Abnormal In Vitro Proliferation and Differentiation of T Cell Colony-forming Cells in Patients with Tropical Spastic Paraparesis/Human T Lymphocyte Virus Type I (HTLV-I)-associated Myeloencephalopathy and Healthy HTLV-I Carriers

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Summary

T cell colonies were generated from the peripheral blood mononuclear cells (PBMC) of 10 patients with tropical spastic paraparesis/human T lymphocyte virus type I (HTLV-I)-associated myeloencephalopathy (TSP/HAM), two healthy HTLV-I carriers, and 17 healthy HTLV-I-seronegative subjects. PBMC were cultured in methylcellulose in the absence of added growth factors (spontaneous T cell colonies), or in the presence of phorbol myristate acetate and interleukin 2 (induced T cell colonies). PBMC T cell colony-forming cells (T-CFC) from all TSP/HAM patients and HTLV-I carriers were able to grow in the absence of added growth factors and/or mitogenic stimulation. Pooled spontaneous and induced colonies were composed of cells bearing $CD3^+$, $CD4^+$, $CD8^+$, and $CD1^+$ antigens. Colonies from normal HTLV-I-seronegative subjects displayed mature cells bearing the $CD3^+$, $CD4^+$, $CD8^+$, and $CD1^-$ surface phenotype. In addition, spontaneous and induced T cell colonies expressed HTLV-I antigens in 18–38% of the cells from TSP/HAM patients and HTLV-I carriers. These results demonstrate that HTLV-I infection is associated with an abnormal proliferation and differentiation of T cell progenitors in vitro and that the T-CFC from HTLV-I-seropositive individuals are infected, suggesting that T-CFC abnormalities may play a predominant role in the pathophysiology of HTLV-I.

A number of assays have been developed in semisolid media to detect T cell colony-forming cells (T-CFC).[1] These T-CFC have been used to determine the number of T cell progenitors and to study the differentiation capacity of peripheral blood and bone marrow T lymphocytes in normal subjects and in patients with a variety of pathological conditions (1–8). In AIDS, results with the T-CFC assays have demonstrated that the T-CFC are infected with HIV-1 and that abnormalities in proliferation and differentiation were associated with a worse prognosis, suggesting a relation with disease progression (9–14).

Growth of blood and bone marrow T cell colonies from healthy individuals requires stimulation with PHA and IL-2; these colonies are called induced, in contrast to spontaneous T cell colonies, which grow without PHA and IL-2. Spontaneous T cell colonies are not observed in normal circumstances. Induced colonies from healthy individuals are comprised of mature T cells with mainly a $CD3^+CD4^+$ surface phenotype. However, some $CD3^+CD8^+$ cells have also been detected in the colonies of these T cell progenitors (9–13). T-CFC have either an immature ($CD2^-CD3^-$) phenotype (displaying neither receptors for sheep erythrocytes, nor CD3 membrane antigens), or more frequently a presumably mature ($CD2^+CD3^+$) presentation (9–13). $CD1^+$ cells are not observed among the progeny of T-CFC in normal individuals (9). On the other hand, $CD1^+$ cells are found in the colonies of T cell progenitors in vitro (9–13) and in the peripheral blood in vivo of patients with AIDS (our unpublished data).

HTLV-I is a type C human retrovirus (15, 16) etiologically linked with adult T cell leukemia/lymphoma (17), a lymphoproliferative malignancy characterized by a clonal expansion of $CD4^+$ lymphocytes and by a monoclonal integration of the provirus(es) in the tumor cells (18, 19). The presence of specific antibodies in serum and cerebrospinal fluid of patients has also linked HTLV-I with a chronic myeloencephalopathy, called tropical spastic paraparesis/HTLV-I-

[1] *Abbreviations used in this paper:* A, adherent; α-MEM, α-modified Eagle's medium; IFA, indirect immunofluorescence assay; T-CFC, T cell colony-forming cells; TSP/HAM, tropical spastic paraparesis/HTLV-I-associated myeloencephalopathy.

associated myelopathy (TSP/HAM) (20–24). Recently, spontaneous proliferation of PBMC from patients with TSP/HAM and from healthy HTLV-I-infected carriers has been reported (25–30). The reverse transcriptase PCR has also shown HTLV-I transcripts in the PBMC from patients with TSP/HAM (29–32). Whether and at what level spontaneous lymphocyte proliferation and HTLV-I expression are related to each other remain to be determined.

The present study demonstrates for the first time that spontaneous T cell colonies can be generated from PBMC of all TSP/HAM patients and asymptomatic HTLV-I carriers examined. Furthermore, induced and/or spontaneous colonies were composed of $CD1^+$, $CD4^+$, and $CD8^+$ cells, indicating an abnormal in vitro T-CFC differentiation. In addition, 18–38% of these T cell colonies express HTLV-I viral antigens, and HTLV-I virus could easily be isolated from these cells.

These results support the conclusion that HTLV-I infection is associated with an abnormal proliferation and differentiation of T cell progenitors in vitro, as evidenced by the spontaneous T cell colony growth and the $CD1^+$ cells in the T cell colonies. Furthermore, the T-CFC from HTLV-I carriers and TSP/HAM patients are infected, raising the possibility that the T-CFC may be an important reservoir or target for infection in vivo, in contrast to the assumption that the mature $CD4^+$ T cells are the chief HTLV-I targets, because the leukemias due to HTLV-I almost always involve $CD4^+$ T cells.

Materials and Methods

Patients. Heparinized peripheral blood was obtained from 10 patients with TSP/HAM, two healthy HTLV-I-seropositive carriers, and 17 HTLV-I- and HIV-1-seronegative donors. All patients fulfilled the TSP/HAM criteria (20–23, 31, 32).

Cell Separation. PBMC were separated on Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden). Interphase cells were washed twice with PBS (Advanced Biotechnologies, Inc., [ABI], Columbia, MD) and resuspended in growth medium (α-modified Eagle's medium [α-MEM]; GIBCO BRL, Gaithersburg, MD). Their viability, as tested by trypan blue dye exclusion, was always >90%. In some experiments, cells were further separated on the basis of rosette formation with 2-amino-ethylisothiouronium hydrobromide (AET; Sigma Molecular Bio, St. Louis, MO)-treated SRBC (ABI) (33) and a second Ficoll-Paque density-gradient centrifugation. Thereafter, interphase cells were treated with anti-CD3 mAb (Ortho, Raritan, NJ) (50 μl/10^6 cells in a final dilution of 1:400; vol/vol) at 4°C, followed by rabbit complement (70–90 μl/10^6 cells in a final dilution of 1:8; vol/vol) (Cedarlane Laboratories, Hornby, Ontario) at 37°C. This cell fraction contained <2% of $CD2^+$ and $CD3^+$ cells and will be referred to as E^-CD3^- cells. The phenotypic characterization of E^-CD3^- cells showed 1% $CD3^+$ cells, 1% $CD2^+$ cells, 0% $CD1^+$ cells, 1% $CD20^+$ cells, 0% $CD56^+$ cells, 5–8% $CD34^+$ cells, and 35–40% $CD71^+$ cells. Staining of the E^-CD3^- cells with fluorescein-conjugated goat anti-mouse antibody did not reveal any positive cells, demonstrating that the CD3 determination on E^-CD3^- cells was not blocked by the anti-CD3 mAb that was used to deplete T cells. E^+ cells were obtained from the pellet of the second density centrifugation after hypotonic lysis of the SRBC. Their viability, as tested by trypan blue dye exclusion, was >90%. Adherent (A^+) and nonadherent (A^-) PBMC were obtained after cell adherence to plastic petri dishes: PBMC (10^6 cells/ml) were incubated in α-MEM supplemented with 10% (vol/vol) FCS and 2 mM glutamine for 2 h in an incubator gassed with 5% CO_2 at 37°C. The nonadherent cells (A^-) were collected, centrifuged, and stained for naphthyl esterase. They always revealed <5% positive cells. Adherent cells were obtained by extensive vigorous washing of the dishes.

T Cell Colony Assay. Fractionated or unfractionated PBMC (5×10^5 cells/ml) were seeded in 0.8% methylcellulose (Fluka Chemie AG, Buchs, Switzerland) in α-MEM supplemented with 20% FCS, 2 mM glutamine, and antibiotics in the presence (induced T cell colonies) of PHA (1%; vol/vol) and 10 U/ml of human rIL-2 (Biogen, Geneva, Switzerland), in the absence of PHA and rIL-2 (spontaneous T cell colonies), and in the presence of rIL-2 without PHA. In some experiments, 5×10^5 cells/ml were incubated for 1 h at 4°C or 37°C with 10–20 μg/ml of anti-Tac (Coulter Immunology, Hialeah, FL), anti-p75 (Mik β 1; Endogen, Inc., Boston, MA) or both anti-Tac (CD25) and anti-p75 mAbs, which recognize the IL-2R α (CD25) and p75 β chains, respectively. The cells were washed extensively and seeded in methylcellulose in the presence or absence of growth factors. In addition, unfractionated PBMC, A^-, E^+, A^-E^+, and $A^-E^-CD3^-$ cells (5×10^5 cells/ml) were incubated for 1 h at 4°C or 37°C with 10–20 μg/ml of anti-CD3, anti-MHC class I, and class II mAb (Amac, Inc., Westbrook, ME). Cells were washed extensively and seeded in methylcellulose in the absence of growth factors. Also, unfractionated PBMC, A^-, A^-E^+, E^+, and $A^-E^-CD3^-$ cells (5×10^5 cells/ml) were cultured with different concentrations of autologous adherent cells or adherent cells from allogeneic healthy donors (2×10^5, 5×10^5, and 1×10^6 cell/ml) and seeded in methylcellulose without growth factor. 0.1 ml of cell-containing methylcellulose preparation was seeded per well in 96-well flat-bottomed microtest plates and incubated at 37°C in 5% CO_2 in air for 5–7 d. Aggregates containing at least 50 cells were counted under an inverted microscope as colonies. Colonies of the same morphology and size were picked up individually and pooled. The cells were dissociated, washed, and immunophenotyped as described below.

Self-Renewal Capacity. The clonogenic capacity of T-CFC was studied by two culture procedures. First, PBMC (10^6 cells/ml) were incubated in growth medium supplemented with 10% FCS and 2 mM glutamine for 4 d at 37°C in 5% CO_2 in air. 5×10^5 washed viable cells/ml were seeded in methylcellulose as described above (delayed plating). The second method was to pick individual primary spontaneous colonies, and pool and dissociate them by pipetting. After washing, 5×10^5 viable cells/ml were seeded as for primary colony growth in the absence or in the presence of added growth factors.

Phenotypic Studies and Expression of Viral Proteins. Cell phenotype was determined by indirect immunofluorescence assays (IFA) using a panel of mAbs against the CD1, CD3, CD4, CD8 (Ortho Diagnostic Systems, Raritan, NJ), and CD25 (Coulter Immunology) molecules. Moreover, IFA was also used to detect the expression of HTLV-I proteins with a gag p19 mAb, an anti-Tax_1 antiserum, and polyclonal sera from TSP/HAM patients. FITC-coupled goat anti-mouse or anti-human IgG antibodies (Nordic Immunology, Tilburg, The Netherlands) were used as a second reagent. The cells were examined using an epifluorescence microscope (E. Leitz, Inc., Rockleigh, NJ).

Cell Infection. In some experiments, pooled colony (spontaneous or induced) cells were irradiated with 2,500 cGy and cocultured with cord blood mononuclear cells in RPMI 1640 supplemented

with 20% FCS and rIL-2 (10 U/ml). Every 3 d, the cultured cells were tested for the expression of gag p19 and CD1 antigens. Furthermore, two-color immunofluorescence studies were performed as reported (34) using mAb coupled to rhodamine and fluorescein.

Electron Microscopy. Samples were taken from T cell colonies after 3, 6, and 9 d of culture, fixed in 1.25% glutaraldehyde and 0.1 M sodium cacodylate in 0.5 M PBS, and stained with 1% aqueous uranyl acetate. After dehydration, the samples were embedded in epoxy resin. Ultrathin sections of samples were examined using an electron microscope (Hitachi).

Statistical Analysis. Statistical comparison of the mean values for T cell colony growth of different fractions and growth conditions was performed using Fisher's analysis of variance, with 95% confidence limits.

Results

PBMC Phenotype. The phenotypic characterization of primary, uncultured PBMC from TSP/HAM patients showed 58–78% $CD3^+$ cells, 48–56% $CD4^+$ cells, 16–30% $CD8^+$ cells, 0% $CD1^+$ cells, and 0% HTLV-I gag $p19^+$ cells (Table 1). The expression of CD25 was increased (9–28%) in PBMC of both TSP/HAM patients and healthy HTLV-I carriers, indicating that T cells are already activated in vivo as known from earlier studies (see Discussion).

T Cell Colony Formation. Not only induced, but also spontaneous, T cell colonies were grown from the PBMC of all TSP/HAM patients and healthy HTLV-I carriers. In contrast, only induced T-CFC could be generated from the PBMC of HTLV-I-seronegative controls (Tables 2 and 3). In the presence of 10 U/ml of rIL-2, and without additional PHA or mitogen stimulation, the growth of T-CFC was significantly inhibited as compared with the spontaneous colony formation in all cases of TSP/HAM patients and healthy HTLV-I carriers (Table 3).

Table 2. *Induced PBMC T Cell Colony Growth in TSP/HAM Patients, HTLV-I Carriers, and HTLV-I-seronegative Controls*

Patients	Diagnosis	PBMC T-CFC
1	TSP/HAM	220 ± 20
2	TSP/HAM	298 ± 12
3	TSP/HAM	262 ± 26
4	TSP/HAM	332 ± 28
5	TSP/HAM	421 ± 23
6	TSP/HAM	380 ± 10
7	TSP/HAM	244 ± 45
8	TSP/HAM	245 ± 17
9	TSP/HAM	303 ± 47
10	TSP/HAM	377 ± 33
11	Carrier*	310 ± 12
12	Carrier*	351 ± 11
HTLV-I seronegatives (n = 17)		370 ± 179

Results are expressed as the mean number of colonies ± SD per 5 × 10^4 seeded cells from triplicate cultures.
* Healthy HTLV-I carriers.

To determine whether the interaction of IL-2 with its receptor (IL-2R) is involved in the spontaneous colony growth, cultures were tested after incubating PBMC with anti-Tac or anti-p75 mAbs alone or with both mAbs together. As shown in Fig. 1, spontaneous T cell colony formation could strongly be inhibited after the treatment with the anti-Tac

Table 1. *Immunological Characteristics of HTLV-I-infected Individuals*

		Percent positive PBMC					
Patients	Diagnosis	p19	CD1	CD25	CD3	CD4	CD8
1	TSP/HAM	0	0	20	75	56	30
2	TSP/HAM	0	0	17	78	58	28
3	TSP/HAM	0	0	21	ND	ND	ND
4	TSP/HAM	0	0	18	69	54	29
5	TSP/HAM	0	0	15	71	55	21
6	TSP/HAM	0	0	19	62	52	18
7	TSP/HAM	0	0	22	72	63	19
8	TSP/HAM	0	0	19	ND	ND	ND
9	TSP/HAM	0	0	28	61	48	18
10	TSP/HAM	0	0	26	74	49	23
11	Carrier*	0	0	22	58	50	16
12	Carrier*	0	0	9	ND	ND	ND

* Healthy HTLV-I carriers.

Table 3. *Spontaneous and rIL-2-induced T Cell Colony Formation*

Patients	Spontaneous	rIL-2
1	35 ± 5	12 ± 3
2	52 ± 7	14 ± 2
3	85 ± 7	51 ± 5
4	45 ± 5	17 ± 3
5	57 ± 4	15 ± 9
6	45 ± 7	23 ± 6
7	40 ± 2	28 ± 3
8	111 ± 9	58 ± 12
9	29 ± 2	20 ± 4
10	96 ± 6	78 ± 7
11*	20 ± 2	15 ± 7
12*	96 ± 14	46 ± 6
13 seronegative HTLV-I controls (n = 5)	0	0

Results are expressed as mean ± SD of colonies per 5 × 10^4 seeded cells from triplicate cultures.
* Healthy HTLV-I carriers.

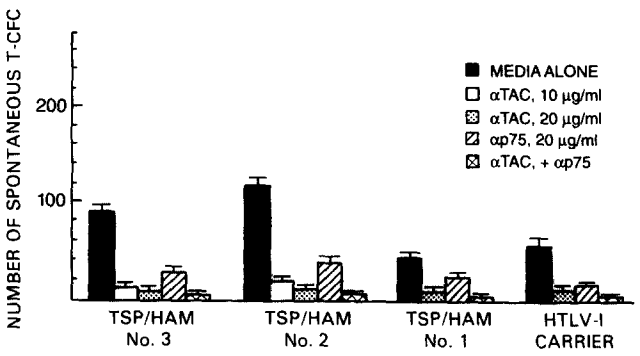

Figure 1. Inhibition of spontaneous T cell colony formation with mAbs directed against the Tac p55(CD25) and/or p75 chains of IL-2R. Results are expressed as in Table 2 (α, anti-).

and anti-p75 mAbs. These IL-2R antibodies inhibited the spontaneous T cell colony growth formation of PBMC from the three TSP/HAM patients and the one healthy HTLV-I carrier tested.

To determine more precisely the nature of the clonogenic T-CFC, such as mature (E^+ $CD3^+$) and immature (E^- $CD3^-$) T-CFC, the following PBMC fractions were also tested for T cell colony growth; E^+, adherent negative (A^-), A^-E^+, E^-CD3^-, $A^-E^-CD3^-$, and A^+. As shown in Table 4, PBMC with (E^+ and A^-E^+) or without T cell markers (E^-CD3^- and $A^-E^-CD3^-$) were able to generate spontaneous colonies, demonstrating that spontaneous T cell colonies originated from both mature and immature T-CFC. A^-, E^+, or A^-E^+ cell fractions generated a significantly lower number of spontaneous T cell colonies than unfractionated PBMC (Scheffe F test, p = 0.0018), suggesting that adherent cells are needed for spontaneous T cell colony formation. The differences were less marked for the immature T-CFC ($A^-E^-CD3^-$ or E^-CD3^-). Adherent cells (A^+) from TSP/HAM patients did not generate spontaneous T cell colonies. Moreover, spontaneous T cell colony formation from A^- cells could be restored by the addition of autologous but not allogeneic A^+ cells (Fig. 2). This suggests that A^+ cells are required to generate spontaneous T cell colonies from mature T-CFC. As shown in Fig. 3, spontaneous T cell colony formation from unfractionated PBMC, A^-E^+, and $A^-E^-CD3^-$ cells could not be inhibited or stimulated after treatment with anti-CD3, or anti-MHC class I or II mAbs. In addition, the number of spontaneous T cell colonies from all fractions examined was not affected by the addition of allogeneic adherent cells.

Phenotypic Characterization of T Cell Colonies. Induced as well as spontaneous T cell colonies generated from PBMC from TSP/HAM patients and HTLV-I carriers were composed of activated T cells, with Tac (CD25) antigens on their surface membrane (Table 5). In addition, 18–38% of the cells expressed HTLV-I antigens, as demonstrated by IFA using

Table 4. *Spontaneous Colony Formation from Different Cell Fractions of TSP/HAM Patients' and Healthy Carrier's PBMC*

Patient	Unfractionated PBMC	A^-	E^+	E^-CD3^-	A^-E^+	$A^-E^-CD3^-$	A^+
2	110 ± 12	7 ± 4	6 ± 2	55 ± 6	3 ± 1	70 ± 11	0
10	96 ± 6	6 ± 1	20 ± 2	74 ± 8	6 ± 2	42 ± 7	0
13	76 ± 5	11 ± 4	43 ± 5	112 ± 16	45 ± 4	83 ± 5	0
5	82 ± 3	11 ± 3	10 ± 1	72 ± 6	11 ± 4	74 ± 7	0
11*	124 ± 8	17 ± 5	12 ± 2	93 ± 8	24 ± 5	98 ± 5	0

Results are expressed as mean number of colonies ± SD per 5 × 10^4 seeded cells from triplicate cultures.
* HTLV-I carrier.

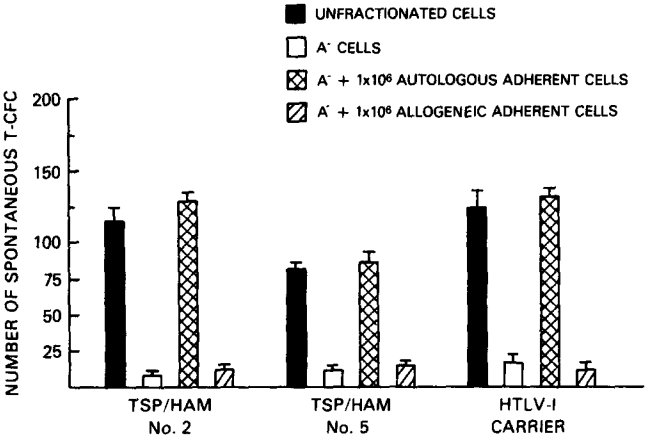

Figure 2. Effect of A^+ (autologous or allogeneic adherent cells) on spontaneous T cell colony formation from TSP/HAM patients and HTLV-I carrier. Results are expressed as in Fig. 1.

a gag p19 mAb (Table 5), and polyclonal sera from TSP/HAM patients (data not shown). In addition, 28–34% of the cells displayed a $CD1^+$ phenotype, as determined by IFA, indicating an aberrant in vitro differentiation of the T-CFC. Not more than 4% $CD1^+$ cells were observed in T cell colonies induced from PBMC T-CFC of healthy seronegative individuals. The cells expressing viral antigens seem to be T cells with abnormal in vitro differentiation. Indeed, two-color immunofluorescence revealed that 19–30% of $CD1^+$ cells, 5–8% of $CD4^+$ cells, and 5–7% of $CD8^+$ cells were also stained by the HTLV-I gag p19 antibody. In addition, spontaneous T cell colonies generated from E^-CD3^- cells from TSP/HAM patients were composed of cells with similar phenotypic features: 71–79% were $CD3^+$ cells, 59–64% $CD4^+$, 26–31% $CD1^+$, 21–27% $CD8^+$, 24–34% $CD25^+$, and 33–50% HTLV-I gag p19 (Table 5). Electron microscopy revealed retroviral particles, indistinguishable from HTLV-I,

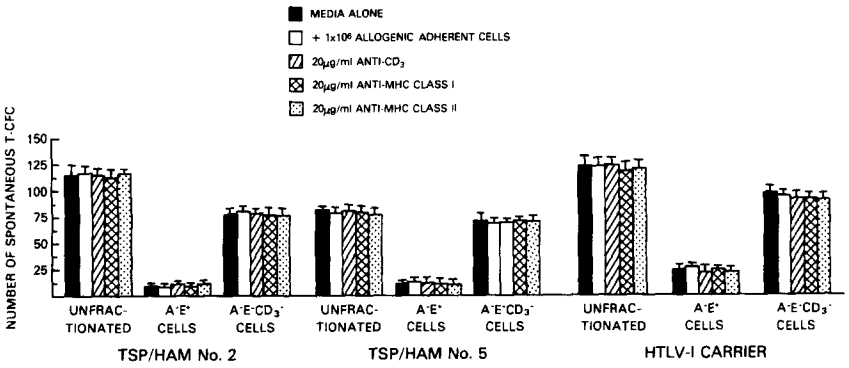

Figure 3. Effect of allogeneic adherent cells, anti-CD3, anti-MHC class I, and MHC class II mAbs on spontaneous T cell colony formation from unfractionated PBMC, A^-E^+, and $A^-E^-CD3^-$ cells from TSP/HAM patients and HTLV-I carrier. Results are expressed as in Fig. 1.

Table 5. *Phenotypic Characterization of Induced and Spontaneous T Cell Colonies from PBMC and E^-CD3^- Cells of TSP/HAM Patients, HTLV-I Carrier, and HTLV-I-seronegative Controls*

	Percent positive cells																	
	Induced colonies from PBMC						Spontaneous colonies from PBMC						Spontaneous colonies from E^-CD3^-					
Patient	Tac	CD1	CD3	CD4	CD8	p19	Tac	CD1	CD3	CD4	CD8	p19	CD25(Tac)	CD1	CD3	CD4	CD8	p19
2	27	28	76	62	25	21	30	31	78	67	23	28	37	30	76	61	21	37
3	31	24	74	62	29	31	26	25	71	65	24	25						
4	22	32	78	65	21	21	29	27	78	60	21	18						
5	30	28	79	61	31	30	27	25	74	58	30	24	31	26	70	59	24	33
9	31	32	82	73	28	24	24	30	80	72	24	15						
6	22	31	75	63	20	19	20	24	74	61	22	19						
11*	19	34	78	59	21	21	22	35	78	65	21	13	25	25	74	67	23	39
-(n = 3)‡	36	3	80	62	37	0												

Individually picked colonies were pooled, washed, and dissociated, and colony cells were stained by indirect immunofluorescence with the corresponding mAb.
* HTLV-I carrier.
‡ HTLV-I-seronegative controls.

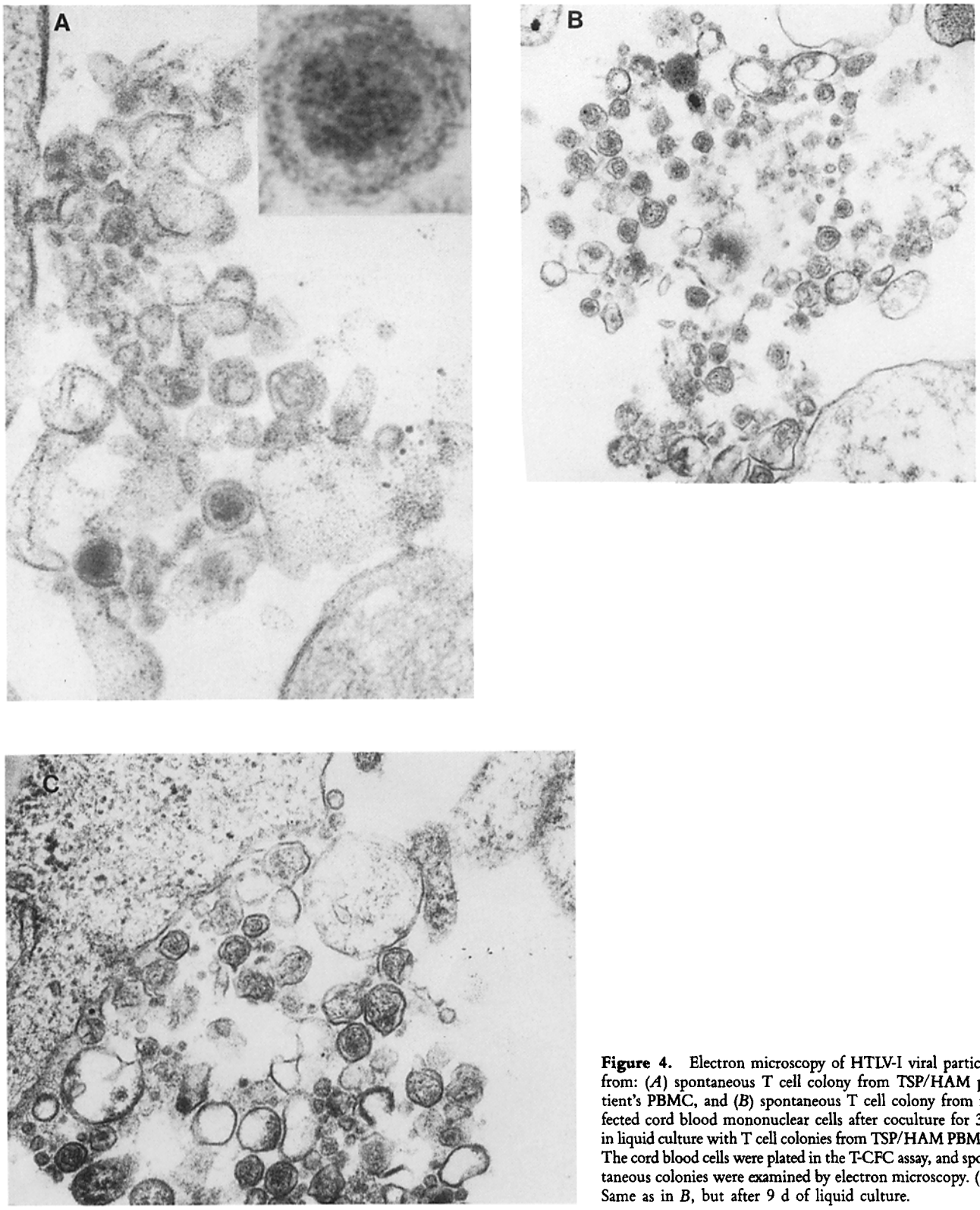

Figure 4. Electron microscopy of HTLV-I viral particles from: (*A*) spontaneous T cell colony from TSP/HAM patient's PBMC, and (*B*) spontaneous T cell colony from infected cord blood mononuclear cells after coculture for 3 d in liquid culture with T cell colonies from TSP/HAM PBMC. The cord blood cells were plated in the T-CFC assay, and spontaneous colonies were examined by electron microscopy. (*C*) Same as in *B*, but after 9 d of liquid culture.

Table 6. *Clonogenic Capacity of T-CFC from TSP/HAM Patients, HTLV-I Carrier, and HTLV-I-seronegative Controls*

	Immediate plating		Delayed plating	
Patient	Spontaneous	Induced	Spontaneous	Induced
4	45 ± 5	332 ± 28	51 ± 12	280 ± 18
5	57 ± 4	421 ± 23	49 ± 6	300 ± 24
2	52 ± 7	298 ± 12	67 ± 9	285 ± 20
6	45 ± 7	380 ± 10	38 ± 4	290 ± 16
11*	20 ± 2	310 ± 12	28 ± 8	296 ± 12
(n = 3)‡	0	284 ± 27	0	140 ± 29

Cells (5×10^5/ml) were seeded in methylcellulose after 72 h of liquid culture in RPMI 1640 with 10% FCS in the absence of added growth factors or mitogens. Results are expressed as mean number of colonies per 5×10^4 seeded cells from triplicate culture.
*HTLV-I carrier.
‡ HTLV-I-seronegative controls.

in the induced and spontaneous T cell colonies from the PBMC of both the healthy HTLV-I carrier and the TSP/HAM patient examined (Fig. 4).

Clonogenic Capacity of T-CFC. The proliferative capacity of PBMC T-CFC was also tested after incubating primary PBMC in liquid culture for 3–5 d, in the absence of added growth factors or mitogens, before seeding in methylcellulose. This delayed plating has been proposed as a measure of the proliferative capacity of the clonogenic cells (9–13). As shown in Table 6, delayed plating of spontaneous and induced T-CFC from TSP/HAM patients and asymptomatic carriers was similar to immediate plating.

Infection of Normal T-CFC by Coculture. HTLV-I gag p19 expression by the cells in the T cell colonies indicates that at least a portion of them are infected by HTLV-I. To further confirm this, pooled T cell colonies from spontaneous or induced T-CFC were irradiated with 2,500 cGy, and cocultured with pooled T cell colonies of normal bone marrow T-CFC or with normal PHA-activated cord blood mononuclear cells. Virus replication was monitored by the expression of HTLV-I gag p19 and electron microscopy. Gag p19 antigen was detected at 3, 6, 9, 15, and 60 d of the liquid cocultures. In addition, cells that were replated in methylcellulose 3, 6, and 9 d after the start of coculture could generate spontaneous T cell colonies. Spontaneous T cell colonies, obtained after 3 and 9 d of liquid coculture, also showed HTLV-I particles by electron microscopy (Fig. 4).

Discussion

The initial intent of these investigations was to study quantitatively and qualitatively the peripheral blood T-CFC of TSP/HAM patients and healthy HTLV-I-infected individuals. It has previously been demonstrated (25–29) that an unusual spontaneous T lymphocyte proliferation exists in TSP/HAM patients and healthy HTLV-I carriers. This study allowed us to determine that the spontaneous T lymphocyte proliferation also occurs at the level of the T-CFC, which is an indicator of the T cell progenitor population. Our results, as well as previous studies, demonstrate that there is an increased CD25 expression on PBL of TSP/HAM patients and healthy HTLV-I carriers (25–31). This suggests that there is abnormal activation of T cells in vivo since CD25 is an epitope of the IL-2R α chain. The mechanism through which spontaneous T lymphocyte proliferation and spontaneous T cell colony formation arise appears to occur at least partly through the interaction of IL-2 with its receptor. Spontaneous proliferation of TSP/HAM T lymphocytes in liquid culture can be inhibited by antibodies directed against the IL-2R (29). Moreover, an expression of IL-2 has been demonstrated in these cultures, strongly suggesting an autocrine IL-2/IL-2R loop in TSP/HAM. We have extended these observations to the level of the T-CFC, which in TSP/HAM and healthy carriers not only show an abnormal spontaneous proliferation, but also an expression of the IL-2R on the T cell colonies they generate. Furthermore, the spontaneous T cell colony formation of PBMC from HTLV-I-infected individuals is strongly inhibited by antibodies directed against the IL-2R α and β chains. In contrast to the healthy HTLV-I-seronegative individuals, the results reported here indicate that T-CFC from all TSP/HAM patients and healthy HTLV-I carriers examined showed abnormal proliferative properties. These spontaneously proliferating T-CFC of TSP/HAM patients and HTLV-I carriers possessed great self-renewal capacity in the absence of added growth factors. Conversely, spontaneously proliferating T-CFC from HIV-1-infected individuals have lost their self renewal capacity, which is a property of primitive progenitor cells (9–13).

To determine more precisely the clonogenic capacity of presumably mature and immature T-CFC, various PBMC fractions, E^+ ($CD2^+$) ("mature" T lymphocytes) or E^- ($CD2^-$) $CD3^-$ (immature T lymphocytes), were tested for spontaneous T cell colony growth with or without adherent cells. Spontaneous T cell colonies originated from both mature (E^+) and immature (E^-CD3^-) T-CFC. Preincubation with anti-CD3 mAb did not stimulate (or inhibit) the growth of spontaneous T cell colonies, originating from unfractionated PBMC, E^+, or E^-CD3^- fractions. Since the anti-CD3 mAb did not stimulate the growth of spontaneous colonies of specimens that were not depleted of mature T lymphocytes, it is highly unlikely that the generation of T cell colonies from E^-CD3^- is due to a mitogenic effect of the anti-CD3 antibody on any residual T cells.

A^-, A^-E^+, and E^+ cells from peripheral blood of HTLV-I-infected individuals generated significantly lower number of spontaneous T cell colonies than unfractionated cells, strongly suggesting that the generation of spontaneous T cell colonies from mature T-CFC requires cellular cooperation between T-CFC and adherent cells. We were not able to block the generation of spontaneous T cell colonies by mAbs directed against MHC class I or class II molecules, suggesting that the interaction with adherent cells does not occur via a MHC class I- or II-restricted mechanism. In-

terestingly, only autologous but not allogeneic adherent cells were able to restore spontaneous T colony formation in adherent cell–depleted PBMC. It is unclear why autologous adherent cells from HTLV-I-infected individuals behave differently from allogeneic adherent cells from healthy seronegative donors. It may be due to a higher level of activation of adherent cells in HTLV-I-infected individuals.

In contrast, the removal of adherent cells did not result in a decrease of the number of spontaneous T cell colonies from fractionated immature T cells ($A^-E^-CD3^-$ vs E^-CD3^-), suggesting that cellular cooperation between adherent cells and T-CFC is not necessary for the generation of spontaneous T cell colonies from immature T-CFC. The reason why immature T-CFC, as opposed to mature T-CFC, do not require cooperation with adherent cells for spontaneous T cell colony formation is not clear, but could be due to a higher level of activation of immature T-CFC

Surprisingly, when the T-CFC were cultured in the presence of IL-2 without PHA or mitogens, the growth of spontaneous T cell colonies was significantly inhibited ($p < 0.0001$), as compared with cultures without PHA or IL-2. We do not yet understand this phenomenon, but one possibility is that IL-2 stimulates suppressor T cells to produce factors inhibiting the proliferation of T-CFC.

In TSP/HAM patients and healthy HTLV-I carriers, pooled spontaneous and induced T cell colonies were composed of a high proportion of cells bearing the $CD1^+$ surface phenotype. Conversely, T cell colonies generated from healthy seronegative individuals displayed <4% $CD1^+$ cells (9–13) The $CD1^+$ antigen is characteristic of immature T lymphocytes (34–36), and its presence on cells of the T cell colonies may indicate a block of the in vitro differentiation of HTLV-I-infected cells. There appears to be a relationship between $CD1^+$ expression and HTLV-I infection since CD1 was expressed on the membrane of ~30% of HTLV-I gag $p19^+$ antigen-positive cells in both induced and spontaneous T cell colonies of TSP/HAM patient and HTLV-I carrier PBMC. This suggests that $CD1^+$ immature T cells may be a target of HTLV-I.

Direct evidence of the infection of T-CFC was shown by the capacity of pooled irradiated T cell colonies of TSP/HAM patients to transmit HTLV-I to normal cord blood or bone marrow mononuclear cells. Moreover, when these cocultured cells were replated in methylcellulose, we observed spontaneous growth of T-CFC from the infected bone marrow and cord blood cells. Recently, it was demonstrated that an early event in HTLV-I infection promotes the spontaneous growth of T-CFC (37). It is possible that HTLV-I triggers a molecular system(s) necessary for the proliferation of T-CFC.

Three important conclusions can be drawn from this study. First, in contrast to PBMC from normal individuals, PBMC from HTLV-I-infected individuals contain T cell progenitors with abnormal proliferative properties, since they can grow in the absence of added growth factors (spontaneous T cell colony formation). Second, T-CFC from TSP/HAM and HTLV-I carrier PBMC show abnormal differentiation in vitro, as evidenced by the $CD1^+$ of part of the cells in the T cell colonies. Third, T-CFC from HTLV-I-infected individuals seem to be infected in vivo, raising the possibility that the T-CFC may be the main target and an important reservoir for HTLV-I infection. One of the most remarkable findings of this study is the rapid appearance of HTLV-I-expressing cells after cultivation in vitro. This is in contrast to previous reported studies, where liquid suspension cultures of HTLV-I-infected cells only led to a gradual and slow selection of cells expressing viral antigens (38) Our interpretation is that culture in semisolid medium allows for the rapid selection of the target cell of infection by HTLV-I (HTLV-I-infected T-CFC) from the rest of the cell population.

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