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Human T-Cell Leukemia-Lymphoma Virus (HTLV): A Progress Report

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A. Introduction

Since type-C retroviruses are known to be involved in naturally occurring leukemias of many animal species [26], a similar viral etiology has been sought in human leukemias. Some of the animal models provide important insight for consideration of human leukemias. For example, while most virus-induced animal leukemias and lymphomas are associated with abundant virus production in the tumor cells, bovine leukemia virus (BLV), the causative agent of bovine leukemias, was not detected until the leukemic cells were cultured in vitro (see review by Miller and Van Der Maaten [13]). This brings out the importance of long-term culture of the appropriate target cells for virus detection and isolation. In 1976, our laboratory reported the discovery of a factor termed T-cell growth factor (TCGF) [15]. Following interaction with an antigen, different subsets of mature T cells respond by making and releasing TCGF or making a receptor to TCGF. The TCGF binds to the receptor-bearing T cells and induces cell growth. Addition of exogenous TCGF can maintain growth of activated mature T cells for long periods [6, 26]. When TCGF was added to T cells obtained from patients with mature T-cell leukemias and lymphomas, some cells directly responded without prior activation in vitro [18]. Some of those samples released a retrovirus which we call human T-cell leukemia-lymphoma virus (HTLV) ([19, 20]; Popovic et al., in preparation). The morphology of HTLV is typically type C. Figure 1 shows an electron micrograph of some HTLV particles. Like all retroviruses, HTLV contains reverse transcriptase, has a high molecular weight RNA genome, and buds from cell membranes. It is distinct from all other known animal retroviruses [9, 16, 22, 23] and to date is the only unequivocal human retrovirus. (The retrovirus later isolated independently in Japan [14, 31] and called ATLV is, in fact, HTLV.) Furthermore, it is specifically associated with certain forms of human leukemia and lymphoma [4]. Here we wish to describe some of the new isolates of HTLV and report on some recent findings on the nature and distribution of HTLV and its transmission to and biological effects on normal T-lymphocytes in vitro.

B. Identification of New HTLV Isolates in Established Cell Lines Derived from Patients with T-Cell Leukemias/Lymphomas

Cell lines derived from patients with leukemia/lymphomas of mature T cells from geographically different parts of the world have been established in culture in the presence of TCGF as described previously for normal mature human T cells [15, 26] and neoplastic mature T cells [18]. These cell lines were analyzed for HTLV by (a) competition radioimmunoprecipitation assay (RIPA) for the major core protein p24 [9], (b) indirect immune fluorescence assay (IFA) using highly specific monoclonal antibody for another HTLV antigen, p19 [24], (c) reverse transcriptase activity in the culture fluids, and (d) electron microscopy. In addition to the positive cell lines CR [19]

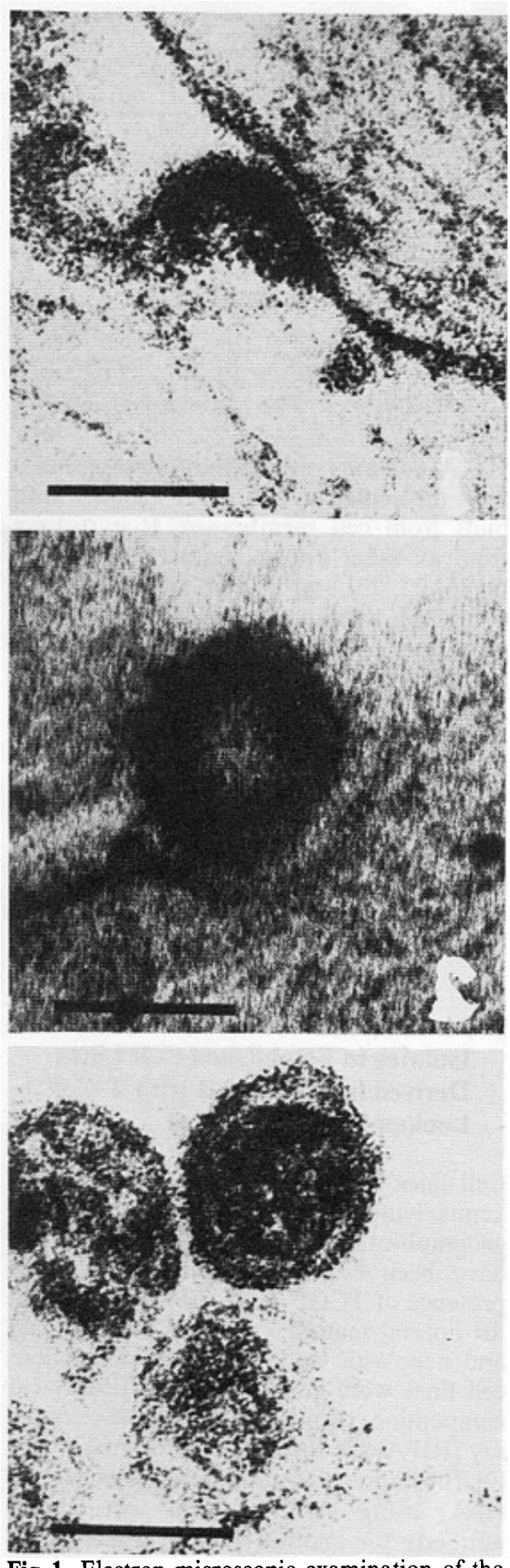

Fig. 1. Electron microscopic examination of the cell line MB, showing extracellular and budding HTLV particles. *Bar* represents 90 μ

and MB [20] published earlier, seven of eight recently established T-cell lines fully expressed HTLV (Table 1) and one showed partial expression. These patients include four individuals from the United States, one from Israel, and three from a single Japanese family from the northwest part of the Honshu Island in Japan. In this family, the patient SK with acute T-cell lymphoma (ATL) and both his parents are virus positive. The father (MK) is clinically healthy and the mother has persistent lymphocytosis, which is considered to be a preleukemic

Table 1. Expression of new HTLV isolates in T-cell lines derived from patients with adult T-cell leukemia/lymphomas

Cell line[d]	p24[a] (ng/mg)	p19[b] (% positive cells)	RT activity[c] (pmol/ml extract)	EM
MJ	128	85	9.3	+
UK	1941	71	4.5	+
MI	1503	63	16.8	+
WA	1076	78	19.2	+
PL	683	23	6.2	+
SK	174	39	5.8	+
TK	2700	54	33.0	+
HK	400	38	13.5	+

[a] Detected by competitive radioimmunoprecipitation assay (RIPA) in cell extract

[b] Indirect immunofluorescence assay (IFA)

[c] Reverse transcriptase activity (RTA) in culture fluids was measured with $(dT)_{15}(rA)_n$

[d] Cell lines were derived from peripheral blood (PB) or bone marrow (BM) of different patients as follows: (a) MJ from PB of a 50-year-old white male with mycosis fungoides, from Boston, Massachusetts; (b) UK from PB of a 45-year-old white male with diffuse histiocytic lymphoma, from Jerusalem, Israel; (c) MI from PB of a 32-year-old black female with T-cell lymphosarcoma cell leukemia, from Granada, West Indies; (d) WA from BM of a 24-year-old black male with diffuse mixed lymphoma, from Augusta, Georgia; (e) PL from PB of a 27-year-old black female with T-cell diffuse mixed lymphoma, from Ovita, Florida; (f) SK from PB of a 21-year-old male with adult T-cell leukemia, from Akita perfecture, Japan; (g) TK from PB of a 45-year-old female (mother of patient SK) who has 7% abnormal cells in her blood, from Akita perfecture, Japan; and (h) HK from PB of a 49-year-old male (father of patient SK) who is normal, from Akita perfecture, Japan

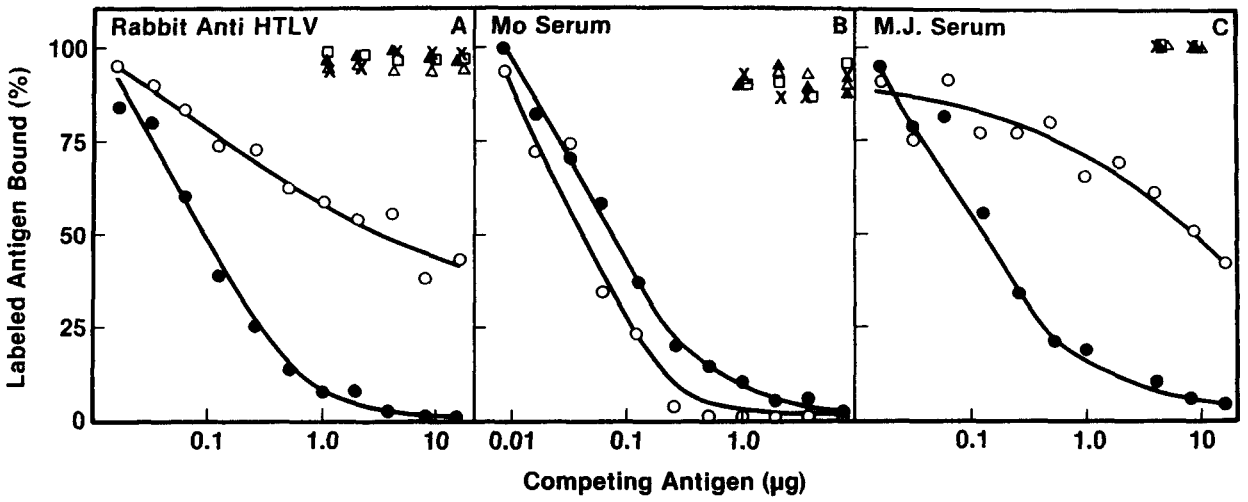

Fig. 2A–C. Homologous and heterologous competition radioimmunoassays of HTLV p24. Assays were carried out as described [9] using ^{125}I-labeled HTLV$_{CR}$ p24 and a limiting dilution of hyperimmune rabbit antibody to HTLV$_{CR}$ or sera from patients MO and MJ. **A** Competition RIA using rabbit anti-HTLV$_{CR}$. **B** Competition RIA using MO serum. **C** Competition RIA using MJ serum. Virus extracts used for competition were: ●–●, HTLV$_{CR}$; ○–○, HTLV$_{MO}$; ×–×, Mason Pfizer monkey virus; △–△, bovine leukemia virus; □–□, Rauscher murine leukemia; ▲–▲, simian sarcoma virus

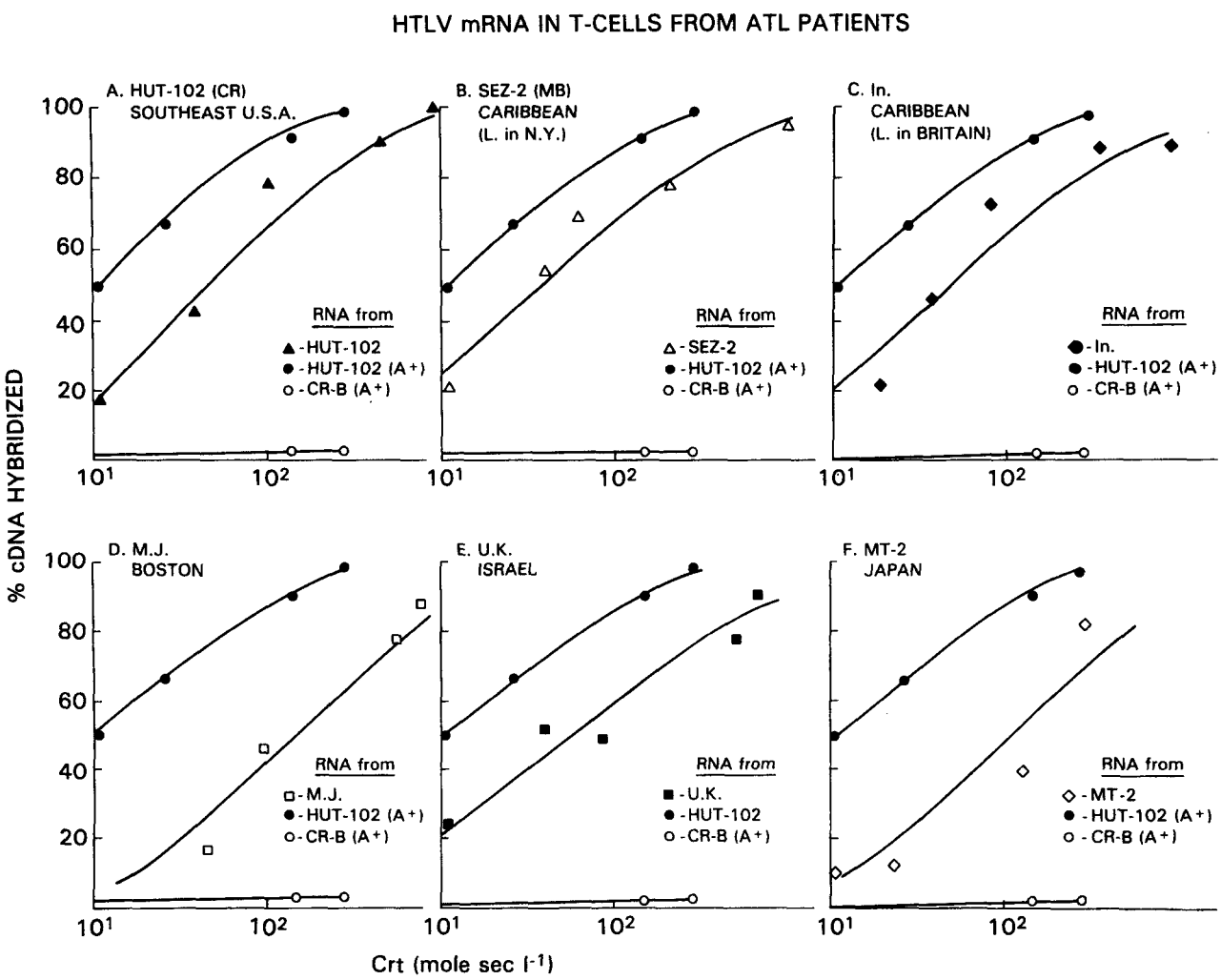

Fig. 3A–F. Relationship of different HTLV isolates by RNA hybridization. ^{3}H-HTLV$_{CR}$ cDNA was synthesized by calf thymus DNA primer [22] and hybridized to cellular RNA from different HTLV-positive cell lines. Results with the controls with poly(A)+RNA from CR-T (HUT 102) and CR-B cells are superimposed on each panel for comparison with the others

state. All cell lines have karyotype and HLA patterns that match those of the donors, and the HLA profiles are different for all eight cell lines. In addition, a T-cell line established by Golde and colleagues from a patient (MO) with hairy cell leukemia [27] was positive vor HTLV p19 and p24 [11]. We propose to designate each virus isolate with a subscript of the patient's initials, e.g., $HTLV_{CR}$, $HTLV_{MB}$, etc. All isolates except $HTLV_{MO}$ are highly related to each other as assayed by competitive radioimmunoassay with p24 (Fig. 2) and hybridization of viral cDNA to mRNA of the producer cell lines (Fig. 3). By these assays, the virus of Japanese ATL is indistinguishable from the prototype HTLV as exemplified by the earlier isolates $HTLV_{CR}$ and $HTLV_{MB}$. On the other hand, $HTLV_{MO}$ competes poorly in the p24 assay (Fig. 2), and nucleic acid sequence homology with $HTLV_{CR}$ was detected only under very nonstringent hybridization conditions (our unpublished data). Therefore, this virus may form a distinct subgroup in the HTLV family. We propose to group them as HTLV-I_{CR}, etc. versus HTLV-II_{MO}.

C. HTLV Provirus in Neoplastic T Cells: Evidence for Exogenous Infection

We had reported earlier that HTLV sequences are present in the infected cells and not in normal uninfected human cells [22], suggesting that HTLV is not an endogenous human virus. In the case of the patient CR, we also had the opportunity to find out whether he was infected pre- or post-zygotically [5]. Several T-cell lines, some clonal derivatives of these lines and a B-cell line have been established from CR. These cells were shown to have originated from the same individual by HLA typing. HTLV proviral DNA was detected in some but not all of the independently established T-cell lines of CR and not in the B cells. An example of the DNA hybridization kinetics is shown in Fig. 4. Furthermore, the surface phenotype OKT3−, OKT4+, and OKT8− appears to correlate with the presence of HTLV. These results indicate that HTLV was acquired by CR by horizontal transmission and suggest that only a subtype of T cells is the target for HTLV infection.

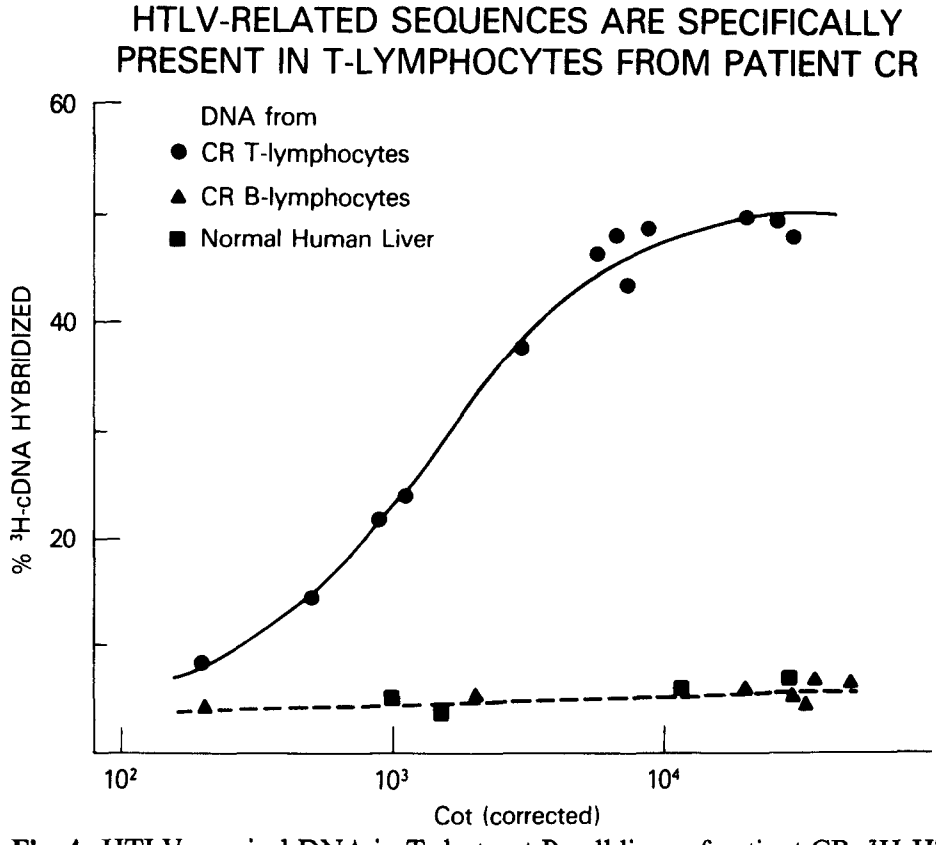

Fig. 4. HTLV proviral DNA in T- but not B-cell lines of patient CR, ^{3}H-HTLV cDNA was annealed to cellular DNA from CR-T lymphocytes (●), CR-B lymphocytes (▲), and PHA-stimulated normal human peripheral blood T-lymphocytes (■)

Recently, molecularly cloned sequences representing the 5′ and 3′ ends of HTLV have been obtained in our laboratory [12 a]. These clones have been used as probes for Southern hybridization of fresh leukemic DNA from patients with HTLV-positive diseases [30]. These revealed one or few copies of HTLV integrated at a site which is unique for a given patient but varies from patient to patient. DNA from normal people did not contain hybridizing sequences. A similar observation has been made by others [31]. These results suggest that the infected cells are of clonal origin, so infection must have occurred prior to disease development. This feature is also found in animal leukemia-lymphomas induced by chronic leukemia retroviruses.

D. Clinical Features of HTLV-Positive Diseases

Seroepidemiological studies have identified HTLV-positive patients from many regions of the world with at least three major areas that appear to be endemic: Southwestern Japan [4, 8, 9, 25], the Caribbean [1], central South America (see also Blattner et al., this volume), but only sporadically in the United States [21]. Similar clinical features are found in the diseases associated with these areas, i.e., Japanese adult T-cell leukemia (ATL) and T-cell lymphosarcoma cell leukemia (T-LCL) in the West Indian Blacks from the Caribbean. Both are represented by an aggressive course an frequent association with lymphadenopathy, hypercalcemia, hepatosplenomegaly, and cutaneous manifestations [2, 28]. The tumor cells are all mature, lack terminal deoxynucleotidyl transferase and express differentiated functions. Typing with monoclonal antibodies as well as functional studies showed that the cells may be either of the helper-inducer or suppressor-cytotoxic phenotype. Histologically, the cells are pleomorphic, often with highly convoluted nuclei. Almost all patients with ATL and T-LCL are HTLV positive. These observations led to the hypothesis that HTLV is associated with a subtype of adult T-cell malignancy which may include an aggressive form of cutaneous T-cell lymphoma (CTCL) found in patients CR and MB. In fact, the presence of HTLV may be of practical importance in disease classification. However, at least two HTLV-positive patients have relatively benign diseases: MJ with Sezary syndrome and MO with T-cell hairy cell leukemia. It should be noted, however, that at least the virus in MO is significantly different from the prototype HTLV.

E. Infection and Transformation of Human Cord Blood T Cells by HTLV In Vitro

Seven of the HTLV isolates described above have been successfully transmitted into fresh human cord blood T cells by cocultivation (Popovic et al., in preparation). The virus-positive neoplasic cells used as donors were first treated with mitomycin-C or X-irradiation before cocultivation with recipient cord blood cells. After 4 weeks, assays for T-cell markers, HTLV, karyotype, and HLA-typing were performed. As shown in Table 2, all recipient cord blood are mature T cells, positive for HTLV provirus, and express various levels of HTLV antigens (p19, p24, and RT). Karyotype and HLA typing consistently matched the recipient cells. Since cord blood T cells from the same donors were consistently negative for HTLV markers and the plasma from their cord blood were also negative for HTLV antibodies, we conclude that the virus was transmitted from HTLV-producing neoplastic T-cell lines into cord blood recipient T cells.

To characterize further whether a target for HTLV could represent a certain subset of mature T cells, phenotypes of HTLV-producing cells were analyzed by a series of monoclonal antibodies specific for helper/inducer and suppressor/cytotoxic T cells. We found that a majority of HTLV-producing T-cell lines consistently exhibited only helper-inducer phenotype. Two established T-cell lines, SK and TK, both from Japanese patients and two HTLV-infected cord blood T cells (C1 and C5) revealed "double" phenotype. However,

Table 2. Transmission of HTLV into human cord blood T cells

Cocultured cells (recipient X donor)[a]	Infected cells	Karyotype after coculti-vation[b]	HTLV proteins	Expression of HTLV proteins			EM
				p19 (%) positive cells	p24 (ng/mg)	RTA (pm/ml extract)	
C1(F) X None			–	0	<1		
C1(F) X MJ(M)	C1/MJ	XX	+	90	385	3.3	+
C4(F) X None			–	0	<1		
C4(F) X UK(M)	C4/UK	XX	+	81	540	34.1	+
C21(M) X None			–	0	<1		
C21(M) X MI(F)	C21/MK	XY	+	47	235	60.4	+
C6(F) X None			–	0	<1		
C6(F) X WA(M)	C6/WA	XX	+	53	502	1.7	–
C8(F) X None			–	0	<1		
C8(F) X SK(M)	C8/SK	XX	+	47	1000	8.7	+
C7(M) X None			–	0	<1		
C7(M) X TK(F)	C7/TK	XY	+	75	685	84.2	+
C36(F) X None			–	0	ND		
C36(F) X MO(M)	C36/MO	XX	+	47	500	30.3	ND

F, female; M, male; ND, not done
[a] Mitomycin treated or X-irradiated
[b] 50–100 mitoses were analyzed

none of the T-cell lines exhibited pure suppressor/cytotoxic phenotype. Unlike HTLV-infected cord blood T cells, PHA-stimulated cells (control) consist of 70% helper/inducer and 30% of T cells with suppressor/cytotoxic phenotype. Thus, these data from T-cell phenotype characterization of HTLV-infected T cells again suggest that a certain subset of mature T-cells is the target for HTLV. HTLV infection studies with cord blood cells deprived of T-cell population with helper/inducer of suppressor/cytotoxic phenotype are currently being carried out.

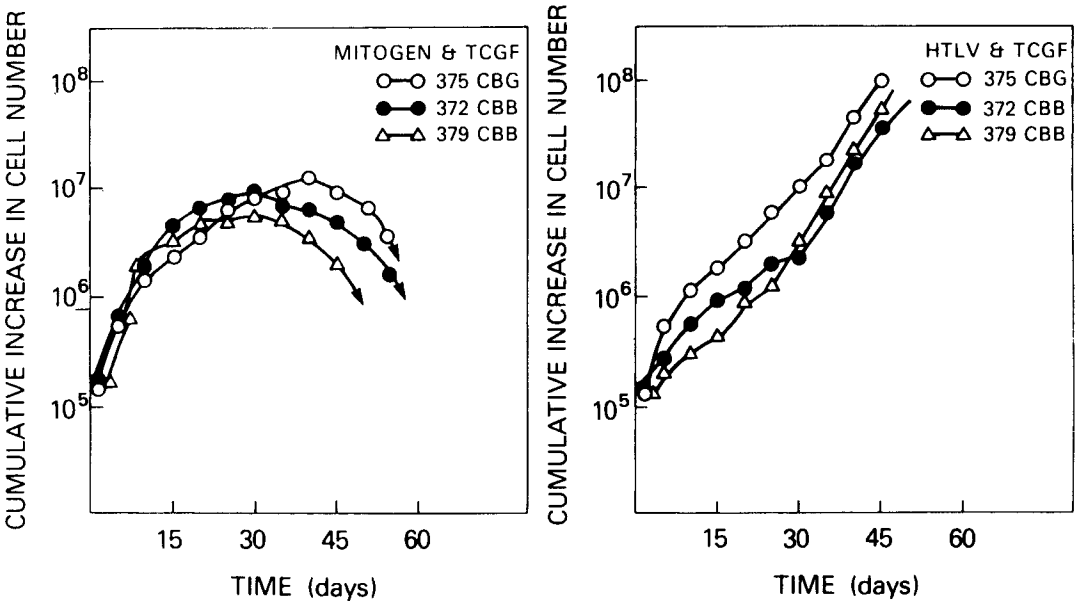

Fig. 5. Growth curves of uninfected and HTLV-infected human cord blood T cells in vitro. *Left panel,* mitogen-stimulated cord blood T cells. *Right panel,* HTLV-infected cord blood T cells. C6/WA and C7/TK cell lines are primary cocultures (for details see Table 2). C5/MJ cells were obtained in three successive transmissions of $HTLV_{MJ}$ isolate into cord blood cells

HTLV-infected cord blood T cells differ from mitogen-stimulated cord blood T cells in several growth properties and cell surface characteristics, the infected cells resembling more the neoplastic cells transformed in vivo by HTLV (see Sarin et al., this volume for details). The most striking feature of HTLV-infected cord blood T cells is their potential for indefinite growth as shown in Fig. 5. In contrast, mitogen-stimulated cord blood T cells from the same patients consistently exhibited growth "crises" after 1 month in culture, even in the continued presence of TCGF. Furthermore, the infected cells, like the neoplastic cells, had the tendency to form clumps in culture. When analyzed by electron microscopy, the cells were seen to have convoluted nuclei (not shown) while the mitogen-stimulated cells did not. Another important and reproducible difference is the decrease in requirement for TCGF by the infected cells. In fact, some of the infected cells are completely independent of exogenous TCGF (see Sarin et al., this volume). Other changes of the infected cells include alteration in their HLA profile and expression of receptors for transferrin, TCGF, and HAA (human-activated lymphocyte antigen detected by monoclonal antibodies) in a high percentage of cells. The data indicate that HTLV is also capable of causing morphological transformation of cord blood T cells in vitro (see Sarin et al., this volume).

F. Possible Molecular Mechanism of Transformation by HTLV

As mentioned earlier, analysis of HTLV-positive leukemic T cells showed that the cells are of clonal origin with respect to the provirus integration sites. In animal systems monoclonality has also been shown to be a common feature of leukemias induced by retroviruses which are chronic leukemia viruses but not those induced by retroviruses which are acute leukemia viruses. Consequently, in spite of its high efficiency to transform T cells in vitro, HTLV probably does not carry on *onc* gene. Several chronic leukemia viruses are known to induce leukemia by activating cellular *onc* genes (*myc* in B-cell lymphomas and *erb* in erythroleukemias) ([7]; Kung, personal communication) by integrating in the proximity of these genes. Activation of these genes is brought about by providing either a viral promotor or viral nucleotide sequences dubbed "enhancer" [12, 17], the real function of which is still unknown. Since HTLV specifically transforms mature T cells, it is likely to affect expression of genes that are important in T-cell proliferation. A model has been proposed for the mechanism of leukemogenesis by HTLV [4]. Briefly, the HTLV envelope protein interacts with the population of T cells normally designed to make TCGF receptors, mimicking an antigen stimulation of blastogenesis. These cells then synthesize receptors for TCGF. Simultaneously, the HTLV provirus integrates in the vicinity of the TCGF gene or a gene that exerts a pleiotropic effect on TCGF expression and activates this gene either by direct promotion or enhancement. The production of TCGF by a cell bearing a TCGF receptor may result in autostimulation and increased cell proliferation. As an approach to study the gene(s) activated by HTLV infection, we have recently identified and isolated a gene that is expressed at high levels in all HTLV-positive neoplastic T cells and in normal cord blood T cells after infection with HTLV but not the uninfected counterparts [12 b]. Study of the expression pattern of this gene in uninfected human hematopoietic cells suggests that its expression may be linked to TCGF production. Experiments are in progress to determine if HTLV integrates at a preferred locus in the human chromosome and affects transcription of specific cellular genes in the vicinity, including this gene in question.

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