-AVTS</td>
<td>Marmoset fibroblasts</td>
<td>55</td>
<td>–</td>
<td>3.1</td>
<td>55</td>
<td>–</td>
<td>0.4</td>
</tr>
<tr>
<td>Vero</td>
<td>African green monkey kidney</td>
<td>31</td>
<td>–</td>
<td>1.3</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>FL</td>
<td>Human amnion</td>
<td>23</td>
<td>–</td>
<td>&lt;0.2</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>RD</td>
<td>Human rhabdomyosarcoma</td>
<td>31</td>
<td>–</td>
<td>3.1</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

* Abbreviations: EM, electron microscopy; RDDP, RNA-dependent DNA polymerase, pmol [3H]TMP incorporation with poly(rA)-(dT)₆ as template; NT, not tested; –, no HTD particles detected.
† Number of passages of indicator cells after co-cultivation.
(a) 80 ~ 60 40~ 20
(b) 100 ~ 80 60 ~ 40

Fig. 3. Radioimmune competition assays against iodinated p30 virus core proteins of BEV (a) and SSAV (b). Hyperimmune goat antisera to the purified p30s of SSAV (a) or RD114 virus (b) were employed. 100% antigen precipitation is the maximum precipitation when no cell lysate is added. Lysates from the following cells were used for competition: △, ▽, ▲, ○, ●, induced GH, HL, ER, Tera-1 and Tera-2 lines, respectively. △, ▽, ▲, ○, BEV-superinfected GH, HL, Tera-1 and Tera-2 lines, respectively. The following cell lines were used as controls: ●, uninfected human embryonic lung cells (HEL); ○, uninfected human embryonic fibroblasts (HEF); ○, uninfected human amnion cells (AV-3); X, A204 human rhabdomyosarcoma cells infected with BEV; ◊, normal rat kidney cells infected with SSAV.

Lack of demonstrable exogenous RDDP activity

In extensive RDDP assays with the three best-growing and highly differentiated TC cell lines, exogenous enzyme activity could not regularly be detected, regardless of whether MnCl₂ or other divalent cations (Mg²⁺, Zn²⁺, Co²⁺, Fe²⁺, Ni²⁺) were employed and titrated to stimulate the activity. No other templates, e.g. poly(rC)·(dG)₁₂₋₁₈ and poly(rCm)·(dG)₁₂₋₁₈, revealed convincing RDDP activity. In contrast, supernatants of TC cells previously superinfected with BEV yielded low levels of exogenous RDDP activity (R. Löwer et al., unpublished results). As HTD and BEV particles are produced in roughly equal amounts in superinfected Tera-1 and GH cells, exogenous RDDP activity should have been measurable in particle preparations from the original TC cells, if the corresponding HTD-1 and HTD-3 particles had contained such activity.

DISCUSSION

Viruses are still being classified and defined primarily by their electron microscopical morphology and by their mode of replication. By these criteria, HTD particles resemble C-type retroviruses (Boller et al., 1983), except for the consistent morphological differences briefly mentioned above.
Contamination by animal retrovirus strains has to be ruled out rigorously whenever the claim is made that retrovirus-like particles can be detected in human cells cultured in vitro. In our Institute, the TC cultures are handled in laboratories physically separated from all other laboratories and by people not involved in other retrovirus projects. More decisive evidence, however, is the results presented in this communication, namely that by criteria concerning morphology, host range and immunology HTD particles can be distinguished from all the other retrovirus strains tested. As previously discussed (Boller et al., 1983), HTD particles do not seem to be related to the human T-cell lymphoma viruses (HTLV).

It is not yet known whether the lack of infectivity is due to a peculiar host range, which would not be surprising as some retrovirus strains replicate exclusively in specific cell types, or whether it is due to defects or immaturity of HTD particle RNA or proteins. The study of these problems has been initiated with 40 litre tissue culture batches from which HTD particles can be banded.
Fig. 4. Endogenous RDDP activity exemplified by sucrose density gradient-banded HTD-3 particles. Peaks of enzyme activity measured as ct/min (■) are demonstrable at densities (▲) 1.16 g/ml and, less regularly, at 1.21 g/ml. These peaks correspond to densities characteristic of intact retroviruses and virus cores, respectively. Protein concentration (●) was determined by the Lowry method.

There is still no satisfying explanation for the lack of convincing exogenous RDDP activity of HTD particles, especially as one would expect a polymerase molecule that is functional in the endogenous assay also to be effective with exogenous template–primers. In this context, it is interesting to note that superinfection of TC cell lines with BEV leads to easily detectable BEV replication, as judged by electron microscopy and BEV rescue experiments (data not shown). On the other hand, exogenous RDDP activity is low, albeit demonstrable, in preparations of BEV grown in dome- and vesicle-forming cultures, as compared to BEV grown in a variety of other host cells.

We have recently obtained evidence that TC cell cultures with high proportions of domes and vesicles and few fibroblastic and epithelioid cells produce a factor that is very effective in the inhibition of the exogenous RDDP activities of all animal retrovirus strains tested so far. This inhibitor is presently being purified to determine its nature and mode of action. It remains to be seen whether it has a relationship to the inhibitors of RDDP activity described in preparations of human trophoblasts (Nelson et al., 1981), in bovine seminal plasma (Reddy et al., 1983), and in lysates of a series of cultured cells (Rokutanda et al., 1982).

As mentioned above, the HTD particles very much resemble in their morphology the human placenta particles, which can hardly be considered to be oncogenic and which may well be of endogenous origin. On the other hand, various so-called non-producer animal tumour cell lines have been established by exogenous infection, which subsequently produced non-infectious avian or mammalian sarcoma viruses (Demsey et al., 1980; Hanafusa & Hanafusa, 1968; Kawai & Hanafusa, 1973; Ramsay & Hayman, 1980; Yoshinaka et al., 1980). Some of these defective
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virus strains morphologically resemble HTD particles. This coincidence, however, should not be regarded as evidence that HTD particles are sarcomagenic.

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