Quantitative Assay of Human T-Cell Leukemia/Lymphoma Virus Transformation


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ABSTRACT

The in vitro transformation of normal T-lymphocytes by human T-cell leukemia/lymphoma virus (HTLV-I) is possible utilizing cocultivation techniques. We now report on a quantitative assay for HTLV-I transformation. Transformed cell lines were produced by cocultivation of either preactivated (phytohemagglutinin and T-cell growth factor) or nonactivated peripheral blood mononuclear cells with an equal number of lethally irradiated HTLV-I-positive donor cells (MT-2). After 14 days in liquid culture, transformed cells were plated in a 2-layer soft agarose system with or without T-cell growth factor (TCGF). Colony formation among 50 normal controls was observed at varying efficiencies with a mean number of 179 colonies (range, 6–599) in the presence of TCGF (up to a 2-log difference). The day 14 T-cell cultures demonstrated relatively low colony-forming efficiencies (<0.1%) and enhanced colony formation in the presence of TCGF. Day 14 after cocultivation was chosen for this assay based on a dose-response relationship between colony formation and the virus-positive donor cell inoculum and the known kinetics of colony growth of normal activated T-cells. An analysis of individual colonies indicated that they were of target cell origin and HTLV-I positive. Recombinant β-interferon in increasing concentrations caused a decrease in colony formation as measured in this assay. Long-term cell cultures (2–18 months) showed higher colony-forming efficiencies (up to 1.0%) which were not enhanced by TCGF. The ability to quantitatively evaluate transformation via colony counts will provide an opportunity to study differences in transforming efficiencies attributable to varying target cells, donor cells, or blocking factors such as interferons, drugs, or anti-HTLV-I antibodies.

INTRODUCTION

HTLV-I2 is a novel type C retrovirus isolated initially from a patient with mature T-lymphocytic neoplasia (1) and is associated with human adult T-cell leukemia and lymphoma (2, 3). HTLV-I demonstrates tropism for T-cells, usually of OKT4 phenotype, and has the ability to transform T-cells in vitro (4–6). In 1982 Yamamoto described a technique for producing HTLV-I-transformed umbilical cord and adult cell lines by cocultivation with lethally irradiated MT-2 cells as the source of virus (7). By modifying this process, Meri et al. (5) showed that transformation of both adult and umbilical cord T-cell lines could be routinely obtained when target cells were “preactivated” with PHA and TCGF. This additional step allowed for the production of both adult and umbilical cord transformed T-cell lines independent of exogenous TCGF from both high- and low-titer HTLV-I-producing donor T-cell lines.

As a biological assay for the detection of virus-containing cells, the isolation of HTLV-I from fresh specimens or, as a test for the relative transforming ability of different virus-positive cell lines, the preactivated liquid culture transformation assay necessarily results in an “all or none” effect. A virus-transformed cell line is either produced or not. Moreover, indicators of the “efficiency of transformation” are generally qualitative, such as consumption of media, time to passage of cultures, and the time required to achieve TCGF-independent growth. A level of quantitation can be achieved by determining the minimum number of lethally irradiated MT-2 cells required for transformation in liquid culture (7). However, liquid culture assays do not readily allow for measuring the quantitative effects of antiviral agents such as suramin (8). Since routine cell-free transformation of T-lymphocytes by HTLV-I has been difficult to date (6, 9) and it has not been possible to develop an assay that measures direct virus-transforming units, we elected to use a cocultivation system. In an attempt to quantify “the frequency of transformation” in such a system we modified a 2-layer soft agarose system which had been successfully adapted to support the growth of normal T-lymphocytes with the formation of clonogenic colonies (10, 11). We were interested in the potential of this assay to provide a quantitative assessment of various parameters such as the efficiency of HTLV-I transformation at different inocula of virus-positive cells, the susceptibility to HTLV-I transformation of various target cells, and the effects on transformation of various blocking factors such as interferon, drugs, or anti-HTLV-I antibodies. The current report describes our initial experience with this assay.

MATERIALS AND METHODS

Cocultivation and Transformation. Approximately 40 ml of blood were collected in sterile heparinized tubes from 50 normal donors. PBMC were obtained by ficoll-hypaque centrifugation (LSM; Bionetics Laboratory Products). All normal donors were negative for HTLV-I antibodies (see below). A portion of the cells were cultured in RPMI 1640 medium (GIBCO Laboratories) with 20% FCS (GIBCO Laboratories), penicillin (100 units/ml), and streptomycin (100 μg/ml). The rest of the cells from each sample were placed in culture with similar media with 20% delectinated, partially purified TCGF (Cellular Products, Inc.) and activated with 5 μg/ml of PHA (Difco Laboratories).

After 24 h, the preactivated cells (those cultured with PHA and TCGF) were centrifuged at 400 × g for 10 min, the PHA-containing supernatant was removed, and the cells were resuspended in media containing 20 μg/ml TCGF at a concentration of 1 × 105 cells/ml. The nonstimulated cells were adjusted to a similar concentration without TCGF. One-half ml of the respective suspensions (5 × 105 cells) was placed in 25-cm2 culture flasks. These represented the target cells.

The HTLV-I donor cell line used was MT-2 (6), a HTLV-I-transformed cord blood T-cell line obtained from Dr. I. Miyoshi. The donor cells were lethally irradiated to 90 Gy (Gammarad 1000) and then centrifuged at 400 × g for 10 min and resuspended in fresh media at a concentration of 1 × 105 cells/ml. One-half ml of the suspension of donor cells (5 × 105 cells) was added to the aliquots of target cells (5 × 105 cells). The 25-cm2 culture flasks were maintained on end until a total of 5 ml of cell suspension was present in the flask, thus allowing for close contact of target and donor cells. Control samples of target cells alone and irradiated MT-2 cells alone were maintained. Fresh cell culture media, RPMI 1640 plus 20% FCS, was added 3 times per week. No additional TCGF was added before plating in the semisolid assay.

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In addition to plating the cultures in semisolid media the initial 11 cultures (Table 1) were maintained in liquid culture. Transformation in liquid culture was qualitatively defined as being positive if continuous proliferation of target cells could be maintained independent of exogenous TCGF for >4 weeks. The last 39 cultures were plated in semisolid media at day 14 and were not maintained in liquid culture.

Quantitative Assay. After 14 days of growth in liquid culture, the T-cell suspensions were plated in triplicate in 35-mm plastic Petri dishes using a 2-layer semisolid media system. The underlayer consisted of a 1-ml mixture of 0.5% agarose, RPMI with 10% FCS, and antibiotics with or without 20% TCGF. For the overlayer, single T-cell suspensions were easily obtained with a Pasteur pipet. Cells were counted in a hemocytometer and 5 x 10^5 viable cells of the heaviest T-cell suspensions were plated in triplicate in 35-mm plastic Petri plates. The cell suspensions three times per week. The cells were passaged in Linbro plates until the cultures could be transferred to 25-cm^2 culture flasks. The resultant cultures were tested for karyotype and HLA haplotype. Target cells of the gender opposite the donor cells were chosen to facilitate identity of the transformed cells by karyotype.

Karyotype. Cytogenetic examination was performed on colchicine-fixed Giemsa-trypsin G-banded metaphases (13) and chromosomal analysis was carried out according to the methods in ISCN, 1978 (14).

Nucleic Acid Hybridization. Transformed cells were tested for the presence of integrated HTLV-I proviral DNA by slot blot molecular hybridization (15). DNA was extracted, isolated, and purified as previously described (16). DNA was transferred to nitrocellulose membranes and prehybridized in the following solution, 5x SSC (1x SSC = 0.15 M NaCl-0.015 M sodium citrate, pH 7.0), 50% formamide, 0.2% sodium dodecyl sulfate, 0.2% Ficoll, 0.2% bovine serum albumin, 0.2% polyvinylpyrrolidone, and 50 mM Tris-HCl (pH 7.5), then hybridized at 37°C overnight in the same solution plus 10% dextran sulfate, 50 μg/ml calf thymus DNA, and 10 x 10^6 cpm of 32P-labeled HTLV-I env/px DNA fragment, pH, kindly provided by Dr. V. S. Kalyanaraman at the Centers for Disease Control, Atlanta, GA. After hybridization, the filters were washed in 2x SSC-0.2% sodium dodecyl sulfate at 60°C and exposed to x-ray film with intensifying screens at -80°C (15).

HLA Antigens. HLA antigens were determined by a standard 2-stage cytotoxicity assay using approximately 118 antisera capable of identifying 93 HLA specificities (36A and 57B antigens) (17).

Anti-HTLV-I ELISA Assay. Serum from all normal donors was tested for IgG and IgM antibodies to purified, disrupted HTLV-I viral proteins utilizing an ELISA assay described elsewhere (18).

Mixed Lymphocyte Culture. To assess whether colony formation was the result of a mixed lymphocyte culture phenomenon, cocultivation of normal target cells was carried out with irradiated PBM from a normal donor of different HLA type. The cocultivation procedure was the same as described above except the irradiated PBM were substituted for viral donor cell lines. Cells were plated in semisolid media at day 14 as described above.

Long-Term Liquid Cultures. Cells from the first 11 transformation assays which were not plated were placed back in 25-cm^2 culture flasks and maintained in liquid culture with RPMI 1640 plus 20% FCS. In addition, 2 HTLV-I-transformed normal adult (UMC-A5+/MT2 and UMC-A4+/HUT102) and 2 umbilical cord (UMC-C10+/MT2 and UMC-C2+/MT2) cell lines were plated after 2-18 months in culture.

RESULTS

To determine the optimal donor cell concentration for plating at day 14 and to assess the effect of the inoculum of virus-

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<tbody>
<tr>
<td>A+/MT-2</td>
<td>+ TCGF</td>
<td>+ TCGF</td>
<td>+ TCGF</td>
<td>+ TCGF</td>
</tr>
<tr>
<td>UMC-A20/MT-2</td>
<td>0</td>
<td>0</td>
<td>173 ± 12</td>
<td>165 ± 6</td>
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<tr>
<td>UMC-A21/MT-2</td>
<td>0</td>
<td>0</td>
<td>68 ± 8</td>
<td>110 ± 6</td>
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<tr>
<td>UMC-A22/MT-2</td>
<td>0</td>
<td>0</td>
<td>3 ± 1.7</td>
<td>190 ± 18</td>
</tr>
<tr>
<td>UMC-A23/MT-2</td>
<td>21 ± 3.5</td>
<td>32 ± 1.5</td>
<td>6 ± 3.3</td>
<td>114 ± 14</td>
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<tr>
<td>UMC-A24/MT-2</td>
<td>38 ± 6.5</td>
<td>65 ± 10</td>
<td>229 ± 8.5</td>
<td>419 ± 11</td>
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<tr>
<td>UMC-A25/MT-2</td>
<td>0</td>
<td>0</td>
<td>149 ± 17</td>
<td>169 ± 18</td>
</tr>
<tr>
<td>UMC-A26/MT-2</td>
<td>0</td>
<td>0</td>
<td>356 ± 26</td>
<td>256 ± 9.6</td>
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<tr>
<td>UMC-A27/MT-2</td>
<td>153 ± 28</td>
<td>71 ± 30</td>
<td>532 ± 113</td>
<td>419 ± 33</td>
</tr>
<tr>
<td>UMC-A5+/MT2</td>
<td>61 ± 17</td>
<td>36 ± 14</td>
<td>118 ± 32</td>
<td>88 ± 34</td>
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<tr>
<td>UMC-A14/MT2</td>
<td>2 ± 1.5</td>
<td>40 ± 5</td>
<td>41 ± 7.5</td>
<td>480 ± 73</td>
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<tr>
<td>UMC-A28/MT2</td>
<td>0</td>
<td>0</td>
<td>79 ± 10</td>
<td>133 ± 49</td>
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<tr>
<td>UMC-A29/MT-2A67/MT-2</td>
<td>2.3 ± 6.2</td>
<td>2.5 ± 11.9</td>
<td>189 ± 118</td>
<td>166 ± 124</td>
</tr>
<tr>
<td>Totals: 50 normal donors</td>
<td>6.8 ± 24</td>
<td>6.2 ± 18</td>
<td>183 ± 127</td>
<td>175 ± 125</td>
</tr>
</tbody>
</table>

* Data of first 11 cultures provided in detail. The last 39 cultures were plated at day 14 and not maintained in liquid culture (UMC-A29/MT-2 to UMC-A67/MT-2).

* UMC is an institutional prefix. A refers to adult target cells. The superscript + indicates preactivation of target cells with PHA and TCGF. The superscript - indicates no preactivation. The source of virus is indicated after the slash. NT, not tested.

* PBM were plated in triplicate and colony counts are expressed as the mean ± SD.

* Qualitative assessment of transformation based on continuous proliferation of target cells independent of TCGF for greater than 4 weeks.

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positive cells on colony formation, varying concentrations of MT-2 were cocultivated with a constant number of normal target cells (5 x 10⁶ cells/ml) (Fig. 1). The number of colonies increased with an increase in the inoculum of MT-2. Maximal colony formation occurred from 2 x 10⁵ to 1 x 10⁶ cells/ml. At 5 x 10⁶ cells/ml there was a decrease in colony formation. Virus-positive donor cells at a concentration of 5 x 10⁵ cells/ml were chosen for further cocultivation experiments.

PBM were harvested from 50 normal controls and were cocultivated with a lethally irradiated HTLV-I-producing cell line (MT-2). All normal controls were found to be seronegative for IgG and IgM HTLV-I antibodies by ELISA assay. Control PBM not exposed to a source of virus as well as the irradiated MT-2 cells exhibited no growth in liquid culture after 14 days as expected. The initial 11 normal PBM cocultivated with MT-2 were maintained in liquid culture and each resulted in the establishment of a TCGF-independent HTLV-I-positive T-cell line (Table 1).

At day 14, equal volumes of comparably passaged cell suspensions were plated in semisolid media with or without 20% TCGF and colony formation occurred with varying efficiencies in all target cells exposed to HTLV-I (Table 1). Fig. 2A shows the appearance of a single cell suspension after plating on day 1. After 14 days of incubation, only rare colonies were observed in control plates with target or irradiated donor cells alone. The mean number of colonies was: A⁺, 0.2; A⁻, 0.5; MT-2, 1.4; A⁺ + TCGF, 2.9, A⁺ + TCGF, 0.2; MT-2 + TCGF, 2.2 (see Table 1 for abbreviations). Those samples cocultivated with MT-2 routinely formed colonies at day 14 (Fig. 2B). The day 14 T-cell cultures demonstrated relatively low plating efficiencies (≤0.1%), with mean numbers in the presence of TCGF of 183 colonies (range, 6–542) and 175 colonies (range, 12–599) in the nonactivated (A⁻/MT-2) and preactivated (A⁺/MT-2) state, respectively (Table 1). The mean number of colonies formed in the absence of TCGF were 6.8 (range, 0–153) and 6.2 (range, 0–73) in the nonactivated (A⁻/MT-2) and preactivated (A⁺/MT-2) state, respectively (Table 1). The majority of cocultivations, 37 of 50 (74%), failed to form colonies in the absence of TCGF. Preactivation with PHA and TCGF resulted in enhancement (greater than 5-fold increase) of colony formation in 4 cell lines, UMC-A22/MT-2, UMC-A23/MT-2, UMC-A38/MT-2, and A14/MT-2 (Fig. 3), but when all 50 cultures are considered, no statistically significant difference was seen between the nonactivated and preactivated state (Table 1). Cocultivation of normal target cells with irradiated normal HTLV-I-negative PBM of different HLA haplotypes was performed in 6 separate experiments with no colony formation (data not shown).

Cells from individual colonies in semisolid media were transferred back to liquid suspension culture and grown continuously with TCGF. These proved to be of target cell origin by virtue of karyotype and HLA antigen haplotype analysis (Table 2). A unimodal chromosome number of 46 with an XX sex chromosome complement was present in all metaphase chromosomes examined. No structural abnormalities were observed. All colonies tested contained HTLV-I homologous DNA sequences by slot blot hybridization analysis (Fig. 4).

The effect of recombinant β-interferon on colony formation at day 14 was measured in 4 separate experiments (Fig. 5). A reproducible, progressive decrease in colony count was seen with increasing concentrations of β-interferon. Complete inhibition of colony formation was observed at a β-interferon concentration of 800 IU/ml.
were plated (Fig. 6). Compared to those obtained when short-term (day 14) cultures were formed only rare colonies. Preactivation with PHA and TCGF (UMC-A14*/MT-2) enhanced colony formation over nonactivated target cells (UMC-A14~/MT-2). The addition of TCGF (Table 3) enhanced colony formation of day 14 transformed cell lines.

2). The addition of TCGF enhanced colony formation over nonactivated target cells (UMC-A14~/MT-2) enhanced colony formation over nonactivated target cells (UMC-A14~/MT-2). These colonies were also larger than those formed when short-term (day 14) cultures were plated (Fig. 6).

DISCUSSION

In an attempt to quantitate transformation, we modified a 2-layer semisolid agarose system optimized for normal T-cell colony growth (11). In that study it was apparent that colony formation in semisolid media was a function of previous time in liquid suspension culture prior to plating. The colony growth of normal activated lymphocytes peaked at approximately 5 days in liquid culture and fell gradually so that no normal T-cell colonies were observed in liquid cultures maintained for at least 14 days prior to plating. Also, from previous studies we know that the HTLV-I-positive, lethally irradiated donor cells required for transformation can no longer be detected after 14 days of liquid culture and would not complicate the interpretation of the results. Hence, the 14-day period of prior liquid culture was chosen in this semisolid HTLV-I transformation assay to assure that only the transformed cells would grow.

Furthermore, the mean number of colonies formed at day 14 in this assay approximates that suggested for optimal interpretation of the human tumor cloning assay which has been utilized primarily for chemosensitivity and cytotoxicity assays of various malignancies (19, 20).

In the current report transformed cells routinely formed colonies in semisolid media with varying plating efficiencies. We proved that the colonies were of target cell origin by virtue of karyotype and HLA typing and were HTLV-I-positive by nucleic acid hybridization analysis (Table 2; Fig. 4). We hypothesize that each colony represents a clone of a transformed, infected target T-cell and thus the number of colonies is related to the number of target cells initially infected by cocultivation. It is likely that some of the colonies may represent progeny of cells infected during the early days of liquid culture, and thus this assay cannot necessarily be construed to measure for HTLV-I infectious units. The clonal nature of each of the colonies and the polyclonal or oligoclonal nature of the initial HTLV-I infection awaits further analysis by surface phenotype and T-cell antigen receptor gene rearrangement studies (21) and Southern blot analysis of proviral integration sites. Studies of cell-free transformation suggest an initial polyclonal infection which becomes monoclonal only after long-term culture (≥60 days) (9). Thus, even with some degree of amplification in liquid culture, which should be minimal at day 14, the assay provides a useful numerical assessment of HTLV-I transformation.

It is apparent that there is variation in the efficiency of transformation depending on donor and target cell characteristics (5). In the present study, the results of the quantitative transformation assay are not due solely to a mixed lymphocyte reaction because cocultivation of irradiated normal donor mononuclear cells with target mononuclear cells produced no colony formation. Target cells transformed by MT-2 demonstrated quantifiable colonies after 14 days of cocultivation. Colony formation by these early T-cell cultures was characterized by relatively low colony-forming efficiency (≤0.1%) and was usually dependent upon the presence of TCGF in the semisolid media (Table 1). As time in liquid suspension culture increased, so did colony-forming efficiency. Established, long-term transformed T-cell lines demonstrated the highest colony-forming efficiencies (up to 1.0%) which were independent of TCGF (Table 3). The higher colony-forming efficiencies observed in established cell lines as compared with those seen in early cultures may be accounted for by the greater number of integrated proviral DNA copies per haploid genome found in the later cell lines (data not shown) and/or by increased expression of the gene(s) that are responsible for HTLV-I transformation (22, 23) or simply clonal selection. It is obvious then that time in liquid culture must be a controlled variable for this

Table 2 Confirmation of target origin of transformed cell lines of UMC-A22/MT-2*

<table>
<thead>
<tr>
<th>Colony</th>
<th>HLA antigens</th>
<th>Karyotype</th>
<th>Transformed cells</th>
<th>HLA antigens</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>A1, A2, B8, B12 (44)</td>
<td>46,XX</td>
<td>A1, A2, B8, B12 (44) [A34, A28, B62, B17, B18, B27]</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>A1, A2, B8, B12 (44)</td>
<td>46,XX</td>
<td>A1, A2, B8, B12 (44) [A28, A31, A32, B62, B16, B17, B18]</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>A1, A2, B8, B12 (44)</td>
<td>46,XX</td>
<td>A1, A2, B8, B12 (44) [A34, A28, B17, B18, B55, B42]</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>A1, A2, B8, B12 (44)</td>
<td>46,XX</td>
<td>Insufficient viability</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>A1, A2, B8, B12 (44)</td>
<td>46,XX</td>
<td>A1, A2, B8, B12 (44) [A34, A28, A11, A31, A29, B14, B62, B17, B18, B42]</td>
<td>46,XX</td>
<td></td>
</tr>
</tbody>
</table>

* Donor cells, MT-2; karyotype, 46,XY.
* Five separate colonies resuspended in media and grown until a sufficient number of cells were present for analysis.
* Data in brackets, extra HLA antigens determined in the transformed cells.
assay to be used in comparative studies of HTLV-I transformation.

The data in Fig. 1 demonstrate that the number of HTLV-I-transformed colonies observed in this assay system correlates with the number of virus-positive donor cells initially added. However, the effect was not linear and no colony formation was observed at an inoculum of less than $5 \times 10^4$ MT-2 cells. Hence the number of virus-positive donor cells used in comparing HTLV-I transformation is also critical for subsequent interpretation. In the present studies we have chosen to use a high inoculum of MT-2 cells to maximize transformation.

From the previous transformation studies it is clear that certain target cells are highly efficient transformers characterized by very early, rapid proliferation independent of TCGF (5, 7). When assayed in the present system after 14 days of liquid culture and a high inoculum of MT-2, up to a 2-log (10) difference in colony formation was observed among 50 different samples. Also, in the absence of added TCGF, colonies were formed in only 13 of 50 target cell samples, while all cultures demonstrated enhanced colony formation in the presence of TCGF. The reasons for this are uncertain at this time but it is reasonable to speculate that those colonies formed independent of TCGF represent a more transformed state than those that are TCGF dependent. Comparative studies on both types of colonies would offer an opportunity to better understand the molecular mechanisms of HTLV-I-induced T-cell transforma-

<table>
<thead>
<tr>
<th>Table 3 Relative colony-forming efficiency of established T-cell culture lines in the presence or absence of TCGF</th>
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<tr>
<td>Cell line</td>
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<tr>
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</tr>
<tr>
<td>UMC-A5+/MT-2</td>
</tr>
<tr>
<td>UMC-A5+/MT-2</td>
</tr>
<tr>
<td>UMC-A4+/MT-2</td>
</tr>
<tr>
<td>UMC-A4+/MT-2</td>
</tr>
<tr>
<td>UMC-C10+/MT-2</td>
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<tr>
<td>UMC-C10+/MT-2</td>
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<tr>
<td>UMC-C2+/MT-2</td>
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<tr>
<td>UMC-C2+/MT-2</td>
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</tbody>
</table>

* UMC is an institutional prefix. C and A refer to cord and adult target cells, respectively. The superscript + indicates preactivation of target cells with PHA and TCGF. The superscript − indicates no preactivation. The source of the virus is indicated after the slash.* T-lymphocytes were plated in triplicate and colony counts are expressed as the mean ± SD. 

This semisolid assay provides a quantitative assessment of HTLV-I transformation. In this study, 4 of 50 normal adult T-cells showed much higher colony formation with activation by PHA and TCGF (Table 1; Fig. 3). However, no significant difference between nonactivated and preactivated conditions was observed for the group as a whole. The reasons for this are unclear but it is possible that using MT-2, a relatively high virus-producing donor cell line, at a high inoculum, abrogated the effect of other factors. Indeed, MT-2 was able to transform 5 of 5 nonactivated cord and 6 of 7 nonactivated adult target cells in a previous study (5). Perhaps using donor cells which are lower producers of HTLV-I would show an enhancement of colony formation with activation.

Clearly, this semisolid assay provides a quantitative assessment of HTLV-I transformation. One potential use is evaluation of the relative differences in the transforming ability of different virus-positive donors. More importantly, the assay
may be useful in establishing host factors important in the susceptibility to HTLV-I infection. Factors such as HLA haplotype, absolute lymphocyte count, helper/suppressor T-cell ratio, and the role of intercurrent viral infections could be investigated. Finally, it enables one to quantitate the effect of blocking factors such as anti-HTLV antibodies, interferon (Fig. 5), and other cytotoxic or antiviral drugs.

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