Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

Transmission of Human T-Cell Leukemia Virus (HTLV) into Human Cord Blood T Cells

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The isolation of HTLV, a type C retrovirus, was first reported from our laboratory from adult patients with T-cell malignancies [4, 10, 11]. HTLV has now been isolated from a number of T-cell leukemia/lymphoma patients from various parts of the world, including the United States, Caribbean, Israel, and Japan [14]. An identical or extremely closely related type C retrovirus was subsequently isolated by Japanese workers from patients with adult T-cell leukemia (ATL) but has been called by another name, ATLV [5, 9, 18]. Seroepidemiological studies indicate that antibodies to the internal antigens (p19 and p24) of this virus are present in a large number of T-cell leukemia/lymphoma patients and some healthy normal blood donors resident in the endemic and nonendemic areas [1, 2, 5, 6, 17]. Nucleic acid hybridization studies and high incidences of association of this virus with T-cell leukemia indicate that this virus is acquired by exogenous infection ([3]; Gallo et atl., this volume; [15, 18]). HTLV and ATLV have been shown to be identical or extremely closely related by competition radioimmunoassays and radioimmunoprecipitation of the internal antigens (p19, p24) and by nucleic acid hybridization studies [13]. HTLV is to date unique to forms of the adult T-cell leukemia/lymphoma (Gallo et al., this volume). Elsewhere in this book (Gallo et al.) we describe the transmission of HTLV into human cord blood T cells and we show the HTLV-induced changes in cell growth and surface phenotype. We also show evidence for changes in expression of certain genes. Here we show the morphological changes in HTLV-infected T cells, and we report on the decreased requirement for T-cell growth factor (TCGF) after transmission of the virus. The features of the HTLV-infected and transformed cord blood human T cells are remarkably similar to the primary tumor cells of HTLV-associated T-cell malignancies.

A. Transmission of HTLV into Human Cord Blood Cells and Characteristics of the Infected Cells

HTLV was transmitted into the cord blood T cells from the HTLV-positive cell lines by cocultivation of the HTLV-positive cell lines with fresh human cord blood T cells or in a few instances by addition of cell-free virus particles. Briefly, the cord blood leukocytes were purified on Ficol/Hypaque, washed three times with RPMI-1640 containing 10% fetal calf serum, and mixed with HTLV-positive cell lines (MJ, UK, TK, etc.) that have been either exposed to X-rays (6000 rads) or to mitomycin-C (100 μg/ml for 20 min at 37°) and washed three times with RPMI-1640 containing 10% fetal calf serum. The cord blood cells and the X-irradiated HTLV-positive cells were mixed at a ratio of 4 : 1 and incubated at 37 °C in the presence of 5% CO_2 in the presence or absence of 5% TCGF. After 3 weeks, 5 weeks, and 7 weeks of coculture, the cells and the conditioned medium were tested for the expression of HTLV-related proteins (p19, p24) and reverse transcriptase. The cells were also examined by electron microscopy for detailed morphologic characteristics and for the expression of type C virus.

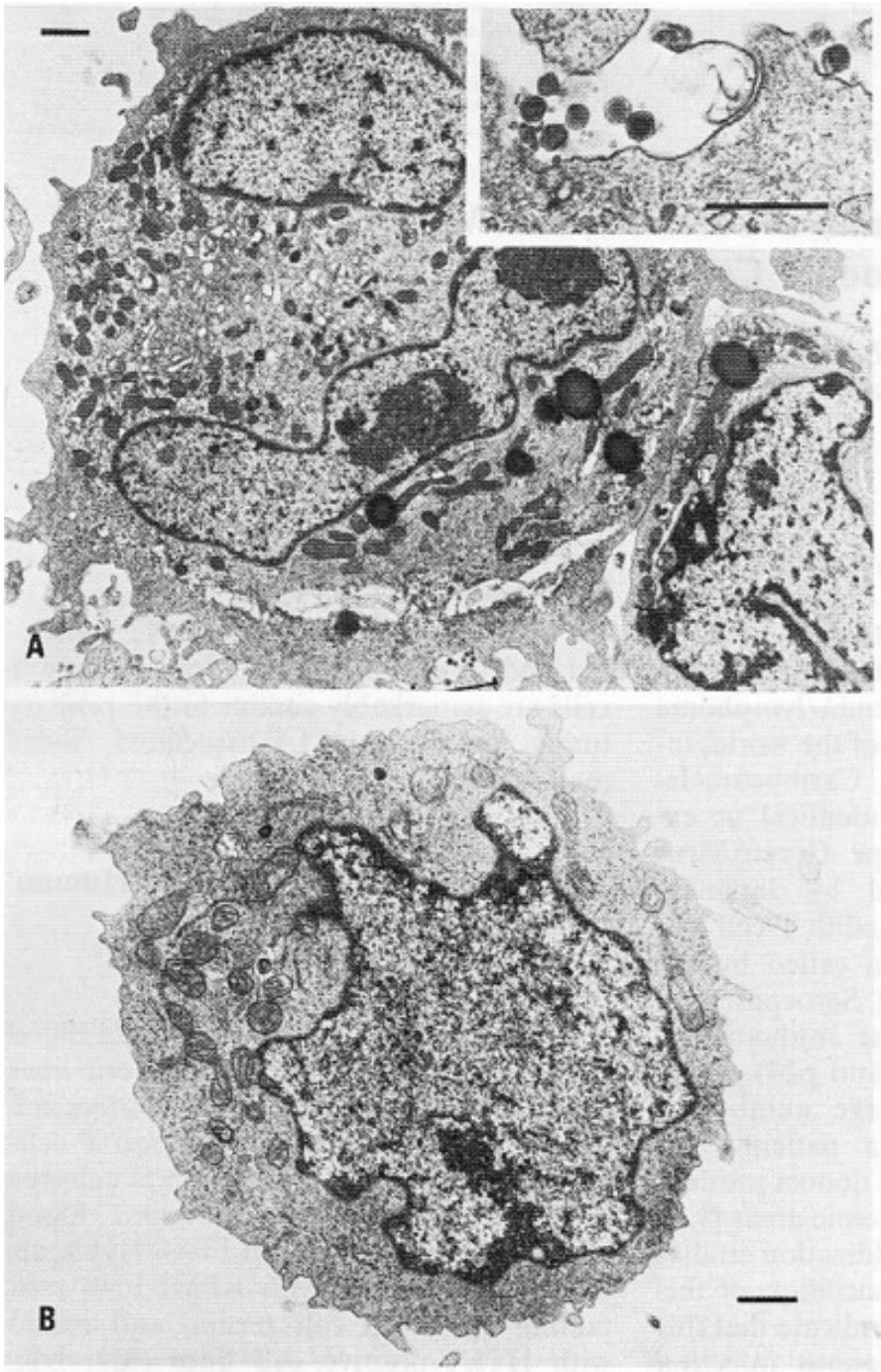

Fig. 1 A, B. Electron microscopic examination of human cord blood T cells before and after infection with HTLV. **A** HTLV-infected cord blood T cells (*insert*). Typical type C virus particles. **B** Uninfected cord blood cells

Nine HTLV-positive cell lines were successfully transmitted into cord blood T cells as observed by the expression of p24, p19, and reverse transcriptase. A representative example of the expression of HTLV into cord blood T cells as seen by electron microscopy is shown in Fig. 1. After infection with HTLV many cord blood T cells develop lobulated nuclei (Fig. 1 A) similar to the morphology of the nuclei in many HTLV-associated primary malignant cells. The *insert* in Fig. 1 A shows the presence of type C virus particles associated with infected cells. A typical normal cord blood T cell is shown in Fig. 1 B. The infected cord blood cells grow as multinucleated giant cells (Fig. 2 C). The presence of multinucleated cells is a common feature of the HTLV-infected cord blood cells. The donor HTLV-positive cell lines also contain multinucleated cells but the size of the HTLV-infected cord blood cells is generally larger and in some cases up to 30 nuclei have been seen in a giant cell.

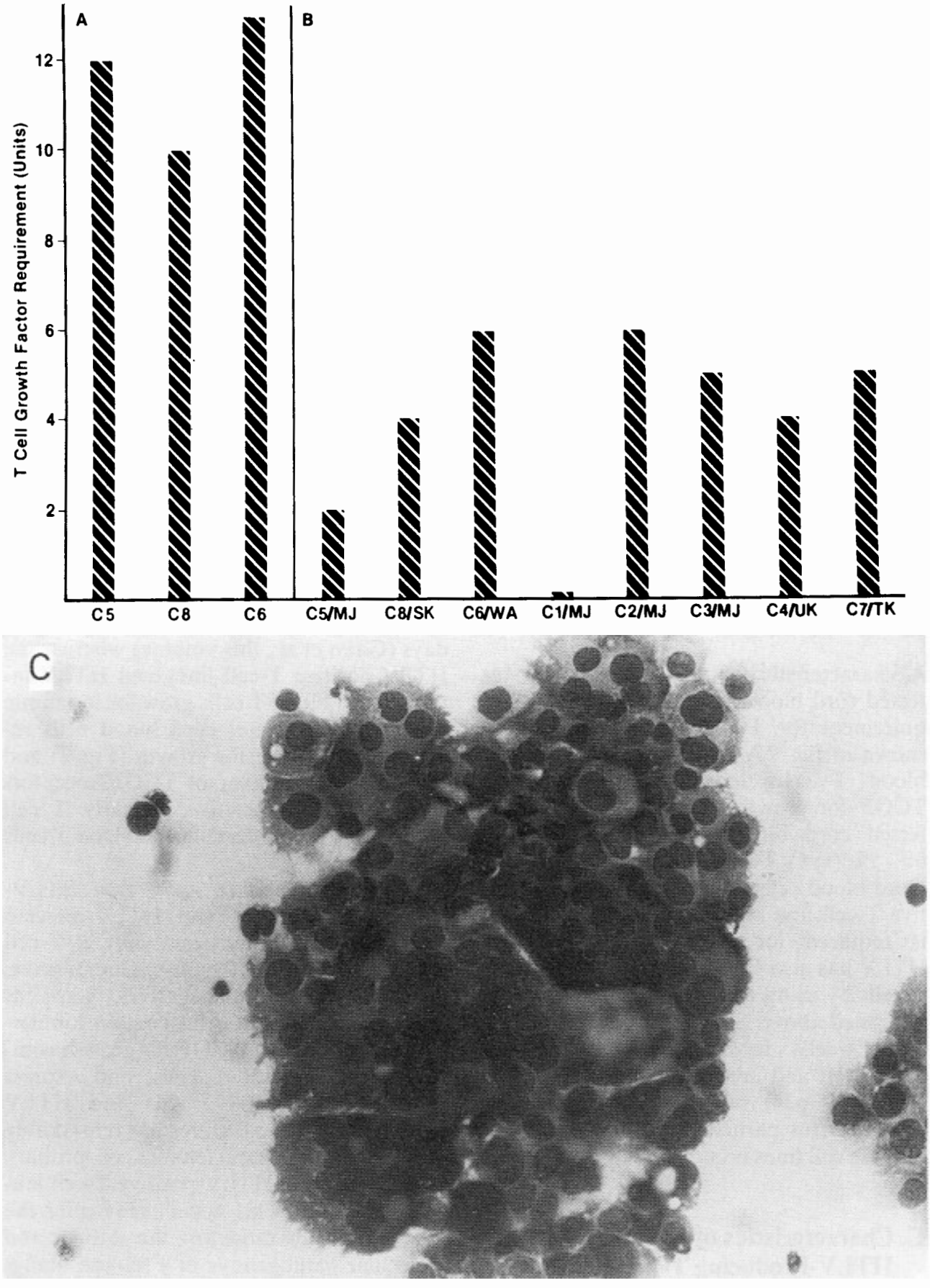

Fig. 2A–C. T-cell growth factor (TCGF) requirement of the uninfected and HTLV-infected cord blood T cells and morphology of the infected cells. **A** TCGF requirement of uninfected cord blood cells. A unit of TCGF is defined as the half maximal incorporation of ^{3}H-TdR in a TCGF microassay. **B** TCGF requirement of HTLV-infected cord blood T cells. **C** Presence of multinucleated giant cells in HTLV-infected cord blood T cells

Table 1. Comparison of the characteristics of HTLV-positive human neoplastic T cells with normal uninfected and HTLV-infected human cord blood T cells

Property	HTLV positive Neoplastic T cell lines	Cord blood T cells	
		HTLV infected	Lectin stimulated
1. In vitro growth	> 180 days	> 180 days	< 50 days
2. Requirement for exogenous TCGF (v/v)	0% – 5%	0% – 5%	10% – 12%
3. TCGF receptors (TAC)[a]	+ + +	+ + +	+
4. E-rosette	+	+	+
5. S-IgG, EBNA, TdT[b]	–	–	–
6. Cell phenotype			
(a) Inducer/helper (OKT4, Leu 3)	10/10	9/9	NT[c]
(b) Suppressor/cytotoxic (OKT8, Leu 2A)	2/10	2/9	NT[c]
7. HTLV p19, p24, and RT[d] expression	+	+	–
8. Type C virus particles (EM)	+	+	–
9. Cell morphology			
(a) Presence of multinucleated giant cells	+	+	–
(b) Presence of lobulated nuclei	+	+	–

[a] TCGF receptors determined by cell sorter with TAC antibody [8, 19]
[b] TdT, terminal deoxynucleotidyl transferase
[c] NT, not tested
[d] RT, reverse transcriptase

B. The TCGF Requirement for Growth

A characteristic feature of the HTLV-infected cord blood cells is the decreased requirement for TCGF for growth [4]. As shown in Fig. 2 A and 2 B the normal cord blood T cells require 10%–12% (v/v) TCGF for growth whereas the HTLV-infected cord blood T cells can grow in 0%–5% (v/v) TCGF. In one case C_1/MJ (cord blood cells infected with HTLV-positive T-cell line MJ) less than 1% of TCGF is required for growth. More recently HTLV has also been transmitted into adult T cells by using conditions similar to those described above. Some of the HTLV-infected T cells have become TCGF independent and are expressing HTLV proteins (p19, p24), reverse transcriptase, and type C virtus particles. Further evaluation of these cell lines is currently in progress.

C. Characteristics of Normal and HTLV-Producing T-Cell Lines

The characteristic features of HTLV-positive primary cell lines obtained from patients with T-cell leukemia and the cord blood T cells before and after infection are summarized in Table 1. The normal cord blood T cells reach a crisis period at 45–50 days (Gallo et al., this volume) whereas the HTLV-positive T-cell lines and HTLV-infected cord blood T cells grow for indefinite periods. The normal cord blood cells require more TCGF for growth (Fig. 2) and have a lower number of TCGF receptors than the HTLV-positive primarly T cell lines and HTLV-infected cord blood T cells (Table 1).

The current studies show that HTLV-positive T-cell lines and HTLV-infected cord blood cells possess mature T-cell markers (OKT4 positive, E-rosette positive, terminal transferase negative), grow as multinucleated giant cells, contain lobulated nuclei, need less TCGF for growth compared with normal T cells, and express HTLV antigens (p19, p24) and HTLV particles. All these features are remarkably similar to the characteristics of primary tumor cells from HTLV-positive T-cell leukemia patients. This system may offer the possibility of investigating the cellular and molecular pathogenesis of a human malignancy in vitro in a manner not previously available for a human cancer.

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