Efficient Transformation of Previously Activated and Dividing T Lymphocytes by Human T Cell Leukemia-Lymphoma Virus


Modifying previously reported techniques, we attempted to increase the efficiency of human T cell leukemia-lymphoma virus (HTLV) transformation of human T lymphocytes. Lethally irradiated donor cells (DCs) were cultured with target mononuclear cells (TMCs). DCs included ten HTLV + T cell lines with varying degrees of virus expression or seven cell lines that do not express HTLV. TMCs were prepared from 20 cord and 16 adult peripheral blood samples, including eight patients with acquired immunodeficiency syndrome (AIDS). TMCs were either added directly to the DCs or were first stimulated with phytohemagglutinin (PHA) (5 μg/mL) and grown in T cell growth factor (TCGF) prior to exposure to DCs. The presence of integrated HTLV proviral DNA in the transformed cells was determined by dot blot hybridization, utilizing a cloned probe to the HTLV-I genome. HTLV production by transformed TMCs was assessed for HTLV p19, reverse transcriptase, and virus particles. No transformation occurred with T cell donor lines that do not express HTLV. Low virus expressor DCs could only, with rare exception, transform preactivated TMCs. High-titer virus-producing DCs could transform activated and nonactivated cord blood cells and activated adult TMCs. Only MT-2 could routinely transform nonactivated normal adult and activated AIDS TMCs. HUT 102 B2 could transform only one activated AIDS sample, the cells of which initially expressed HTLV-like proteins and virions. Transformed cell lines contained subsets of mature T lymphocytes with variable HTLV expression. Prior activation and culture of the T lymphocytes increases the probability and rate of transformation by HTLV, allowing for biologic detection of low HTLV-producing cells and for in vitro expansion of T lymphocyte subsets from selected patients.

THE HUMAN T CELL leukemia-lymphoma virus (HTLV) is a novel type C retrovirus associated with mature T cell neoplasia throughout the world. The detection of HTLV has been made possible by the availability of purified T cell growth factor (TCGF), which permits long-term culture of T lymphocytes. Methods for determining the presence of HTLV have included analyzing cultured T lymphocytes for reverse transcriptase (RT), virus particles, HTLV core proteins, and integrated HTLV genomic sequences. In order to detect a small percentage of virus-positive cells, as may occur in vivo, a means of amplifying the number of infected cells to be evaluated or a sensitive biologic assay is necessary.

Previous attempts at transformation of normal adult T cells by cocultivation with virus-positive cell lines have met with limited success. Early passages of HUT 102, a cell line derived from the neoplastic cells of the first HTLV + patient, were unsuccessfully used as the source of transforming virus in the first experiments. However, a report in 1982 described a technique for producing TCGF-dependent transformed adult and umbilical cord cell lines utilizing irradiated MT-2 cells as the source of virus. MT-2 is a transformed cord blood cell line that expresses the adult T cell leukemia virus (ATLV), a highly related, if not identical, retrovirus to HTLV. Modifying this procedure by activating the target cells with phytohemagglutinin-P (PHA) and TCGF has resulted in our ability to routinely create T cell lines independent of exogenous TCGF from adult and umbilical cord lymphocytes with both high- and low-titer HTLV-producing cell lines. As such, this technique could be utilized as a biologic assay for the detection of virus-containing cells and for the subsequent isolation of HTLV from fresh specimens.

MATERIALS AND METHODS

Transformation Assay

Blood was collected in syringes rinsed with preservative-free heparin from 16 adults (one with T cell prolymphocytic leukemia, three children of a patient with a HTLV-positive T cell malignancy, four normal individuals, and eight patients with acquired immunodeficiency syndrome [AIDS]) and from nine human umbilical cords. Target cells of the opposite gender of the donor cells were chosen to facilitate karyologic documentation of the target cell origin of transformed cell lines. The lymphocytes from patients with AIDS were included in an attempt to establish continuous T cell lines from these patients. Cord blood was incubated with 6.5 g/100 mL Dextran T500 (Pharmacia, Piscataway, NJ) in normal saline at a ratio of 8:1 in 5% CO2 at 37 °C. The leukocyte-rich supernatant was washed in Hanks’ balanced salt solution without calcium or magnesium (GIBCO Laboratories, Grand Island, NY) and centrifuged at 400 g for ten minutes. Mononuclear cells were obtained from the resultant pellet or adult blood by Ficoll-Hypaque centrifugation.
(LSM, Bionetics Laboratory Products, Kensington, Md). A portion of the cells were cultured in RPMI 1640 medium (GIBCO Laboratories) with 20% fetal calf serum (FCS) (GIBCO Laboratories) and antibiotics. The rest of the cells from each sample were cultured in similar media with 20% TCGF (Cellular Products, Inc, Buffalo) and activated with 5 μg/mL of PHA (Difco Laboratories, Detroit).

Twenty-four hours later, the activated cells were centrifuged at 400 g for ten minutes, the PHA-containing supernatant was removed, and the cells resuspended in media containing 20% TCGF at a concentration of 10^6 cells/mL. The concentration of unstimulated cells was similarly adjusted in media without TCGF. One-half milliliter of the respective suspensions was placed in 25 cm² culture flasks. In five experiments, the PHA-stimulated target cells were grown in TCGF-containing media for an additional two weeks prior to cocultivation.

The donor cells were obtained from ten T cell lines with varying degrees of HTLV production (low-titer virus expressors: five HTLV-positive cell lines transformed in vitro and the two early passages of HUT 102, HUT 102 p13, and HUT 102 p81; high-titer producers: HUT 102 B2, CTCL-11 [a cell line derived from the neoplastic cells of a patient with cutaneous T cell lymphoma (17,18)], and MT-2). Low-titer virus-expressing cell lines are defined as cells expressing less than five virions per cell by electron microscopy, incorporating less than 15 pmol/GMP/h/mL extract in a reverse transcriptase assay (10 mmol/L Mg with the template primer poly rC oligo dG), and less than 40% p19 positive. High-titer producing cell lines were greater than 75% p19 positive and expressed greater than 25 virions per cell and incorporated greater than 15 pmol/GMP/hr/mL extract in a reverse transcriptase assay. Levels of HTLV production by each cell line were further analyzed by quantitating the level of reverse transcriptase in sucrose-purified particles (density 1.12 to 1.18 g/mL) from 5 L of cell culture conditioned media. Dot blot hybridization of the HTLV-I probe, pATK32, to total genomic DNAs of the donor cells indicated that, on the average, the high producing cell lines had five to ten times as much integrated HTLV proviral DNA as the low producing cell lines. All donor cell lines were tested on three common non–PHA-activated cord blood target cells to assess their relative production of infectious virions. Control donor cells that do not express HTLV included five normal adult T cell cultures and the non–HTLV-transformed T cell lines Molt 4 and HUT 78. All donor cells were irradiated to 90 gray (Gammacell 1,000). The cells were then centrifuged at 400 g for ten minutes and resuspended in fresh media at a concentration of 10^6 cells/mL. One-half milliliter of the suspension was added to the aliquots of target 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 donor cells alone were maintained. Fresh cell culture media, consisting of RPMI 1640, 20% FCS + TCGF, was added three times per week to cultures exhibiting growth. Only those samples with PHA-stimulated (activated) target cells received media with TCGF. After one day to two weeks, media without TCGF were added to all growing samples. Transformation was defined as continuous proliferation of target cells independent of exogenous TCGF for greater than four weeks.

All initial samples and subsequent transformed lines were analyzed for T cell surface antigens, HLA antigens, and karyotype to assure that transformed lines were of target cell origin. Also, target cells prior to cocultivation and resultant cell lines were assessed for HTLV production by assaying for extracellular reverse transcriptase and intracellular HTLV p10 core protein and by electron microscopy for virions. The presence of integrated HTLV proviral DNA in the transformed cells was determined by dot blot hybridization using a cloned DNA probe to the gag-pol regions of HTLV-I. All work with viable virus was carried out under p2 biologic containment guidelines, as established by the NCI for work with HTLV.

**T Cell Surface Markers**

T cell phenotypes were determined with mouse monoclonal antibodies. Differentiation antigens (Coulter, Hialeah, Fla; Ortho, Raritan, NJ) and fluorescein-conjugated goat anti-mouse immunoglobulin (Cappel, Cochranville, Pa). Lymphocytes were determined by indirect immunofluorescence with rabbit anti-Ia (Alpha-Gamma Labs, Sierra Madre, Calif). TCGF receptors were determined with mouse monoclonal antibody to T cell activation antigen. Lympocytes were counted with an Epics V Flow Cytometer (Coulter).

**HLA Antigens**

HLA antigens were determined by a standard two-stage cytotoxicity assay, using approximately 70 antisera capable of identifying 45 HLA specificities (18 A and 27 B antigens). The HLA antigens were determined without previous knowledge of the cell type or line.

**Karyotype**

Cytogenetic examination was performed on colcemid-arrested, Giemsa-banded metaphases from cultured cells.

**Nucleic Acid Hybridization**

Genomic DNA was extracted from the cultured cells and transferred onto nitrocellulose filters following published procedures. The nucleic acids were analyzed by hybridization with 11β-radiola beled cloned HTLV-I probe, pATK32.

**Reverse Transcriptase Assay**

Assessment of reverse transcriptase activity under conditions specific for HTLV (using the template primer poly rC oligo dG with 10 mmol/L magnesium) was carried out as previously described.

**Electron Microscopy**

Cell cultures were treated with 60 μg/mL iododeoxyuridine (IDUR) for 24 hours. The cells were centrifuged at 400 g for ten minutes, the IDUR-containing supernatant removed, and fresh culture media were added. Three days later, the cells were pelleted by centrifugation at 400 g for eight minutes and fixed with 2.5% glutaraldehyde. The fixed material was processed for electron microscopy by previously reported techniques.

**Monoclonal p19 Antibodies**

Indirect immunofluorescent assays for HTLV p19 were performed by modifying a previously described technique. Cells, after treatment with IDUR as above, were fixed either to slides with 50% methanol in acetone or in suspension with 70% ethanol in phosphate-buffered saline (PBS). Slides or cell suspensions were preincubated with a 1/100 dilution (in PBS) of control P3 x 63 ascites or α-HTLV p19 ascites for 30 minutes at room temperature. The α-HTLV p19 monoclonal antibody is obtained from a hybridoma derived from a nonsecreting clone of the P3 x 63 cell line, nont specific mouse immunoglobulin secreted by P3 x 63 is used as the negative control antibody. A 1/400 (for cytofluorometry) or 1/200 (for fluorescent microscopy) dilution of FITC-conjugated sheep anti-mouse IgG F(ab)′, (Cappel), purified by affinity chromatography, was layered onto the cells for 30 minutes. The cells were then washed with PBS for one to two hours (for slides) or overnight (for suspension). Slides were examined by fluorescent microscope, and suspensions were assessed with the Coulter Epics V cytofluorometer.
vated lymphocytes were utilized, rapid transformation was noted in 1 of 13 experiments. When the high-titer virus-producing cell lines, HUT 102 B2, CTCL-11, MT-2, were employed as the source of HTLV, a very high frequency of transformation was noted (54/65). MT-2 readily transformed virtually all target cells (28/29). Although HUT 102 B2 could transform activated and nonactivated cord blood cells (11/12), only two of 11 nonactivated adult T cell cultures were transformed. When preactivated normal adult T cells were used, routine transformation (11/11) occurred. Using the preactivated T lymphocytes from patients with AIDS as target cells, eight of eight cultures were transformed by cocultivation with HUT 102 B2. However, only one of four cultures could be transformed by HUT 102 B2. Those lymphocytes were obtained from a patient with pro- dromal disease, that is, a patient with generalized lymphadenopathy and a normal OKT4:OKT8 ratio (67% OKT4:37% OKT8).

Figure 1 shows the growth in two sets of cell lines being transformed by cocultivation with HUT 102 B2. In each case, there were more cells and a greater rate of rise in cell number when the target cells had been preactivated with PHA and TCGF. As shown, while the nonactivated cord blood cells eventually were transformed, the nonactivated adult target lymphocytes were not. Successful transformation was noted regardless of the timing of the PHA stimulation of target cells, and was not simply a result of increasing the number of target cells by PHA stimulation. In five experiments, common adult target/donor cell combinations were cocultivated (all at the same density of target and donor cells) under various conditions of target treatment: (a) without PHA activation; (b) with 24 hours of PHA activation; or (c) with PHA activation and two weeks of continuous culture with depleted TCGF. No TCGF was added after the cells were cocultivated. None of the nonactivated targets were transformed, while both sets of activated cycling targets were transformed with equal efficiency.

Figure 2 shows the results of RT assays performed on density-banded HTLV virions from equal quantities of media conditioned by four HTLV-positive cell lines. The cell line, UMC-A4+/HUT 102 B2, is a very low producer of HTLV compared to the other cell lines and did not transform nonactivated target cells. The fact that it could easily transform (3/3) these same target cells when they are activated with PHA emphasizes the sensitivity of this assay.

Confirmation of Target Origin of Transformed Cell Lines

All transformed cell lines, evaluated by karyotype and HLA antigens, proved to be of target cell origin. Additional HLA antigens were occasionally detected on the transformed cells (Table 2). That these antigens were on target cells was confirmed by the fact that

| Table 1. HTLV Transformation of Nonactivated and Activated Normal T Lymphocytes |
|-----------------|-----------------|-----------------|-----------------|
|                  | Transformed Target Cells/Attempts |                 |                 |
|                  | Nonactivated     | Activated       |                 |
| Donor Cells      | Cord  | Adult | Cord  | Adult |
| Negative HTLV expression | 0/5  | 0/5  | 0/20  | 0/5  |
| Low HTLV expression | 2/11 | 0/2  | 9/11  | 2/2  |
| High HTLV expression |                 |                 |                 |
| MT-2             | 5/5   | 6/7  | 5/5   | 12/12 |
| CTCL-11          | 1/1   | 0/0  | 1/1   | 0/0  |

RESULTS

The results of the cocultivation experiments, with or without activated normal target cells, are presented in Table 1. Donor cells that do not express HTLV did not transform any target cells (0/35). Donor cells expressing small amounts of HTLV transformed only two of 13 nonactivated target cell cultures, but when preactivated lymphocytes were utilized, rapid transformation was noted in 11 of 13 experiments. When the high-titer virus-producing cell lines, HUT 102 B2, CTCL-11, and MT-2, were employed as the source of HTLV, a very high frequency of transformation was noted (54/65). MT-2 readily transformed virtually all target cells (28/29). Although HUT 102 B2 could transform activated and nonactivated cord blood cells (11/12), only two of 11 nonactivated adult T cell cultures were transformed. When preactivated normal adult T cells were used, routine transformation (11/11) occurred. Using the preactivated T lymphocytes from patients with AIDS as target cells, eight of eight cultures were transformed by cocultivation with HUT 102 B2. However, only one of four cultures could be transformed by HUT 102 B2. Those lymphocytes were obtained from a patient with prodromal disease, that is, a patient with generalized lymphadenopathy and a normal OKT4:OKT8 ratio (67% OKT4:37% OKT8).

Figure 1 shows the growth in two sets of cell lines

Fig 1. Rate of transformation with activated and nonactivated target cells. Starting with 1 - 10^6 viable T lymphocytes, the number and rate of increase in number of cells were greater when target cells were preactivated and cultured prior to cocultivation. Fresh medium was added as necessary. The dashed lines indicate that the cultured cells were deliberately repassaged at a concentration of 1 x 10^6 cells/mL and continued in culture. Viability was determined by trypan blue exclusion. See Fig 2 for abbreviations.

Fig 2. Reverse transcriptase assays of conditioned media from four HTLV-positive cell lines. Virions from cell-free media from each cell line were density banded in a 22% to 65% continuous sucrose gradient, and each fraction was assayed for reverse transcriptase activity using the template primer poly dG in 10 mmol/L magnesium, as previously described. Reverse transcriptase activity is expressed as picomoles guanine monophosphate incorporated per hour. O-O, MT-2; CBI+/MT-2; HUT 102 B2; A A4+/HUT 102 B2.
Data are expressed as percent of cells positive for surface antigens detected by the monoclonal antibodies OKT3, OKT4, or OKT8.

**Table 2. Confirmation of Target Origin of Transformed Cell Lines**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Target Cells</th>
<th>HLA Antigens</th>
<th>Karyotype</th>
<th>Transformed Cells</th>
<th>HLA Antigens</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMC-CB1*/MT-2</td>
<td>A28, A3, B7, B12</td>
<td>46,XX</td>
<td></td>
<td>A28, A3, B7, B12 (Aw30, Aw33, B40, Bw21)</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>UMC-CB3*/MT-2</td>
<td>A2, A9, B15, B37</td>
<td>46,XX</td>
<td></td>
<td>A2, A9, B15, B37 (B17, Bw38, Bw45)</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>UMC-A1*/MT-2</td>
<td>A2, A9, B12, B16</td>
<td>46,XX</td>
<td></td>
<td>A2, A9, B12, B16 (Aw31, B18, Bw22, Bw49)</td>
<td>46,XX</td>
<td></td>
</tr>
<tr>
<td>UMC-A3*/HUT 102</td>
<td>A9, A32, B5, B12</td>
<td>46,XX</td>
<td></td>
<td>A9, A32, B5, B12 (Aw31, B17, Bw35, Bw49)</td>
<td>46,XX</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3. T Cell Surface Markers**

<table>
<thead>
<tr>
<th>Control</th>
<th>Transformed Line</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT3</td>
<td>OKT4</td>
</tr>
<tr>
<td>A7</td>
<td>92</td>
</tr>
<tr>
<td>A8</td>
<td>11</td>
</tr>
<tr>
<td>A9</td>
<td>45</td>
</tr>
<tr>
<td>A10</td>
<td>70</td>
</tr>
<tr>
<td>A11</td>
<td>97</td>
</tr>
<tr>
<td>CB3</td>
<td>ND</td>
</tr>
<tr>
<td>CB1</td>
<td>ND</td>
</tr>
<tr>
<td>HUT 102 B2</td>
<td>0</td>
</tr>
<tr>
<td>MT-2</td>
<td>90</td>
</tr>
</tbody>
</table>

*Data are expressed as percent of cells positive for surface antigens detected by the monoclonal antibodies OKT3, OKT4, or OKT8.
†ND, not done.

Confirmation of the Presence of HTLV in Transformed Cell Lines

All transformed target cells contained integrated HTLV-I sequences, usually in a relatively low copy number compared to HUT 102 B2 and MT-2 (Fig 3A). All initial target samples and subsequent transformed lines were assessed for intracellular HTLV p19 protein and extracellular RT. The non-AIDS target cells, prior to cocultivation, showed no p19 reactivity or significant reverse transcriptase activity. Although it was not known at the time of cocultivation, the initial target cells from four of the patients with AIDS were significantly positive for p19 (80%). An electron micrograph of those cells revealed virus particles. This cell sample was the only AIDS sample that could be transformed by HUT 102 B2. Assessment of HTLV expression in representative samples of the transformed lines is presented in Table 4. An electron micrograph of the cord blood cells transformed by HUT 102 p13, revealing extracellular virus particles, is shown in Fig 3B.

DISCUSSION

Various attempts have been made to infect mononuclear cells with HTLV. Unsuccessful earlier approaches involved the use of mitomycin-treated virus-producing cells to spontaneously transform adult T cells. In 1982, a technique employing lethally irradiated MT-2 cells cocultured with unstimulated or resting adult and umbilical cord mononuclear cells was reported. Although there was a high rate of infection, most of the resultant cell lines required exogenous karyotypes of the transformed cells were always the gender of the target cells (Table 2).

**T Cell Surface Markers**

Table 3 shows the T cell surface markers of four patients with AIDS (A7, A8, A9, A10), one patient with T suppressor cell prolymphocytic leukemia (A11), and ten transformed cell lines. UMC-A8*/MT-2 is 85% OKT8 positive, while UMC-A10*/MT-2 is 93% OKT4 positive. In addition, the neoplastic T cells of A11, which were 94% OKT8 positive, could be transformed by HTLV. In three cases (UMC-A7*/MT-2, UMC-A10*/MT-2, UMC-CB3*/HUT 102), markers were available on transformed lines derived from both the activated and unactivated forms of the same target cells. The relative numbers of OKT4 to OKT8 cells were comparable whether the target cells had been stimulated with PHA or not. All transformed lines were positive for the Ia antigen and the TCGF receptor, as determined by monoclonal antibody to T cell activation antigen (TAC). No transformed cell lines tested positive for surface immunoglobulins or Epstein-Barr virus nuclear antigens.
HTLV TRANSFORMATION OF T LYMPHOCYTES

Fig 3. (A) Dot blot hybridization of cloned HTLV-I DNA, pATK32, to DNA extracted from HUT 102 B2 (lane 1), two normal cord blood lymphocyte cultures (lanes 2 and 3), UMC-A10+/MT-2 (lane 4), UMC-A12+/MT-2 (lane 5), UMC-C9+/C2+/MT-2 (lane 6), UMC-A8+/MT-2 (lane 7), Molt 4/MT-2 (lane 8), and UMC-A1+/HUT 102 (lane 9). The transformed cell lines (lanes 4 through 7, 9) are positive for HTLV-I genomic sequences at a copy number less than that of HUT 102 B2. Control cord lymphocytes (lanes 2 and 3) are negative. Molt 4/MT-2 (lane 8) exhibits a weakly positive reaction. (B) Electron micrograph showing 100-nm extracellular virus particles from a preactivated cord blood cell transformed by HUT 102 p13.

Table 4. HTLV Expression by Selected Transformed Cell Lines

<table>
<thead>
<tr>
<th>Transformed Cell Lines</th>
<th>Reverse Transcriptase (pmol GMP/mL Extract)</th>
<th>Percent Cells Positive for p19</th>
</tr>
</thead>
<tbody>
<tr>
<td>UMC-CB2+/MT-2</td>
<td>22.6</td>
<td>19</td>
</tr>
<tr>
<td>UMC-CB2+/HUT 102 P13</td>
<td>25.6</td>
<td>19</td>
</tr>
<tr>
<td>UMC-CB4+/MT-2</td>
<td>77.1</td>
<td>4</td>
</tr>
<tr>
<td>UMC-CB3+/HUT 102 B2</td>
<td>11.2</td>
<td>30</td>
</tr>
<tr>
<td>UMC-CB7+/HUT 102 P13</td>
<td>12.0</td>
<td>0</td>
</tr>
<tr>
<td>UMC-CB8+/HUT 102 B2</td>
<td>4.4</td>
<td>7.5</td>
</tr>
<tr>
<td>UMC-CB9+/C2+/MT-2†</td>
<td>80.0</td>
<td>93</td>
</tr>
<tr>
<td>UMC-CB9+/C2+/MT-2‡</td>
<td>44.0</td>
<td>92</td>
</tr>
<tr>
<td>UMC-A2+/MT-2</td>
<td>8.8</td>
<td>0</td>
</tr>
<tr>
<td>UMC-A5+/MT-2</td>
<td>8.4</td>
<td>43</td>
</tr>
<tr>
<td>UMC-A4+/HUT 102 B2</td>
<td>6.4</td>
<td>29</td>
</tr>
<tr>
<td>UMC-A8+/MT-2‡</td>
<td>22.4</td>
<td>78</td>
</tr>
<tr>
<td>UMC-A9+/MT-2‡</td>
<td>14.7</td>
<td>72</td>
</tr>
<tr>
<td>UMC-A10+/MT-2</td>
<td>36.4</td>
<td>84</td>
</tr>
<tr>
<td>Nontransformed controls*</td>
<td>4.4†</td>
<td>0†</td>
</tr>
</tbody>
</table>

Positive controls
- HUT 102 B2 16.0 82
- CxCL 11 68.0 96
- MT-2 124.0 96

*Includes three adult and two cord blood T cell cultures prior to cocultivation.
†Represents the mean of five results.
‡These lines were evaluated after four months in culture. All other lines were assessed after two months in culture.

TCGF for continued growth. Subsequent reports demonstrated a high frequency of transformation of cord blood T lymphocytes could be achieved with relatively high HTLV-producing donor cells. However, low-titer virus-producing cell lines were not assessed in these studies. More recent endeavors have attempted to utilize cell-free suspensions of virus. In one report, although transformation did not occur, it was shown that PHA stimulation of the target T lymphocyte increased the probability of infection with cell-free HTLV. In another, only cells from relatives of a patient positive for HTLV could be grown continuously after exposure to the cell-free virus suspension. In this last report, all target cells had been PHA-stimulated, and it is not known whether the unstimulated targets from the relative could be transformed. Also, target cells still required the addition of exogenous TCGF for continuous growth.

In the current report, target cells were or were not activated by PHA and TCGF and then cocultivated with lethally irradiated virus-producing cells. The HTLV-positive donor cell lines were ranked according to relative virus production by assessment of viral particle and protein expression and biologic activity on common cord blood lymphocyte target cells. With nonactivated target cells, the highest virus-producing cell line, MT-2, resulted in routine transformation. Other high-titer virus-producing cell lines, HUT 102
B2 and CTCL-11, could transform both nonactivated and activated cord blood cell cultures. For low-titer virus-producing cell lines or for HUT 102 B2 to transform adult T cells, the use of preactivated target cells was optimal and resulted in the ability to reproducibly transform these cells. Preactivated target cells from patients with AIDS were the only exceptions. While all eight target samples could be transformed by MT-2, only one of four cultures could be transformed by HUT 102 B2. The increased transformability of these cells may relate to preexistent integrated retrovirus, as indicated by the expression of HTLV-like proteins and virions by these target cells.

In addition to the success of transformation, the rate of transformation was increased by the PHA/TCGF activation and stimulated growth (Fig 1). Although the definition of transformation included a period of four weeks of growth independent of exogenous TCGF, at one to two weeks it was possible to predict the successful fulfillment of the transformation criteria. The nonactivated target cells, especially cord blood cells, could be transformed, but it generally took four to six weeks to attain the same degree of growth rate as those cells that had been preactivated. Again, in contrast to earlier reports, it should be emphasized that all transformed cells could be cultured independently of exogenous TCGF. Whether these cell lines produce endogenous TCGF, as has been reported in some, but not all, HTLV-infected cells, is currently under investigation.

The mechanism of PHA and TCGF enhancement of transformation is unknown at this time. PHA has been shown to stimulate lymphocytes, resulting in increased expression of TCGF receptors and other activation antigens. It is possible that target cell activation may result in increased expression of receptors for HTLV envelope glycoproteins, thereby enhancing viral penetration into the cell. Alternatively, the proliferative state induced by PHA and TCGF may, as has been demonstrated in some animal retrovirus systems, result in increased cytoplasmic proviral DNA copies of HTLV or enhanced integration of viral DNA into the host genome.

The improved transformation efficiency seen with PHA-stimulated target cells is not explained by an increased cell number secondary to mitogen-stimulated growth. In five experiments, the PHA-stimulated lymphocytes were cultured in TCGF-containing media for two weeks prior to cocultivation. The TCGF was then removed and the cells cocultivated with HTLV donor cells as before. The removal of TCGF at that point results in approximately three days of reduced target cell growth rate followed by the death of these cells under normal conditions. This lower level of proliferation, although providing activated cycling target cells, would render the number of nonactivated and activated lymphocytes more comparable. The fact that these activated targets could be more efficiently transformed than nonactivated target lymphocytes cocultivated similarly two weeks before suggests that it is the activated state of the T lymphocyte, rather than an increase in target cell number, that accounts for the greater efficiency of transformation. It is conceivable that even the transformation of non–PHA-stimulated cells observed with high HTLV-producing cell lines requires the prior activation of the target T cells by HTLV or cellular antigens. The same additional HLA class I antigens observed in the transformed cells in this report have been found by others. While this result could be explained by coexistent reactivity in the HLA typing sera against the Ia antigen, recent reports suggest that some of this reactivity may be secondary to homology with regions within the envelope glycoprotein of HTLV. These antigens could result in increased activation of a target T cells, even in an autologous cocultivation.

The characteristics of these transformed cell lines are similar to those previously reported. The cultured cells, at first, exhibit a small degree of HTLV p19 and RT expression, but with prolonged time in culture, the number of cells positive for intracellular p19 increases, as does extracellular RT (data not shown). The levels of p19 and RT expression do not necessarily correlate. It is clear that certain target cells resulted in more rapidly proliferating transformed lines and more expression of HTLV but, within the limits of this study, there was no correlation with HLA or T surface antigen phenotype.

This is the first published report of MT-2, a Japanese transformed cord line, and the US isolated lines, HUT 102 and CTCL-11, being assessed by the same assay system. It is clear that although all of these lines are infective, MT-2 results in more efficient transformation when common targets are utilized and can consistently transform unactivated normal adult T cells and preactivated lymphocytes from patients with AIDS. Higher viral production by MT-2 cells with respect to HUT 102 B2 (fivefold in Fig 2) may be responsible for these findings, as comparisons of the molecular weight and the relative amounts of viral proteins between virions produced by MT-2 and HUT 102 are identical (data not shown) and there is considerable homology in the integrated proviral DNA in these cell lines (M. Yoshida, Department of Viral Oncology, Cancer Institute, Tokyo, personal communication, May 1983).
A major advantage of this system is the production of TCGF-independent cell lines. These lines will permit evaluation of endogenous lymphokine synthesis without exogenous contamination. The ability to routinely produce transformed cell lines permits the long-term growth of interesting T cells, such as those from AIDS patients. In addition, the ability to detect low-titer virus is a significant benefit, since the detection of virus, even in low titers, from candidate T cells may now be possible. Finally, this system provides a means for studying the factors that may influence HTLV infectivity, which may ultimately lead to a better understanding of how HTLV causes human disease.

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Efficient transformation of previously activated and dividing T lymphocytes by human T cell leukemia-lymphoma virus

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