Characterization of a Novel HTLV-Infected Cell Line

By H. Phillip Koeffler, Irvin S.Y. Chen, and David W. Golde

A man from Chile developed an aggressive mature T cell leukemia associated with marked eosinophilia. The neoplastic lymphocytes were of T helper surface phenotype, and they expressed the p24 and p19 antigens of human T cell leukemia virus (HTLV). A cell line (ME) was established from the patient's peripheral blood cells that was initially composed of eosinophils and T and B lymphocytes. The B lymphocytes of the cell line are polyclonal and contain Epstein-Barr virus (EBV) DNA. Many of the T lymphocytes, a few of the B lymphocytes, and none of the eosinophils express HTLV p19 and p24 antigens. By 6 months of culture, the ME line no longer contained eosinophils.

A variant line of ME was established; this variant (ME-2) is notable because the cells (> 80%) adhere tightly to the bottom of the culture flask; they do not express T lymphocyte markers, but 30% of the cells contain cytoplasmic μ heavy immunoglobulin chains. These pre-B and null lymphocytes contain p19 and p24 antigens (80% of cells), have the HTLV-I genome, and are able to transform normal T lymphocytes in vitro. We isolated a B lymphocyte clone (11A) from ME that expresses cytoplasmic immunoglobulin (70% of cells) and p19 and p24 antigens (75% of cells), contains the EBV and HTLV genomes, and can transform T lymphocytes from normal volunteers. These data show that B lymphocytes can be infected with HTLV, although no disease of HTLV-infected B lymphocytes has been reported. The T lymphocytes from normal adult peripheral blood were easily immortalized (about 70% efficiency) by cocultivation with lethally irradiated ME cells. Twenty-five of 27 of the transformant lines were composed of T lymphocytes with helper antigens, and two of the lines were of T suppressor antigen phenotype. All the cell lines that were tested constitutively produce lymphokines, including colony-stimulating factor (CSF), erythroid-potentiating activity (EPA), macrophage migration-inhibitory factor (MIF), neutrophil-inhibitory factor (NIF), and differentiation-inducing factor (DIF).

An unusual form of adult T cell leukemia/lymphoma was described by Japanese investigators in 1977,1,2. "Typical features of the disease include: (1) subacute or chronic leukemia often resistant to chemotherapy with rapid terminal progression; (2) lymphadenopathy, hepatosplenomegaly, erythrophagocytosis, or nodular skin lesions, and usually no mediastinal mass; (3) hypercalcemia; and (4) pleomorphic leukemic cells with convoluted nuclei and T cell surface markers. The disease occurs principally in southwest Japan, but a number of cases have also been reported from other parts of the world, including the United States, the West Indies, and Israel.4 The disease is closely associated with a unique retrovirus known as human T cell leukemia virus (HTLV). HTLV was first isolated from cells of a black patient with an aggressive mature T cell neoplasm.5 Subsequently, the virus (also referred to as adult T leukemia virus, ATLV) was isolated from Japanese patients and adults in various parts of the world with leukemia/lymphoma of mature T cells.6-8 The virus has also been isolated from clinically healthy individuals who either had morphologically abnormal lymphocytes in the peripheral blood or lived with a relative who had HTLV-positive T cell leukemia.9 High titer antibodies against HTLV have been detected in the sera of relatives and other close associates of patients with HTLV-associated T cell malignancies.6,7 Proviral sequences have been found in the DNA of HTLV-positive leukemic T cells, but not in DNA from normal uninfected human tissue.9,10 The findings, to date, suggest a causative role of HTLV in these T cell leukemias and lymphomas.

Several T lymphocyte cell lines containing HTLV have been established from patients with mature T cell leukemia.5,11,12 The HTLV-positive cell lines produce viral-related proteins, including a major core protein (p24), another core protein known as p19, and reverse transcriptase.13-15 The p24 and p19 antigens typically are expressed on 5% to 99% of the cells.

We report the development of a HTLV-producing cell line (ME) from a patient who had a mature T cell leukemia and marked eosinophilia. The ME line is composed of both T lymphocytes that secrete lymphokines and of B lymphocytes. The ME cell line can transmit HTLV to normal human adult bone marrow or peripheral blood T lymphocytes and causes transformation and immortalization of these cells.

CASE HISTORY

M.E. was a 39-year-old man who presented to the hospital with a two-week history of weakness and diarrhea. He was born and raised in Chile, lived two years in Venezuela, and visited many areas of the world as a travel agent. Physical examination showed that the spleen...
was enlarged 2 to 3 cm below the left costal margin, and the liver was enlarged 3 cm below the right costal margin. He had diffuse, modest lymphadenopathy. The hematocrit was 29% and the white blood cell count was 157,000/μL, with 14% granulocytes, 40% eosinophils, and 46% abnormal lymphocytes. The lymphocytes varied in size, with irregular, lobulated, and cerebriform nuclei and moderately clumped nuclear chromatin. Occasional inconspicuous nucleoli were identified, and the cells had basophilic cytoplasm. The bone marrow showed evidence of a lymphoproliferative disorder with markedly increased numbers of lymphocytes containing convoluted and folded nuclei. A striking eosinophilia (about 50%) was present in the peripheral blood, with eosinophils in all stages of development. Most of the lymphocytes showed focal reactivity for acid phosphatase and were PAS negative. The peripheral blood and bone marrow lymphocytes rosetted with sheep erythrocytes (86%), and fewer than 3% of the mononuclear cells had surface membrane immunoglobulin. Over 90% of the lymphocytes expressed a pan-T antigen (Leu-1), 80% expressed Leu-3 (antigen associated with helper-inducer T lymphocytes), and less than 1% expressed Leu-2 (suppressor-cytotoxic T lymphocytes).

The serum calcium was elevated at 11.1 mg/dL (normal 9.2 to 10.8). Quantitative immunoglobulins were within normal limits. Antibody titer to Epstein-Barr viral capsid antigen was less than 1:10,000 (within normal limits). Numerous blood and stool cultures were negative for parasites, although an earlier stool culture at an outside hospital had grown Isospora belli. The patient was treated with cyclophosphamide, vincristine, and prednisone, without significant change of medium. Subsequently, the cells were fed biweekly by removing half the medium and replacing it with fresh medium. A continuous cell line (known as ME) developed after three weeks. The cells were cultured initially at a relatively high density centrifugation.
RESULTS

HTLV Cell Line (ME)

The ME cell line has been in culture for over 1 1/2 years, and during that time, evolution in characteristics of the cells has occurred and variants of the line have been established. Table 1 displays the morphological and histochemical evolution of the ME line. The patient had 45% eosinophils in the peripheral blood at the time that cells were taken to establish the line. After 2 1/2 months of culture (passage 10), the ME line continued to have 36% eosinophils at different stages of maturation (Fig 1A). These cells were stained with peroxidase and Luxol blue. By 4 1/2 months (passage 25) and later, no eosinophils were evident, and the cells contained a dimorphic population of lymphocytes with both small cells with scanty basophilic cytoplasm and large cells (> 30 μm) with abundant, clear cytoplasm (Fig 1B). Many of the lymphocytes showed activity for acid phosphatase, β-glucuronidase, and fluoride-resistant α-naphthyl butyrate esterase (NBE) (single large acid phosphatase, β-glucuronidase, and fluoride-resistant α-naphthyl butyrate esterase (NBE) (single large paranuclear area of activity) (Table 1). These staining characteristics are typical of T lymphocytes.

Thin-section electron micrographs demonstrated cells (Fig 1C) with irregular nuclei, prominent mitochondria, and mature and immature virus particles with C type morphology, both budding from the cells and also present in the extracellular space (Fig 1D). The mature particles had an electron-dense core (nucleocytid) surrounded by an outer membrane separated by an electron-lucent region. The particles were approximately 100 nm in diameter. No intracellular type C virus particles were observed.

The patient had predominantly OKT1-positive T lymphocytes in his peripheral blood (Table 1). At passage 10, the ME line contained 50% cells that expressed the pan-T cell antigen (OKT1, Leu-1), formed E rosettes, and reacted with the Leu-5 antibody (E rosette receptor). About 30% of the cells had the helper T cell surface phenotype (OKT4 and Leu-3); none of the cells expressed the suppressor surface phenotype (OKT8 and Leu-2). About 25% of the ME cells were positive for surface membrane immunoglobulin (SMIg) for the first 20 cell passages. The B cells were polyclonal; 84% expressed kappa and 15% expressed lambda immunoglobulin light chains at passage 20. After nearly a year in liquid culture, the ME cells lost many of their cell surface phenotypic markers, with less than 20% of the cells expressing Leu-1, Leu-3, and Leu-5. Approximately 30% of the cells contained SMIg and intracytoplasmic immunoglobulin. The ME cells were HLA-D antigen positive and did not contain terminal deoxynucleotidyl transferase (TdT), nor did they react with monoclonal antibodies against the common acute lymphocytic leukemia antigen (CALLA), B cell antigen, or an antigen found on myeloid leukemic cells (D5/D6).28,29

HTLV Antigen Expression on ME

The expression of the p19 and p24 proteins of HTLV and production of immunoglobulin was examined by indirect immunofluorescence in individual methanol-acetone-fixed cells (Table 1). About 60% of the patient's peripheral blood white cells expressed the p19 and p24 antigens of HTLV after the cells were placed in liquid culture for two days. We examined individual cells from the patient and from ME passage 10 for both the p19 antigen under fluorescence and for intracytoplasmic eosinophilic granules under the light phase of the microscope. Cells that contained the p19 antigen did not contain intracytoplasmic granules, suggesting that the eosinophils did not express the p19 antigen of HTLV. Between 25% and 45% of the cells from the ME line demonstrated brilliant staining for the p24 and p19 antigens in the cytoplasm when examined at different times over 1 1/2 years of culture. The fluorescence was slightly granular or appeared as large clumps in the cytoplasm but not in the nucleus. The p19 and p24 antisera did not react with established T lymphoid (CEM, HSB, MOLT-4), B lymphoid (P3HR-1, BiB), or myeloid (HL60, KG-1, ML-3) cell lines, as previously described.13,14

We investigated the question of whether ME contained B lymphocytes that also express antigens of HTLV. Examination of the ME cell line at different

Table 1. Characteristics of the HTLV-I-Infected Cell Line (ME)
Fig 1. (A) The ME cell line after ten weeks of culture (passage 10), composed of eosinophils (several illustrated by arrows) and small lymphocytes. (B) The ME cell line at 35 weeks (passage 40), composed of a dimorphic population of lymphocytes consisting of small cells (several illustrated by arrows) and large cells. (C) An electron micrograph of the ME cells at passage 20 (18 weeks). Magnification ×2,600. (D) An electron micrograph of ME cell passage 20, showing C type particles budding from the cell membrane and present extracellularly. The bar is 0.1 μm in length.

Passages over 1½ years of culture showed that approximately 20% to 40% of the cells contained surface and intracytoplasmic immunoglobulin. The cells that were smaller in size were usually positive for intracytoplasmic immunoglobulin, while most of the larger cells and almost all of the multinucleated cells expressed the antigens of HTLV and did not contain intracytoplasmic immunoglobulin. Five to nine percent of the cells that were positive for intracytoplasmic immunoglobulin also expressed the p19 antigen. The cells that expressed the dual characteristics were small in size, similar to other B cells in the ME line that contained only intracytoplasmic immunoglobulin. The ME cells were examined for dual markers at passages 10, 20, 30, and 40, and the results were similar, confirming that some B cells were probably infected with HTLV.30

We examined the expression of HTLV antigens in ME cells after exposure to several compounds known to increase expression of DNA and RNA virus-associated antigens. Both IUDR (30 to 60 μg/mL) and TPA (2 to 20 ng/mL) caused a twofold increment in the percentage of cells that expressed the p19 and p24 antigens (data not shown). As expected, we were unable to induce p19 antigen in HTLV-negative lymphoid and myeloid cell lines (CEM, MOLT-4, P3HR-1, KG-1, HL60).
Southern Blot Hybridization

In order to confirm that a population of the ME cells was infected with HTLV-I, the DNA was isolated from ME (passage 20) and from cells of a myeloid line (HL60), digested with EcoRI (HTLV-I contains no EcoRI sites in the proviral genome), and analyzed for sequences complementary to an HTLV-I DNA probe (Fig 2A). The DNA from ME (lane 1) contained the viral genome, while HL60 (lane 2) did not. At least five bands of hybridization were seen in the ME DNA, suggesting that at least five sites of integration of the HTLV occurred and two sites contained HTLV smaller than the whole proviral genome (8.7 kb).

In order to determine if a population of the ME cells was infected with Epstein-Barr virus, the DNA isolated from ME (passage 20) and from cells of HL60 and a Burkitt’s line (P3HR-1) were analyzed for sequences complementary to an Epstein-Barr virus-specific DNA probe (Fig 2B). The DNA from ME (lane C) and P3HR-1 (lane B, positive control) contained the 3.5-kb BamHI band that represents the large internal repeat of the EBV genome. DNA from the promyelocyte, HL60 (lane A, negative control), did not hybridize with the probe.

Miscellaneous Characteristics of ME

Giemsa-banded chromosome analysis of the ME cell line showed a 47XY karyotype with trisomy 14 (20 of 23 metaphases). Several cells (5/23) showed a partial deletion of the short arm of chromosome 10, and others (3/23) showed a partial deletion of the long arm of chromosome 9. Three of 23 metaphases showed a normal XY karyotype. The HLA antigens expressed on ME were A1, A24, B17; the doubling time of the cells in liquid culture was about 48 hours; and the cloning efficiency of the cells in soft agar was 0.1%. The cloning efficiency was not affected when 5 x 10^{-6} mol/L dexamethasone was added to the culture plates, but cloning efficiency decreased by 50% in the presence of 5 x 10^{-5} mol/L dexamethasone (a suprapharmacologic concentration). Recombinant a2-interferon at 1 to 500 μ/mL had no effect on ME cloning efficiency, but 5,000 μ/mL decreased colony formation by 60%.

Pre-B Lymphocyte Subline and B Lymphocyte Clone of ME

A subline (ME-2) of the ME cells was developed within the first 15 passages of ME (Table 2). The ME-2 cells are of very large size, averaging 40 to 50 μm, and over 80% of the cells adhere to the plastic of the culture flasks. The variant was isolated by its ability to firmly stick to plastic. The staining characteristics of these cells is the same as ME, except that 1% to 2% of the cells stain diffusely for NBE, and the staining is partially resistant to sodium fluoride. A total of 6% ME-2 cells were able to phagocytize Candida albicans. The cells were exposed for five days to known inducers of myeloid differentiation, including TPA (10^{-8} mol/L); 1,25(OH)_{2}D_{3} (10^{-6} mol/L); trans retinoic acid (10^{-6} mol/L); DMSO (1.25%); no changes in phagocytosis, morphology, or cytochemistry occurred after exposure to the compounds (data not shown).

The cells do not express either Leu-2, -3, -4, -5, CALLA, Tdt, or SMIg for IgG, IgA, IgM, lambda or kappa, but the cells do express Leu-1 (15%), HLA-D (94%), and μ heavy immunoglobulin chains (30%) in the cytoplasm. The cells express p19 and p24 of Table 2. Characteristics of HTLV- and EBV-Positive Variant and Clone of ME

<table>
<thead>
<tr>
<th>Cell Marker</th>
<th>ME-2</th>
<th>ME Clone 1A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leu-1</td>
<td>15</td>
<td>40</td>
</tr>
<tr>
<td>Leu-2-5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HLA-D</td>
<td>94</td>
<td>92</td>
</tr>
<tr>
<td>SMIg</td>
<td>0</td>
<td>71</td>
</tr>
<tr>
<td>C Ig</td>
<td>31 (μ chain)</td>
<td>NT</td>
</tr>
<tr>
<td>p24</td>
<td>87</td>
<td>76</td>
</tr>
<tr>
<td>p19</td>
<td>93</td>
<td>81</td>
</tr>
<tr>
<td>HTLV-I genome*</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>EBV genome*</td>
<td>NT</td>
<td>+</td>
</tr>
</tbody>
</table>

*HTLV-I and EBV genome detected by Southern blotting (see Materials and Methods).
HTLV, and Southern blotting shows that the cells contain the genome of HTLV. The cells produce little CSF or DIF lymphokines (data not shown). The cells probably are HTLV-infected pre-B and non-B, non-T lymphocytes.

In order to confirm our impression that ME contains HTLV-positive B lymphocytes, we isolated a B lymphocyte clone of ME (ME clone IIA). The ME cells were cloned in sea plaque agarose (Marine colloids); clones were isolated with a Pasteur pipette on day 14 of culture and subcultured in liquid medium. After 3 months in liquid culture, several clones began to grow rapidly. We selected one clone (clone IIA) for detailed analysis. The cells appear similar to the small cells of the ME line, and they grow in small clumps in suspension cultures. Over 70% of the cells express sMig, but do not express Leu-2, -3, -4, or -5 (Table 2). About 40% of the cells express Leu-1 (Leu-1 is a pan-T lymphocyte marker, but the antigen is also expressed on B lymphocytes from patients with chronic lymphocytic leukemia). Over 75% of the cells contain the p19 and p24 antigen of HTLV, and Southern blotting of high molecular weight DNA of clone IIA shows that the cells contain the HTLV-I and EBV genomes. The data show clearly that the ME line contains HTLV-I-infected B lymphocytes.

Lymphokine Production by ME

The ME line produces CSF, DIF, EPA, NIF, and MIF, although MIF production is not constitutive. The cells produce approximately 2,000 μg/mL of CSF after incubation at 1 x 10⁵ cells/mL for five days in alpha medium containing 10% fetal calf serum and about 1,000 μg/mL of CSF when cultured in serum-free medium at 1 x 10⁶ cells/mL for five days. The CSF production was increased 65% to 85% when the ME cells were cultured with 1% phytohemagglutinin (PHA) or 5 x 10⁻¹⁰ mol/L TPA, as compared to flasks containing cells in alpha medium with fetal calf serum alone.

The CSF activity of ME CM was destroyed by exposure to pronase (0.2 mg/mL), trypsin (0.1 mg/mL), or 100°C for 30 minutes; but the CSF activity was not altered after exposure to urea (10 mol/L), dithiothreitol (5 mmol/L), neuraminidase (10 μg/mL), or 75°C for 30 minutes. Partial purification of the ME CSF was performed. The peak ME CSF activity for normal human marrow granulocyte-macrophage colony formation appeared in a fraction equivalent to a molecular weight of 33,000 daltons after filtration on an AcA Ultrogel column. The CSF activity in the ME conditioned medium bound to both lentil lectin and concanavalin A-Sepharose columns, and this activity could be eluted by 0.5 mol/L methyl-α-D-mannoside. These properties are similar to those of CSF from the Mo T cell line.²⁴

We examined the cellular morphology of normal human marrow myeloid colonies that proliferated when cultured with CSF from ME (2% crude CM). The colonies (> 50 cells) were plucked from soft agar on day 12 of culture, placed on glass slides, fixed with methanol, and stained with either Giemsa, α-naphthyl butyrate esterase, or Luxol blue. The CSF from ME stimulated the formation of 6% eosinophil, 22% neutrophil, 34% macrophage, and 38% mixed neutrophil and macrophage colonies. The morphology of the colonies stimulated by ME CM was examined on passages 10, 20, and 40 of culture of ME, and the results did not differ significantly, even though approximately 50% of the ME cells were eosinophils on passage 10.

The ME cells produced differentiation-inducing factor (DIF). The CM (10%) harvested from 1 x 10⁹/mL ME cells at four days was added to HL60 cells (0.75 x 10⁶/mL), and morphology, phagocytosis, and ability to reduce NBT was studied after five days of liquid culture. The CM induced a mean of 36% ± 4% (± SE), 24% ± 6%, and 42% ± 4% of the cells to morphologically mature to macrophages or granulocyte-like cells, to phagocytose yeast, and to reduce NBT, respectively. The HL60 cells from control flasks contained less than 5% cells that were either morphologically mature, could phagocytose, or reduce NBT. These experiments were performed on five separate occasions. The CM of ME was a rich source of EPA, NIF, and MIF. A 10% concentration of CM yielded a twofold stimulation of erythroid burst-forming units (BFU-E). MIF and NIF activities were comparable to those observed in a previously described T cell line.²²₂³

Transformation and Lymphokine Production of Human Adult Lymphocytes After Cocultivation With ME

The ME cells caused high efficiency transformation of normal human adult T lymphocytes in coculture experiments (Table 3). T lymphocytes from normal adult peripheral blood began active proliferation within three to four weeks of coincubation with x-irradiated (10,000 rad) ME cells. Identification of the lines as true transformants was confirmed, as the target cells used were female and the 46,XX karyotype was identified in the transformed cells. In control cultures, the x-irradiated ME cells did not proliferate. We established over 27 transformed T cell lines. The efficiency of transformation was about 70%. All of the transformed lines had similar morphology, and greater than 90% stained positively for acid phosphatase, β-glucuronidase, and fluoride-resistant α-naphthyl
Table 3. Transformation of Adult Human T Lymphocytes With x-Irradiated HTLV-Positive Cells

<table>
<thead>
<tr>
<th>Cell Line Name</th>
<th>HTLV p19-Positive Cells (%)</th>
<th>Leu-3 or OKT4 (%)</th>
<th>Leu-2 or OKT8 (%)</th>
<th>CSF (μ/mL)</th>
<th>EPA (%) Control at 0.1 Dilution</th>
<th>MIF (%) Inhibition</th>
<th>NIF Titer (%)</th>
<th>DIF (% Mature)</th>
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<tbody>
<tr>
<td>S-LB1</td>
<td>90</td>
<td>69</td>
<td>0</td>
<td>3,400</td>
<td>187</td>
<td>58</td>
<td>1/32</td>
<td>NT</td>
</tr>
<tr>
<td>S-LBII</td>
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<td>99</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>S-LBIII</td>
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<td>68</td>
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<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>S-WM</td>
<td>90</td>
<td>56</td>
<td>0</td>
<td>1,400</td>
<td>153</td>
<td>63</td>
<td>1/64</td>
<td>NT</td>
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<tr>
<td>S-WMP</td>
<td>90</td>
<td>12</td>
<td>72</td>
<td>NT</td>
<td>12</td>
<td>56</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>S-KK(II)</td>
<td>90</td>
<td>NT</td>
<td>NT</td>
<td>1,500</td>
<td>253</td>
<td>NT</td>
<td>NT</td>
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</tr>
<tr>
<td>S-KK(III-IV)</td>
<td>90</td>
<td>80</td>
<td>0</td>
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<tr>
<td>S-AH (II-IV)</td>
<td>90</td>
<td>80</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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</tr>
<tr>
<td>S-FS (I-IV)</td>
<td>90</td>
<td>80</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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<tr>
<td>S-BF</td>
<td>54</td>
<td>0</td>
<td>80</td>
<td>3,600</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>42</td>
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<tr>
<td>S-CL</td>
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<td>80</td>
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<td>2,400</td>
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<td>25</td>
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<tr>
<td>S-PM</td>
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<td>S-CP</td>
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<td>S-DP</td>
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<td>NT</td>
<td>NT</td>
<td>46</td>
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<tr>
<td>(S-BF)PM</td>
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<td>46</td>
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<td>2,600</td>
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<td>NT</td>
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<td>28</td>
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<tr>
<td>(S-BF)BF</td>
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<td>0</td>
<td>1,200</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>38</td>
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<tr>
<td>(S-LB)WM</td>
<td>90</td>
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<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>(S-LB)LB</td>
<td>90</td>
<td>80</td>
<td>0</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
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*Surface membrane immunoglobulin was absent on all cell lines.

†Conditioned medium was harvested after four days of cell growth and tested for the presence of various lymphokines. Neutrophil migration-inhibitory factor (NIF) activity is expressed as the last dilution of conditioned medium giving a positive result. Units of colony-stimulating factor (CSF) are the number of CFU stimulated/mL of conditioned medium; in the absence of CSF, no colonies are seen. Erythroid potentiating activity is assayed at 1/10 dilution of CM by enumerating large erythroid colonies (BFU-E), as described. MIF, macrophage-inhibitory factor; DIF, differentiating-inducing factor. The DIF results represent the percent HL6O promyelocytic cells that morphologically matured to macrophages or granulocytes after culture with 1/10 dilution of CM. NT, not tested.

DISCUSSION

We developed an HTLV-positive cell line (ME) from a man with adult T cell leukemia and marked eosinophilia. The neoplastic lymphocytes, but not the eosinophils, of the patient expressed the p19 and p24 antigens of HTLV. The cause of the prominent proliferation of eosinophils in culture for over two months is efficiently as ME (data not shown). T cell growth factor (TCGF) was not added in any of the transformation experiments, indicating that an exogenous source of TCGF was not necessary for transformation. Several of the transformed cell lines had a markedly slow rate of proliferation when cultured at a low cell density (5 x 10⁴ cells/mL), suggesting a possible “autoconditioning” of the medium.

Each of the transformant lines tested produced lymphokines, including CSF, EPA, MIF, NIF, and DIF (Table 3). The S-BF and S-WMP cell lines had a T cell suppressor antigen phenotype, but these cells were also able to produce lymphokines, indicating that the T cell surface phenotype did not correlate with the capabilities of the lines to produce lymphokines.

Butyrate esterase in the paranuclear area. Most of the transformant cells expressed the p24 (data not shown) and p19 antigens (usually 90%) (Table 3). Control peripheral blood mononuclear cells were negative for HTLV antigens.

Most of the transformed lines (25 of 27) had the helper surface phenotype (OKT4, Leu-3a). The S-WMP and S-BF lines expressed a suppressor antigen phenotype. None of the cell lines expressed surface membrane immunoglobulin or TdT. Four secondary T cell lines were established; that is, the initial transformants were irradiated and used to immortalize normal adult peripheral blood [lines: (S-BF)PM, (S-BF)BF, (S-LB)WM, (S-LB)LB] (Table 3). Two of the successive transmissions were produced by coculturing the x-irradiated initial line (S-BF and S-LB) with peripheral blood cells from the same volunteer from which the first line was derived, (S-BF)BF, (S-LB)LB. The S-BF line had the suppressor (OKT4⁺, OKT8⁻) phenotype, but its secondary transformant, (S-BF)BF, was of helper phenotype (OKT4⁺, OKT8⁻). The variant (ME-2) and clone (11A) of ME were able to transform normal volunteer T lymphocytes as efficiently as ME (data not shown).
not clear, but their growth may have been facilitated by production of an eosinophilopoietin by the T lymphocytes of ME. The lymphoid cells of the ME line variant subline (ME-2) and clone (11A) expressed the p19 and p24 antigens of HTLV and produced C type virus, as seen by electron microscopy. During cocultivation of ME cells and normal adult human leukocytes, HTLV was transmitted from ME cells to normal adult mononuclear cells. The majority of the lymphocytes, HTLV was transmitted from ME cells to the target T cells, causing immortalization of these cells.

The patient's neoplastic lymphocytes were mature T cells of helper phenotype. The majority of the lymphocytes from the ME cell line also initially expressed the helper phenotype. With continued passage (approximately 1 1/2 years), most of the ME cells were composed of lymphocytes that contained few identifiable surface phenotypic markers, but continued to synthesize lymphokines. About 30% of the ME cells were B lymphocytes with intracytoplasmic kappa or lambda immunoglobulin, and some or all of these cells were infected with Epstein-Barr virus.

Our studies clearly showed that some cells of ME are HTLV-1-infected B lymphocytes. First, using double-fluorescence labeling for p19 antigen and cytoplasmic immunoglobulin, we found that ME contained 20% to 40% B lymphocytes, and approximately 5% to 9% of these cells expressed the p19 antigen of HTLV. Second, we developed an adherent variant of the ME cell line known as ME-2. The cells have no T cell surface antigens, but 30% of cells contain cytoplasmic heavy chains of immunoglobulin. The cells strongly express p19 and p24 antigens (> 80%) and contain the HTLV genome. Classification of the cells is not clear; but the cells are probably pre-B (30%) and null (non-B, non-T) lymphocytes that are infected with HTLV. Finally, we isolated a B lymphocyte clone from ME (clone 11A). These cells expressed SM1g (> 70%), expressed the p19 and p24 antigens of HTLV (80%), contained the EBV and HTLV-I genomes, and efficiently transformed normal human peripheral blood T lymphocytes. Japanese investigators also established several EBV-transformed cell lines harboring HTLV from patients with adult T cell leukemia. Thirty colonies were isolated from one of the lines after soft-gel culture, and two thirds of the colonies were composed of B lymphocytes that expressed HTLV antigens. We have also isolated an EBV-transformed B lymphocyte line that contains infectious HTLV-II. The combined findings suggest that B cells and/or lymphocyte stem cells that can mature to either B or T cells may be infected by HTLV in vitro. Thus far, no human B cell leukemia has been associated with HTLV, suggesting that although HTLV can infect the B lymphocytes, the virus does not cause malignant transformation of the cells. Likewise, only T lymphocyte lines developed when we cocultured irradiated ME with normal adult mononuclear cells.

We were able to immortalize T lymphocytes from peripheral blood of normal adult volunteers by coculture with x-irradiated ME cells. Other investigators have also immortalized normal T cells, although many have used umbilical cord T lymphocytes in coculture experiments. The efficiency of transformation using x-irradiated ME was high: a total of 27 T lymphocyte lines were developed out of 38 attempts. The development of the lines did not require exogenous T cell growth factor or mitogen. The antigen phenotype of cells from 25 of 27 of the lines was helper T cell surface phenotype. Two of 27 lines were of suppressor T cell surface phenotype. A secondary transformant was established by cocultivation of one of the suppressor lines, S-BF, with cells of the same donor. The resulting T lymphocyte line, (S-BF)BF, was of helper antigen phenotype. The original ME cell line, and all phenotypic helper and suppressor T cell-transformed lines, produced lymphokines, including CSF, DIF, EPA, MIF, and NIF. The transformants with suppressor antigen phenotype constitutively produced lymphokines in amounts comparable to transformants with helper antigen phenotypes. Yamada previously showed a divergence between the surface antigen phenotype and the function of leukemic cells from patients with adult T cell leukemia. They showed that leukemia cells with helper phenotype were found to suppress antibody synthesis of B lymphocytes.

The ease of development of T cell lines by cocultivation with HTLV-positive cell lines may facilitate the purification and study of human lymphokines, the establishment of permanent T cell lines from patients with T cell-related diseases, and possibly, the study of the mechanism of leukemogenesis induced by HTLV.

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Characterization of a novel HTLV-infected cell line

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