A Clonal CD4-Positive T-Cell Line Established from the Blood of a Patient with Sézary Syndrome

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The reported inability to establish long-term T-cell lines from the blood of cutaneous T-cell lymphoma patients with circulating neoplastic T cells has hindered the development of an in vitro system to investigate Sézary syndrome. We have established a rapidly proliferating T-cell line from the peripheral blood of a patient with Sézary syndrome, which expresses a mature helper T-cell phenotype and contains cytogenetic abnormalities and T-cell receptor gene rearrangements identical to those in the patient's blood. The method of establishment and characteristics of this line are described. *J Invest Dermatol 96:31–37, 1991*

Sézary syndrome, a leukemic variant of cutaneous T-cell lymphoma (CTCL), is an intermediate-grade malignant lymphoma characterized by generalized erythroderma, lymphadenopathy, and the presence of T lymphocytes with cerebriform nuclei in the peripheral blood [1]. With a single exception [1], attempts to establish T-cell lines from patients with HTLV-I–negative Sézary syndrome have been unsuccessful [2–7]. For reasons not well understood, Sézary T-cell lines cannot be generated using experimental conditions that are sufficient for the propagation of CD4-positive cell lines from healthy donors. This apparent defect may be due to an inability of Sézary cells to be activated by conventional cell mitogens and/or a requirement for growth factors distinct from interleukin-2 (IL-2) [7].

By combining a factor produced by peripheral blood mononuclear cells (PBMC) of Sézary syndrome patients and recombinant (r) IL-2, we have established an IL-2–responsive, continuously growing T-cell line with structural and genetic characteristics of neoplastic Sézary cells.

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Abbreviations:
- CM: conditioned media
- CTCL: cutaneous T-cell lymphoma
- HTLV: human T-cell lymphotropic virus
- IL-2: interleukin-2
- PBMC: peripheral blood mononuclear cells
- PCR: polymerase chain reaction
- TCR: T-cell receptor
- TdR: [$_3$H] thymidine

MATERIALS AND METHODS

Patient Description Sézary patient number 4 (SZ-4), a 66-year-old black woman who lived in Philadelphia all her life, presented in May 1984 with numerous irregularly shaped patches and slightly infiltrated plaques disseminated over the trunk and extremities. The patient also had enlarged (1–2-cm–diameter) lymph nodes in both axillae and no other signs of extracutaneous involvement. Histopathologic findings on a skin biopsy specimen were diagnostic for an epidermotropic form of CTCL; immunohistochemistry, using parallel sections, demonstrated that the predominant T cell expressed an aberrant helper phenotype (CD2−, CD3+, CD4+, CD5+, CD7−, CD8−) with high expression of some but not all activation markers (HLA DR+, CD25+, and CD30−). A lymph node removed from the left axilla showed dermatopathic lymphadenitis and the remainder of the staging evaluation was unremarkable, although a few small Sézary-like cells (7 cells/100 lymphocytes) were identified on blood smears. Thus, the patient was considered to have plaque-phase mycosis fungoides at stage IIa (T2 N1 B0 M0). Despite systemic chemotherapy and oral methoxsalen combined with long-wave ultraviolet radiation, the patient's skin became diffusely eczematous by October 1985. In November 1986, lymph-node biopsy revealed diffuse effacement of nodal architecture, with relatively small irregularly shaped lymphocytes expressing identical phenotype as that observed in skin biopsy. Her disease was staged at IVa (T4 N3 B1 M0).

In October 1987, the patient died with extensive lymphomatous involvement. Permission for an autopsy was not obtained.

Establishment and Culture of the T-Cell Line Blood was obtained for establishing a cell line in September 1986. PBMC were recovered from heparinized blood from SZ-4 by Ficoll-Hypaque density centrifugation (Phamacia, Piscataway, NJ). 20×10^6 cells were placed in media containing 15 U/ml recombinant r-IL-2, (Amgen, Thousand Oaks, CA) and 10% conditioned media (CM) from concanavalin A (Con A; Sigma, St. Louis, MO)-treated Sézary PBMC. The culture was fed 2–3 times per week with media containing rIL-2, split at a 1 : 2 ratio when necessary, and allowed to grow. When culture growth slowed, as determined by tritiated thymidine incorporation and by viable cell counts, the cells were restimulated. Restimulation consisted of addition of either CM

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alone or CM and gamma-irradiated random allogeneic PBMC. Typically, the line required restimulation every 3 months for the first year of culture.

Conditioned Media (CM) PBMC recovered from another Sézary patient (SZ-1) were cultured at 4×10^6/ml with 2 μg/ml Con A for 48 h; the supernatant was then collected. One milligram per milliliter alpha-methyl mannoside (Sigma) was added to inhibit Con A activity. CM was used at 0.2% final concentration.

Culture Medium Cells were cultured in 15% fetal bovine serum (Flow, McLean, VA), 2% L-glutamine (Gibco, Grand Island, NY), 2% penicillin-streptomycin (Hazelton, Lenexa, KS), 0.5% Hepes buffer (Hazelton), 0.2% gentamycin (Sigma), and 15 U/ml rIL-2 in RPMI 1604 (Hazelton).

Phenotypic Analysis The expression of cell-surface antigens was analyzed by examining the reactivity of the cell line with mouse anti-human leuckocyte monoclonal antibodies (MoAb) whose binding was revealed with a FITC-conjugated goat anti-mouse IgG (Cappel, Malvern, PA). MoAb against CD2, CD3, CD4, CD8, CD15, CD16, and CD20 were the gifts of Dr. Georgia Trinchieri, Wistar Institute; anti-CD7 was a gift from Drs. Tom Palker and Bart Haynes (Duke University, Durham, NC); monoclonals specific for CD5, transferrin receptor (TFN-R), HLA DR, Leu 8, and T-cell receptor beta chain were purchased from Becton Dickinson (Mountain View, CA); and anti BE2 MoAb was the gift of Dr. Carol Berger, (Columbia University, NY, NY). MoAb reactive with T-cell receptor delta chain was purchased from T-Cell Sciences (Cambridge, MA); and anti-CD25 (Tac) MoAb was the gift of Dr. Thomas Waldman (NIH, Bethesda, MD). Analysis was performed on the Ortho (Raritan, NJ) cytofluorograf with log amplification. Negative controls of goat anti-mouse FITC alone were routinely performed. Data shown do not subtract background.

Cytogenetic Analysis Peripheral blood specimens were cultured for 72–96 h with a mitogen combination consisting of phytohemagglutinin (PHA), tetradecanol-O-phorbol-13-acetate (TPA), and interleukin 2 as previously described [8,9]. Standard trypsin-Giemsa-banded chromosome preparations were made [9]. At least 30 counts and 4 karyotype analyses were done to characterize the chromosomally abnormal clone.

Cells of the SZ-4 line were similarly processed without additional mitogenic stimulation.

Southern Blot Analysis DNA from both SZ-4 PBMC or SZ-4 cell line was extracted by cell lysis, proteinase K digestion, phenol extraction, and ethanol precipitation. Cellular DNA was digested with an excess of appropriate restriction enzymes, sized in 0.6% agarose gels, and transferred to nitrocellulose filters as described [10]. A cDNA probe for the constant region of the human T cell receptor beta chain was used to detect rearrangements of the TCR-B locus [11]. The probe was labeled by nick translation [12], using one of several alpha-^{32}P deoxycytosine triphosphates. Hybridization was carried out in 50% (vol/vol) formamide, $4 \times$ SSC ($1 \times$ SSC is 0.15 M sodium chloride/0.015 M, pH 7.0), 0.2 mg of sonicated salmon sperm DNA/ml, $1 \times$ Denhardt's solution (0.02% bovine serum albumin/0.02% Ficoll/ 0.02% polyvinylpyrrolidone) at 37°C for 15 h. After hybridization, filters were washed for 30 min in pre-hybridization solution (as above) at 37°C, followed serially by washes of 0.1 M KH_2PO4 in $2 \times$ SSC for 30 min at 65°C and $0.1 \times$ SSC of 30 min at 65°C. After drying, filters were exposed to Kodak XAR-5 film with intensifying screens.

IL-2 Responsiveness To determine the responsiveness of this line to IL-2, varying amounts of rIL2 were added to 5×10^4 SZ-4 cells that were previously washed free of media in 96-well flat-bottom plates (Costar, Boston, MA), which were cultured for 48 h at

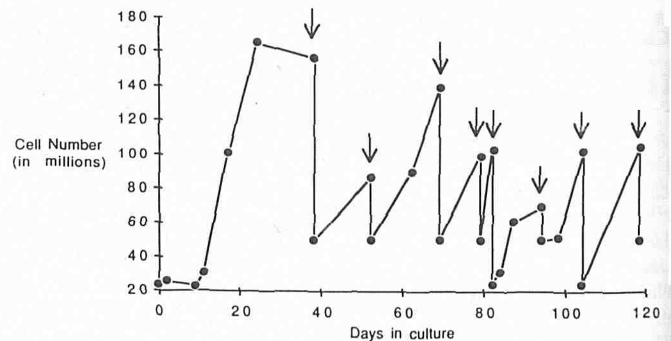

Figure 1. The growth pattern of SZ-4 cell line over a 120-d period is depicted. Twenty-five million SZ-4 cells were placed in culture with 15 U/ml rIL-2 and 0.2% CM until day 3; the cells were then cultured with rIL-2 only. The *arrows* represent removal of cells from the culture. (Reprinted with permission from [24].)

37°C. [^{3}H]-thymidine ([^{3}H]-TdR) (Amersham, Arlington Heights; 60 Ci/mMol) was added for the final 6 h of culture using 2 μCi/well and the cells harvested onto glass filtermats (Skatron, Lier, Norway) using a cell harvester (Flow, McLean, VA).

In IL-2 dependency experiments, SZ-4 cells were washed twice and incubated at 37°C for 45 min, then washed again to rid the cells of any bound IL-2. The cells were cultured for 48 h in 96-well flat-bottom plates as above, in media without IL-2 containing dilutions of goat anti-human IL-2 (produced by Dr. P Wettstein, Wistar Institute) varying from 1:200 to 1:125,000. [^{3}H]-TdR was added to each well for the final 6 h of culture and harvested as above.

Immunohistochemical Staining SZ-4 cell line T cells were processed for immunohistochemical staining by cytocentrifugation on glass slides and fixation in methanol : acetone (1 : 1). After blocking with 30% goat serum, cells were incubated with optimal concentration of MoAb for 1 h, rinsed, and then developed with the Dako (Santa Barbara, CA) alkaline phosphatase–anti-alkaline phosphatase (APAAP) kit. Briefly, linker antibody was added for 1 h, then the APAAP immuno complex [13] was generated by subsequent sequential incubations. The AP substrate (napthol AS-MX phosphate and Fast Red) will yield a bright red positive reaction. Endogenous phosphatase was inhibited by levamisole contained in the buffers. This technique allows the identification of one positive cell in 10^4 cells due to the negligible background and dramatic color contrast between positive and negative cells [14].

Immunoblotting (Western Blot Transfer) Immunoblotting was used to evaluate the presence of antibodies against HTLV-I proteins in patient serum as described [14].

Lysates: Twenty million exponentially growing MT-2 cells, a known HTLV-I infected cell line, were pelleted and washed twice in phosphate-buffered saline (PBS) without Mg^{++} and Ca^{++}. The cells were lysed by the addition of 2 ml lysing buffer containing 1% Triton X100, 1 mM MgC1, 0.1 M Tris, pH 7.5, 1.0 mM dithiothreitol, and 2.0 mM phenylmethylsulfonyl fluoride. The cells were then gently vortexed followed by addition of equal volume of 2% Triton lysing buffer and a 2-h incubation on ice. The lysate was centrifuged at 15,000 rpm in a Spinco centrifuge for 30 min to remove cellular debris.

Electrophoresis: Both commercially available purified HTLV-I proteins and lysate from MT-2 were separated by SDS-polyacrylamide gel electrophoresis using 10% acrylamide 20 V–8 mA/gel for 16 h. After electrophoretic separation, proteins were transferred to nitrocellulose paper (Schleicher and Schuell, Keene, NH) in a Transblot (Biorad, Richmond, CA) at 60 V–0.25 A for 4 h following manufacturer's instructions. The nitrocellulose sheet was cut in strips and washed with blocking buffer (20 mM Tris, 500 mM NaCl [pH 7.5], 3% gelatin) to saturate free binding sites and reacted for 60

Table I. Phenotypic Analysis of SZ-4 Blood and Cell Line

	CD2	CD4	CD8	CD3	CD5	CD7	Leu8	TFN-R	Dr	Tac	BE2	CD16
Blood												
11/85	74	55	19									
4/86	90	81	9									
Line	90	96	4	97	66	38	38	60	95	97	97	7

min with anti-HTLV I antibody or SZ-4 serum. After thorough washing with 20 mM Tris, 500 mM NaCl, 0.05% Tween-20 (TBS), strips were reacted with conjugate (peroxidase-labeled goat anti-mouse or anti-human IgG) for 1 h. Strips were washed again and developed for 10–15 min with freshly prepared solution containing one part of 4-chloro-1-naphtol in methanol (0.3%), five parts of 100 mM Tris [pH 7.6] and H_2O_2 to final concentration 1:3000. This system can detect less than 100 ng of a specific protein. Strips with molecular weight markers are stained with amidoblack.

Reverse Transcriptase Assay Reverse transcriptase activity was detected as performed by Poiesz et al [15] with poly A template, oligo(dT) primer in the presence of 10 mM Mg^{++}.

Electron Microscopy To determine morphologic characteristics, either buffy coat preparations from the blood of patient SZ-4 or the SZ-4 cell line were processed for electron microscopy. Briefly, cells were fixed overnight at 40°C with 2% glutaraldehyde (Electron Microscopy Sciences, Fort Washington, PA) in 0.1 M cacodylate (Electron Microscopy Sciences), pH 7.4 (buffer), and then washed and post-fixed in 1% osmium tetroxide (Electron Microscopy Sciences) in buffer for 1 h, rinsed 2 times with buffer, dehydrated through graded ethanol to 100% and propylene oxide (Ladd, Burlington, VT) and embedded in Epon (Marivar, Nova Scotia). Thin sections were cut on an LKB Ultrotime III, stained with uronyl acetate and lead citrate, and viewed with a Hitachi HU-12A electron microscope.

Analysis of SZ-4 Genomic DNA for HTLV-I Gag Sequences

DNA Extraction: DNA was extracted from SZ-4 cell line by SDS/proteinase-K digestion of cells followed by phenol-chloroform extraction in a nucleic acid extractor (Applied Biosystem, Inc.) The filter containing DNA was dissolved in double distilled water. DNA concentration were estimated by measuring the absorbance at 260/280 nm.

PCR: Two micrograms of DNA was amplified in 25 repetitive, three-step cycles for 1 min each at 95°C and 55°C, and for 2 min at 72°C. All amplifications were carried out in a Perkin-Elmer Cetus Thermal Cycler. Primer pairs were gag 841–864 bp, gag 1353–1375 bp (HTLV-1 proviral sequence HL1PROP from gene bank); RM73 HTLV-I gag genomic DNA was used as a probe in this experiment. The 100 μl of PCR reaction mixture contained 2 μg of sample DNA, 278 μM each dATP, dCTP, dGTP, dTTP, 0.8 μM of each primer, 10 mM Tris (pH8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01% (w/v) gelatin (Sigma), and 2.5 units of Thermus Aquaticus polymerase (Taq) enzyme [1] (Perkin-Elmer, Cetus). The reaction mixture was overlayed with two drops of mineral oil to prevent evaporation and was denatured at 94°C for 7 min before the Taq polymerase was added.

Analysis of Amplified DNA for HTLV-I Gag Homology: Twenty-five microliters of the final amplified reaction product was analyzed by electrophoresis on 1.2% agarose gel (150 v, 2–3 h) and transferred to Nitran nylon membrane (S&S Nitran) by blotting. The filter was soaked with 2 × SSC for 5 min at room temperature and baked at 80°C for 2 h under vacuum. The prehybridization buffer consists of 6 × SSC, 0.5%, SDS, 50% formamide, 5 × Denhardt's solution and 150 μg/ml herring sperm DNA. The filter was prehybridized overnight at 37°C and then hybridized overnight with 12×10^6 cpm of labeled probe in prehybridization buffer. Filters were then washed with 2 × SSC and 0.1% SDS, two times for 20 min at room temperature, then with 0.2 × X SSC, SDS, and 0.1% SDS for 20 min at room temperature, then 0.1 × SSC and 0.1% SDS for 30 min at 37°C. Autoradiography was carried out with two intensifying screens (Cronen Hi Plans, Dupont) at −70° with Kodak X-AR film.

RESULTS

SZ-4 is a T-cell line established from the peripheral blood mononuclear cells of a patient with Sézary syndrome. To examine whether the cell line was dividing in culture, cell counts were performed. The growth characteristics of this cell line after stimulation with CM over a four-month period is depicted in Fig 1. The cell line appears to have a doubling time of approximately 30 h, which is maintained throughout the period shown.

The phenotypic analysis of these cells performed months after initiation indicates that the cell line is a mature helper T cell expressing CD2, CD3, and CD4 but not the cytotoxic/suppressor cell marker CD8 (Table I). This phenotype is characteristically expressed on the majority of atypical lymphocytes in blood of Sézary syndrome patients [16], and correlates well with the lymphocyte subset analysis performed on both the blood and the skin of patient SZ-4, with the exception of CD2. However, diminished CD2 expression on infiltrating T cells in the skin is not an uncommon occurrence [17]. The cell line also expresses a variety of activation markers including HLA-DR, transferrin receptors, CD5 (T10), and CD 25 (Tac, light chain of the IL-2 receptor). In addition, these cells were positive with BE2, a monoclonal antibody that reacts with an activation antigen expressed on normal PHA blasts [18], whose reactivity has also been associated with Sézary cells [19]. The cell line had partial reactivity with Leu 8 (38%), CD7 (Leu 9, 3A1 (38%)), LFA1 (60%), and anti-beta TCR antibody (41%), but was

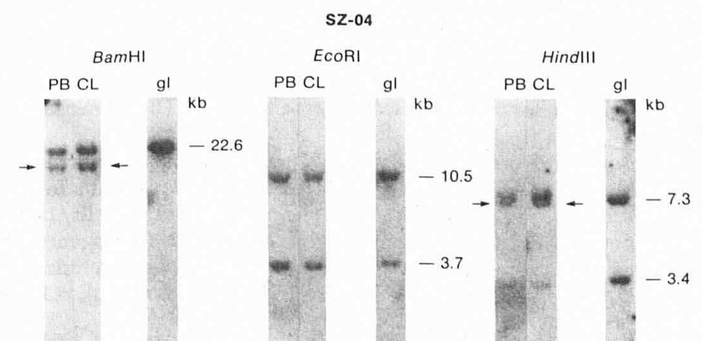

Figure 2. DNA extracted from SZ-4 cell line (*CL*) and the patient's PBMC (*PB*) were subjected to Southern blot analysis using a probe for the C-beta region of the TCR. Bam H1-digested DNA show the germline band (gl) at 22.6-kb and a 10-kb rearrangement in both the blood and cell line. The blood and cell line provide germline configurations in the Eco R1-digested DNA. Hind III digestion produced a 7-kB fragment in the DNA from both cell line and blood. (Reprinted with permission from [24].)

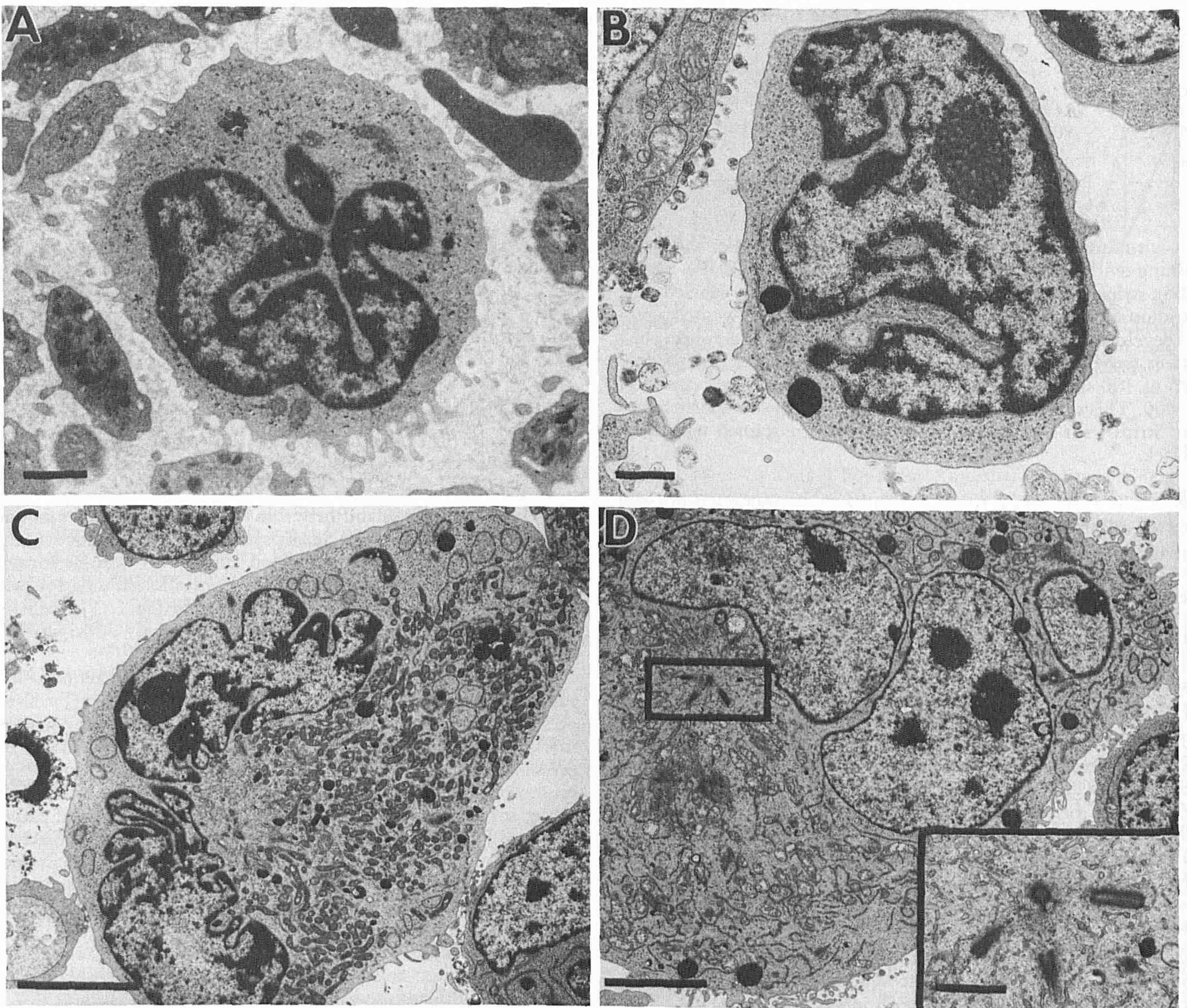

Figure 3. Transmission electronmicroscopy of a typical Sézary T cell from the peripheral blood buffy coat prior to culture (*A*), compared to cultured cells of SZ-4 line (*B–D*). Note the elaborately infolded nuclear contour of a pre-culture Sézary cell (*A*), and retention of these contour anomalies by some of the cultured cells from this patient (*B–D*). Occasional cells in the SZ-4 line were multinucleated (syncytia-like cells); some contained numerous mitochondria and cistternae of smooth endoplasmic reticulum (*C, D*) and clusters of aberrant centrioles centrally located within the cytoplasm (*D*). *A: bar,* 1 μm. *B: bar,* 1 μm. *C: bar,* 5 μm. *D: bar,* 5 μm (*inset bar,* 1 μm).

found negative for *CD 16* (NK marker) and *CD 20* (B cell marker) and TCR delta chain.

Chromosome studies were originally performed on a blood specimen obtained in December 1985. At that time the patient's total leukocyte count was 8,800 (54% lymphocytes, 22 Sézary cells/100 lymphocytes). A pseudodiploid clone with multiple cytogenetic abnormalities was found, with the karyotype partially characterized as: 46,XX, 1p−, 4q−, +?4q−, abn 5, −10, 16q+, iso [17q], −18, 21q+, +mar.

A repeat study was performed on the blood in January 1987. Several related near-diploid subclones were present with a number of the same karyotypic alterations (e.g., 1p−, 16q+, iso [17q], 21q+) still present, as well as additional changes (e.g. 3q−, 6q−), consistent with the patient's clinical progression. In both blood studies, a very small number of near-tetraploid metaphases (<3%) was observed.

Cytogenetic data on the SZ-4 cell line, established in September 1986, were first obtained in November 1986 (day 64). Approximately half of the metaphases were normal, and the remainder constituted a near-tetraploid clone with many of the same structural chromosome abnormalities observed in lymphocytes from the blood: 1p−, 3q−, 4q−, 16q+, iso [17q], 21q+. The cell line was reexamined in December 1987 (day 451), and although only a few usable metaphases were obtained, they all appeared to represent the same near-tetraploid clone.

To confirm the observation made by cytogenetic analysis, we subjected DNA extracted from the cell line and the patient's PBMC to Southern blot analysis using a probe for the C-beta region of the TCR gene as described in *Materials and Methods.* Results in Fig 2 show that cells from both of these sources contain similar clonal rearrangements. Bam H1-digested DNA show a 10-kb rearranged band found in both the blood and cell line. Hind III digestion produced a 7-kb rearranged band in the DNA from both cell line and blood. These data indicate that both the cell line and blood contain a clone with a unique TCR rearrangement that produces identically sized fragments in Bam HI and Hind III digestions.

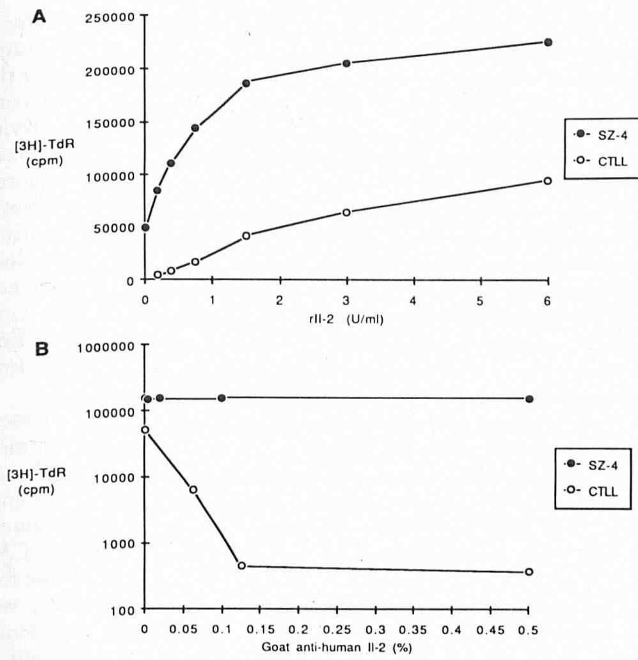

Figure 4. The thymidine (TdR) incorporation of SZ-4 *(closed circle)* and CTLL *(open circle)* cell lines to a titration of rIL-2 is shown in *A*. SZ-4 cell line was cultured in medium without IL-2 for 48 h before initiation of the experiment. IL-2–dependent CTLL cells removed from a rapidly growing culture were used as an IL-2–dependent control. Cells were cultured as described in *Materials and Methods* with 5 × 10⁴ cells/well in 200-μl volume in flat-bottom 96-well plates. Cells were cultured for 40 h, with 2 uCi ^{3}H-TdR provided for the last 4 h of culture. Data shown represent the mean of triplicate cultures. *B* shows the effect of anti–IL-2 antisera on the proliferation of CTLL *(open circle)* and SZ-4 cell line *(closed circle)*. CTLL cells taken from an IL-2–dependent culture were given 12.5 U/ml rIL-2 and various concentrations of goat anti-human IL-2. SZ-4 cells were taken from a culture deprived of rIL-2 for 48 h and then cultured without rIL-2, along with various concentrations of the goat anti–IL-2 antisera. Five × 10⁴ cells/well from each cell line were cultured for 48 h, with 2 uCi ^{3}H TdR provided for the final 4 h. Data represent the mean of triplicate cultures. Response of SZ-4 cell line when given 15 U/ml rIL-2 in this experiment was 239,610 (cpm). (Reprinted with permission from [24].)

Migration patterns such as these have been interpreted by others as signifying identical clones [20,21].

Sézary cells observed in the peripheral blood typically contain convoluted nuclei [22]. The morphologic characteristics of a typical Sézary cell from the peripheral blood of patient SZ-4 (Fig 3*A*) and several of the T cells in SZ-4 cell line (Fig 3 *B, C, D*) are shown in the electron micrographs in Fig 3. Many of the cells observed in the cell line are moderately convoluted in contour, an example of which is shown in *B*. Such cells were indistinguishable from Sézary cells in the peripheral blood. However, not all cultured cells had convoluted nuclei, which may relate to in vitro conditions. Approximately 5% of the cells contained multiple nuclei *C, D*), and aberrant mitoses and centrioles were documented in occasional cells (*D*, inset).

Because T-cell dependence on IL-2 for growth is a hallmark of normal, but not necessarily of tumor, cells [23], we wished to determine if SZ-4 was dependent on IL-2 for its continuous proliferation. To investigate the IL-2 requirements of this cell line, we performed a titration of rIL-2. Figure 4 (*A*) contains data demonstrating that the line is responsive to IL-2 but continues to synthesize DNA, as measured by thymidine incorporation, with no IL-2 present. Data with CTLl, an IL-2–dependent line, are also shown. To confirm the IL-2 independence of SZ-4 and to eliminate the possibility that the line is itself producing enough IL-2 to stimulate the cell division in an autocrine fashion (observed in the "no IL-2" group), we cultured the line for 2 d without IL-2 and then with varying concentrations of goat anti-human IL-2 in media without rIL-2. The data in Fig 4 (*B*) show that the cell line is not affected by the antiserum. In addition, conditioned media from SZ-4 cells grown without IL-2 for 3 d were found devoid of IL-2 when tested in a CTLl assay, suggesting that SZ-4 line does not constitutively produce IL-2 (data not shown). Taken together, these data indicate that this cell line can proliferate in an IL-2–independent manner.

Because the cell line demonstrated IL-2 independence and because HTLV-I infection has been observed with some patients with CTCL, we tested serum from this individual for reactivity against pure HTLV-I proteins on a Western blot. Reactivity against an HTLV-I gag protein (p24) was observed, but little or no reactivity against the env or other mature proteins was found (data not

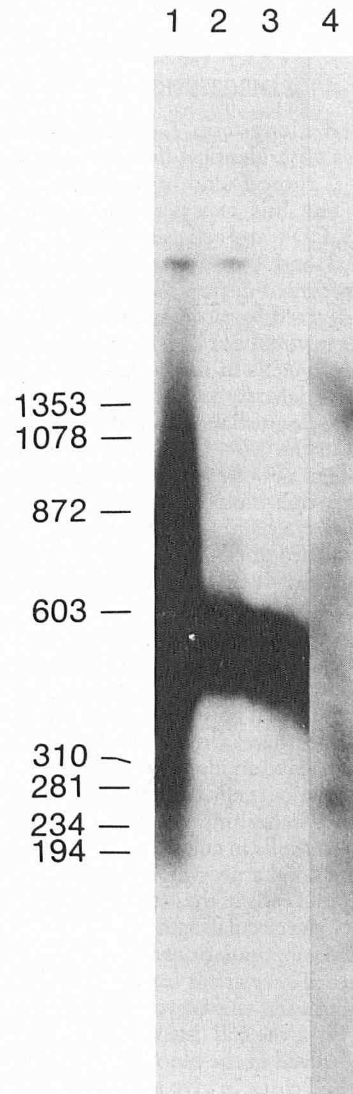

Figure 5. Analysis of SZ-4 amplified with HTLV-1 gag primer pairs. Two micrograms of SZ-4 genomic DNA was amplified for 25 cycles as described in *Materials and Methods* and analyzed for HTLV-I gag sequence *(lane 4)*. The PCR product was analyzed after sizing in a 1.2% agarose gel by hybridization to a labeled HTLV-I gag probe; autoradiography was then performed. Either 20 or 2 ng of DNA from a known HTLV-I–infected cell line (13-4) was amplified as a positive control *(lanes 1–3)*. Two micograms of genomic DNA from 7 healthy donors was also found negative for HTLV-I gag sequences (not shown). Primer pairs used in this experiment were gag 841-864 bp, gag 1353-1375 bp (HTLV-1 proviral sequence HL1PROP from gene bank), and HTLV-I gag genomic probe.

shown). These results are considered negative for HTLV-I infection. In addition, SZ-4 was tested for expression of the gag proteins p19 and p24 and env protein gp46 using the alkaline phosphatase anti-alkaline phosphatase (APAAP) system. The cell line did not express either p24 or gp 46 HTLV-I proteins (data not shown). Furthermore, culture supernatants were found devoid of reverse transcriptase activity (data not shown).

To confirm the lack of HTLV-I infection in this line, we performed polymerase chain reaction (PCR) amplification of SZ-4 genomic DNA as described in *Materials and Methods.* After extraction, 2 μg SZ-4 genomic DNA was amplified utilizing HTLV-I gag primers for 25 cycles and then analyzed for hybridization to an HTLV-I gag-genomic probe. Results shown in Fig 5 indicate that SZ-4 does not contain HTLV-I gag sequences *(lane 4)*, as compared to the amplified product generated from either 200-, 20-, or 2-ng genomic DNA from a known HTLV-I–infected cell line (13-4) *(lanes 1–3)*. Taken together, we conclude that neither the SZ-4 patient nor the cell line were HTLV-I infected.

DISCUSSION

We have shown that a long-term T-cell line has been established that contains T cells with identical morphologic, phenotypic, and genotypic features as those found in the blood of a patient with Sézary syndrome. The line expresses the normal helper T-cell markers CD2, CD3, CD4, and activation antigens HLA-DR, transferrin receptors, BE2, and Tac. To our knowledge, this is the first non–HTLV-I transformed Sézary T-cell line to express all these antigens and to be derived from the neoplastic clone.

In order to examine whether the cells in culture resembled morphologically the Sézary cells in the peripheral blood, electron microscopy was performed. Many of the cells in the SZ-4 cell line were morphologically indistinguishable from the Sézary cells in the patients' blood. Cells in the SZ-4 cell line with slightly convoluted nuclei, although similar in both size and overall morphology to the cells with moderately convoluted nuclei in the culture, may represent either normal cells remaining in the culture or Sézary cells that have lost their convoluted nuclei in culture. If the latter is true, this in vitro model could provide a means to investigate how nuclear morphology is regulated.

To determine if the T cells in our culture were derived from malignant (Sézary) cells in the blood of the patient, we compared the karyotype and genotype of cells from these two sources. Results from these analyses show that both the patient's circulating lymphocytes and the cell line share a number of specific chromosomal abnormalities and also have an identical TCR gene rearrangement, strongly supporting the concept that the cells in culture were derived from the Sézary cell clone in the blood. Interestingly, the predominant neoplastic cells in culture had a near-tetraploid karyotype, whereas in the blood a near-diploid clone was predominant, with rare hypotetraploid cells in these chromosome preparations. In many Sézary patients, the circulating neoplastic clone is a mixture of diploid and tetraploid subpopulations [8,9], with proportions varying at different times. There are at least two possible explanations for the differences between the karyotypes observed in the blood and in the cell line. First, the cell line was derived from the hypotetraploid subclone observed in the blood, which in turn was derived from the pseudodiploid clone in vivo at a time before the aterations to chromosomes 5, 10, and 18 occurred. Alternatively, the hypotetraploid clone grown in vitro was derived from the pseudodiploid clone in vivo; the karyotypic differences between the line and blood represent changes that occurred in culture. It is not clear why the hypotetraploid clone grew more readily in our culture conditions.

Another aspect of this cell line that suggests in malignant neoplastic origin is its ability to proliferate in an IL-2–independent manner. The entry of normal T cells from G1 to S phase of the cell cycle is dependent upon interaction with either endogenous or exogenous IL-2, along with other signals [25–30]. Loss of the requirement for these signals can be observed with aggressive T-cell tumors such as adult T-cell leukemia and suggests that the cells are transformed. Two types of transforming agents for human T cells are the retroviruses HTLV I and II [23]. Although the patient's serum contains antibodies that react with HTLV-I p24 gag protein, reactivity was not observed with products from any of the other genes, including any mature envelope proteins, and therefore must be considered negative. Because the HTLV-I proteins used in this assay also cross-react immunologically with HTLV II, this patient can be considered seronegative for this virus as well. In addition, the cell line does not express either p24 gag, p46, or p62 env HTLV-I antigens, nor does it have reverse-transcriptase activity. Furthermore, we were unable to detect the HTLV-I gag gene in amplified SZ-4 cell line DNA. Taken together, it is unlikely that this patient or her cell line is infected with HTLV-I or II.

Success in establishing this line may relate to the method we used to initiate the culture, and/or to the aggressive nature of this tumor clone. The clinical status of this individual at the time blood was drawn for culture suggests that her tumor was an aggressive variant. This line was established by the addition of mitogen-conditioned media made with Sézary PBMC to the PBMC of this patient. CM generated with PBMC from healthy donors could not substitute for Sézary-derived CM. However, in preliminary experiments, we found that some, but not all, PBMC from other Sézary patients appeared to have similar activity. SZ-1 CM appears to contain a potentially novel factor that we have called Sézary Activating Factor (SAF), which induced functional IL-2R on these cells.* As we and others have failed to grow Sézary tumor cells in culture through the addition of mitogens [2–6], SAF may activate Sézary cells through a different pathway than that utilized by mitogens.

The long-term Sézary T-cell line described here may prove useful in understanding the nature of this disease through examination of its responsiveness to, and production of, lymphokines and cytokines. Although the chromosomal abnormalities in patients with Sézary syndrome appear to be largely random [9], analysis of the specific abnormalities found in this clone may provide clues to its growth advantage and interleukin independence. In addition, determination of the specific V-region family used in this clone's T-cell receptor compared to that used in other cases of Sézary syndrome may provide clues as to whether a subtype of T cell, or a specific antigen, is involved in this disease.

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