Loss of Interleukin-2 Requirement for the Generation of T Colonies Defines an Early Event of Human T-Lymphotropic Virus Type I Infection

By Madeleine Duc Dodon and Louis Gazzolo

Accessory cells and/or soluble factors, together with interleukin 2 (IL2), are required for the proliferation and differentiation of phytohemagglutinin (PHA)-activated T lymphocytes. Human T-lymphotropic virus, type I (HTLV-I), a human retrovirus isolated from patients with adult T cell leukemia, can transform T cells in vitro. We investigated the role of HTLV-I-transformed T cell lines as accessory cells in promoting the growth of T colony-forming cells. We found that T cells isolated by E rosetting and then activated with PHA, when seeded with as few as $5 \times 10^3$ irradiated HTLV-I-producing cells, could generate colonies in the absence of IL2. We analyzed further the effects of HTLV-I virions on T colony formation. Infected T cells with semipurified HTLV-I viral particles promoted colony formation, in the absence of IL2, of accessory cells or soluble factors. The same results were obtained either with monocyte-depleted T lymphocytes, or with T4 or T8 lymphocytes. Furthermore, T lymphocytes in the presence of heat-inactivated HTLV-I (devoid of replicative potential) could form colonies independently of IL2. Finally, experiments with sera positive for HTLV-I antibodies (to abolish binding of viral particles to cellular receptors) indicated that HTLV-I promoted IL2-independent colony formation, only by “touching” T colony-forming cells. These results taken together demonstrate that the loss of the exogenous IL2 (and other growth-helping factors) requirement defines an early event of HTLV-I infection. The results also suggest that viral attachment to T cells possibly supplies an accessory function triggering autocrine secretion of IL2 by these cells.

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THE IDENTIFICATION, isolation and characterization of growth factors have provided new and challenging insights on the mechanisms governing cellular proliferation and differentiation. In particular, lymphokines produced by antigen- or mitogen-activated T lymphocytes are responsible for the multiple effects of cellular immune reactions, by regulating the differentiation of lymphoid cells. Among these lymphokines, IL2 (formerly known as T cell growth factor) is required for the amplification of T cell populations. Upon exposure to antigens, lectins and other mitogenic signals, T lymphocytes express cell surface IL2 receptors. Such activated T cells are now able to produce IL2 which, after interaction with specific high-affinity IL2 receptors, stimulates the growth of these cells. Furthermore, the proliferative response of T cells to activating agents is effective only in the presence of accessory cells.

Studies on the generation of colonies by T cells have provided much valuable information on the parameters controlling T cell proliferation in vitro. Thus, IL2 and accessory cells are both required for PHA-induced T colony formation. Accessory cells, which include monocytes, T cells, and B cells of normal and leukemic origin, are implicated both in the induction of IL2 synthesis and in the expression of functional IL2 receptors allowing IL2 responsiveness by the T colony-forming cells. The same colony-forming ability was also observed when soluble factors released by accessory cells were incorporated in the colony medium.

Because it leads to the amplification of activated T cells, IL2 has been decisive in isolating and characterizing human T-lymphotropic retroviruses (HTLV-I), such as HTLV-I associated with T cell leukemia (ATL) and LAV (lymphadenopathy-associated virus)/HTLV-III associated with the acquired immunodeficiency syndrome.

HTLV-I is able to transform T cells in vitro. T lymphocytes obtained from peripheral blood, umbilical cord blood or bone marrow and infected with HTLV-I are in most cases grown in the presence of IL2 until the overgrowth by transformed T cells which have become independent on this growth factor, several weeks after infection. T cell lines derived from leukemic donors and T cell lines obtained after in vitro infection both continue to express IL2 receptors. Moreover, the majority of these HTLV-I-transformed cell lines do not release IL2, while they are still able to secrete a large variety of other lymphokines.

Several studies have shown that infection of T cells by HTLV-I causes impaired immune functions of T cells. Once infected, these cells might also disturb the growth of normal T cells. Preliminary observations on the role of HTLV-I transformed T cell lines as accessory cells led us to investigate further the involvement of viral particles in T colony formation. The results reported in this paper indicate that HTLV-I infected cells and HTLV-I virions are both able to induce the proliferation of T colony-forming cells in the absence of exogenous IL2.

MATERIALS AND METHODS

Cell separation. Peripheral blood was collected from normal healthy volunteers. Leukocytes (PBL) were obtained after buffy coat sedimentation at unit gravity for 45 minutes using a 1% methylcellulose solution. Mononuclear cells (MNC) were separated by Ficoll-Hypaque density centrifugation (density = 1.077g/mL), washed twice with RPMI 1640 and resuspended in RPMI supplemented with 10% heat-inactivated fetal calf serum (FCS). T cell separation was carried out according to Madsen et al by rosetting with 2-aminoethylisothiouronium bromide (AET)-treated sheep red blood cells.
blood cells (SRBC) and a second density centrifugation was performed through Ficoll-Hypaque. The E rosette-depleted cells (E⁻) were collected from the interface, and the E rosette-enriched cells (E⁺) were recovered from the pellet, after hypotonic lysis of the SRBC in 0.15 mol/L ammonium chloride. The E⁺ cell population contained a majority of T cells (over 95%), as assessed by immunofluorescent labeling with OKT3 monoclonal antibody, and about 5% of nonspecific esterase−positive monocytes and of surface Ig−positive cells.

In specific experiments, purified T lymphocytes were obtained by using a three-step procedure. MNC were depleted of adherent cells by one-hour incubation in a plastic culture flask, followed by the elimination of phagocytic cells by carbonyl iron ingestion at 37 °C for one hour and passage over a magnet. T lymphocytes were then isolated by overnight incubation with AET-treated SRBC and centrifugation over a Ficoll-Hypaque gradient. Almost pure T cells (OKT3-positive) were recovered from the pellet after lysis of the SRBC. These T cells were further separated in T4 or T8 lymphocytes by treatment with the OKT8 or OKT4 monoclonal antibodies, respectively, and then appropriately diluted newborn rabbit complement, with the use of a cytotoxic assay.

Monocytes were prepared after adherence of MNC to plastic culture flasks. After elimination of the nonadherent cells and extensive washings, adherent cells were detected after a 20-minute incubation in 3 mmol/L EDTA. This cell population was found to contain more than 95% nonspecific esterase−positive monocytes and less than 3% OKT3 cells.

T colony-forming assay. The T colony assay was performed according to a previously described technique. Cultures were prepared in 35-mm plastic Petri dishes, by using two layers of semisolid agar medium of 1 mL each. The underlayer contained 0.45% agar, 10% of a mixture of human AB serum and FCS, 40 μg of phytohemagglutinin (PHA)-P (Wellcome) and 10% lactic-free IL2 (Cellular Products) in a minimum essential medium (MEM). This was overlaid with 1 mL of 0.30% agar in α-MEM containing 2 × 10⁶ E⁺ cells, 1 × 10⁶ X-irradiated accessory cells (PBL or E⁺ cells), or conditioned medium from Raji cells, to a final concentration of 40%. Plates were then incubated for seven days at 37 °C in 5% CO₂ humidified atmosphere. On days 1 through 3, colonies were not generally found. On days 4 to 5 after seeding, an increase in the number of colonies was observed. This number reached a peak on day 7 or 8 at which time they were counted using a Nikon inverted microscope. Only clusters containing more than 40 cells were scored as colonies. Each assay was performed in duplicate. The results of at least five separate experiments were expressed as mean ± SD.

Cell lines. All cell lines (kindly provided by Dr R.C. Gallo, Bethesda, MD) used in this study were grown in RPMI 1640 supplemented with 10% FCS. The HTLV-I–transformed T cell lines included HUT102 (derived from a patient with a cutaneous T cell lymphoma) MT2, C91/PL, and C81-66/45. All obtained after transformation of cord blood cells, cocultivated with HTLV-I–producing cells. All these transformed HTLV-I T cell lines express the 42-kilodalton HTLV-I x-for protein product correlated with trans-activation of the long terminal repeat. Finally, the C81-66/45 cell line is nonproductively infected with HTLV-I, since these cells do not express gag antigens, and reverse transcriptase activity and viral particles were not detected in culture medium. Finally, the H9 clone of the HT lymphoblastoid cell line was also used in this study, together with LAV/HTLV-III–infected H9 cells. Before addition to the upper layer with E⁺ cells, these cell lines were extensively washed, resuspended in medium and X-irradiated at 6,000 R.

The conditioned medium from Raji cells (a B cell line) was obtained by cultivating these cells in RPMI 1640 supplemented with 10% FCS for four days at 37 °C. The supernatant was then collected, pooled after centrifugation at 1,000 g for ten minutes and filtered through a 0.45 μm membrane filter. It was stored at −20 °C.

Viral infection of cells. Media containing either HTLV-I or LAV/HTLV-III particles were harvested from the different cells listed above. Following removal of the cells by low-speed centrifugation, the virus containing supernatant was concentrated 20-fold by centrifuging for three hours at 32,000 g in a Beckman type 55 rotor and resuspending the viral pellets in an appropriate volume of RPMI 1640 supplemented with 10% FCS. The virus was frozen at −70 °C, until infection of E⁺ cells: 100 μL of viral suspension and 100 μL of E⁺ cell suspension (at a concentration of 4 × 10⁶ cells per mL) were mixed in a glass tube and incubated at room temperature for 30 minutes. At the end of the incubation period, cells were added to 1,800 μL of agar medium and delivered into two 35-mm dishes, on the top of the hard agar layer. The virus was heat-inactivated by incubation at 56 °C for one hour, just before infection of E⁺ cells.

Sera to be used in neutralization assays were heat-inactivated at 56 °C for 30 minutes. For each assay, one volume of viral suspension and one volume of serum at the desired dilution were mixed and incubated for one hour at room temperature, just before infection of E⁺ cells.

**RESULTS**

**Effect of HTLV-I–producing lines on T cell colony formation.** As indicated in Materials and Methods, the presence of accessory cells or that of soluble factors produced by them was found to be mandatory for colony formation by E⁺ cells under our experimental conditions. No colony formation was observed when E⁻ cells were incubated under the same assay conditions.

Experiments to ascertain the role of HTLV-I–producing T cell lines as accessory cells in T colony formation were performed in the absence or in the presence of IL2. In fact, some of the HTLV-I–transformed cells (MT2, HUT102) were found to secrete low amounts of IL2. As indicated in Table 1, it was surprising to find that IL2 was not necessary for colony formation when irradiated HTLV-I–producing cells were seeded in the upper layer. In fact, T colony–forming cells proliferated when as few as 5 × 10³ TMTC were added in the presence of a mixture of human AB serum and 10% FCS. The conditioned medium from Raji cells (a B cell line) was obtained by cultivating these cells in RPMI 1640 supplemented with 10% FCS for four days at 37 °C. The supernatant was then collected, pooled after centrifugation at 1,000 g for ten minutes and filtered through a 0.45 μm membrane filter. It was stored at −20 °C.

**Table 1. Effect of HTLV-I–Transformed T Cell Lines as Accessory Cells on the Number of Colonies Formed by E⁺ Cells, in the Absence of IL2**

<table>
<thead>
<tr>
<th>Accessory Cells</th>
<th>HTLV-I Production</th>
<th>Number of Cells</th>
<th>Number of Colonies/Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transformed with HTLV-I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C91/PL</td>
<td>+</td>
<td>5 × 10⁴</td>
<td>1312 ± 187</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 × 10⁴</td>
<td>1474 ± 225</td>
</tr>
<tr>
<td>MT2</td>
<td>+</td>
<td>5 × 10⁴</td>
<td>2030 ± 155</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 × 10⁴</td>
<td>TMTC</td>
</tr>
<tr>
<td>HUT102</td>
<td>+</td>
<td>5 × 10⁴</td>
<td>1225 ± 72</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 × 10⁴</td>
<td>1737 ± 75</td>
</tr>
<tr>
<td>C81-66/45</td>
<td>−</td>
<td>5 × 10⁴</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 × 10⁴</td>
<td>0</td>
</tr>
<tr>
<td>Not transformed with HTLV-I</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H9</td>
<td>−</td>
<td>5 × 10⁴</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 × 10⁴</td>
<td>0</td>
</tr>
<tr>
<td>Peripheral blood leucocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>5 × 10⁴</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>20 × 10⁴</td>
<td>0</td>
</tr>
</tbody>
</table>

Accessory cells were X-irradiated at 6,000 R and then seeded in the agar upper layer together with 2 × 10⁴ E⁺ cells. Data expressed as mean ± SD. TMTC, too many to count.
irradiated cells were cocultured with them. With the same number of irradiated normal PBL, colony formation was not observed. The majority of colonies obtained in the presence of irradiated HTLV-I-transformed cells were larger and contained more than 100 cells. That explains why in some cultures T colonies were too numerous to count when 20 × 10⁴ irradiated transformed cells were seeded. In addition, overgrowth of the cultures in some dishes did not favor the development of colonies. The same was true when IL2 was added to these cultures (data not shown).

Only cells transformed by and producing HTLV-I were able to cooperate in the colony growth. The lymphoblastoid T cells (H9, not infected by HTLV-I) and C81-66/45 cells (which are transformed by HTLV-I but defective in the replication of the virus) did not promote colony formation when seeded at 5 × 10⁴ cells/dish. These results suggest that generation of T colonies in the presence of HTLV-I-producing cells, but in the absence of exogenous IL2, may be accounted for by viral particles and/or viral antigens released by these cells.

**HTLV-I infection induces T colony formation in the absence of IL2 and monocytes.** To test this hypothesis, virus was concentrated from the culture supernatant of one HTLV-I-producing cell line (C91/PL). One volume of the viral preparation was added to one volume of the E⁺ cell suspension. After a 30-minute incubation at room temperature, cells were seeded into the agar upper layer at three different concentrations. The results of a representative experiment are shown in Fig. 1. Under standard conditions in which HTLV-I-treated E⁺ cells were seeded at 2 × 10⁵ cells/dish, T cell colony formation was observed in the absence of added IL2. No colony was detected in virus-untreated cultures (control) seeded at this concentration. At lower concentrations of HTLV-I-infected E⁺ cells (1 × 10⁵ cells/dish), there was no promotion of colony growth in the absence of IL2. Only in control E⁺ cultures with more than 3 × 10⁵ cells/dish were few colonies detected when IL2 was omitted. This low number of colonies may reflect the endogenous production of IL2. It should be noted that at this latter cell density (3 × 10⁴), in the absence of exogenous IL2, colonies in HTLV-I-treated cultures were 30 times more numerous than those found in control cultures (6,000 to 200, respectively).

When IL2 was added in the medium, colonies were observed with control as well as with virus-treated cells. However, the number of colonies in HTLV-I-treated cultures was about twofold higher than that in control cultures. Once again, colonies observed in E⁺ cultures incubated with virus in the absence of IL2 were larger than those in control cultures, grown in the presence of IL2. In the absence of PHA, HTLV-I-treated T lymphocytes as well as control T lymphocytes failed to generate colonies.

Further results shown in Fig 2 indicate that (1) no modification in the colony growth was noted when the virus-treated cells were seeded in the presence or absence of conditioned medium (Fig 2A); (2) the number of colonies obtained was proportional to the amount of virus added (Fig 2B); no colony formation could be demonstrated in the virus-free supernatant (obtained after high-speed centrifugation of the C91/PL growth medium (Fig 2C); (3) HTLV-I viral particles concentrated from the culture fluid of other HTLV-I-producing cell lines induced colony formation; conversely, LAV/HTLV-III was unable to promote colony formation without IL2 (Fig 2D); after addition of IL2, the same number of colonies as in the control cultures was observed with LAV/HTLV-III infected T cells (data not shown).

Phenotypic characterization of the colony cells was performed after mass harvesting the colonies of the agar culture. The membrane phenotype studies indicated that colony cells consisted of a mixture of cells bearing either the epitope T4 or T8.

Experiments described above were performed using E⁺ cells incubated with HTLV-I. This cell population was found...
to contain a low percentage of monocytes. To determine whether monocytes were essential to the IL2-independent colony formation, experiments were performed on monocyte-depleted T lymphocytes. It was found that purified T lymphocytes incubated with HTLV-I were still able to generate colonies in the absence of exogenous IL2. However, the addition of 50,000 uninfected monocytes in the agar upper layer was followed by an increase in the number of colonies. The same increase in percentage (around 64%) was observed when uninfected purified T cells were seeded in presence of monocytes (Fig 3).

Finally, it was observed that T colony-forming cells, recruited among monocyte-depleted T4 or T8 lymphocytes, are able to proliferate in the absence of IL2, after incubation with HTLV-I (Fig 3). However, there were twice as many colonies generated with T4 lymphocytes as with those bearing the T8 epitope. Once again, a significant increase in the number of colonies followed the addition of monocytes.

In conclusion, incubation of T colony-forming cells with HTLV-I viral particles promotes a colony-forming ability independent of exogenous IL2, within seven days after plating. Moreover, monocytes, although favoring T colony formation, are not required for the IL2-independent colony generation promoted by HTLV-I.

**Binding of HTLV-I particles to T cells is sufficient for colony formation independent of IL2.** HTLV-I antigen p19 could not be detected in colony cells, indicating that viral replication was not taking place in these cells during the short time of colony formation. These results lead us to anticipate that T colony ability promoted by HTLV-I infection may not be linked to viral integration and replication, but would follow an early event, such as the binding of HTLV-I virions to the surface of T colony-forming cells.

It is well established that early events in the infection of cells by virus, such as binding and penetration, are mediated by specific viral receptors on the surface of target cells. As assayed by flow cytometry, viral receptors of HTLV-I are more numerous on human T lymphocytes than on B lymphocytes. To verify that the induction of T colony formation by HTLV-I particles could be mediated by binding of the virus to receptors on T colony-forming cells, viral particles were heat-inactivated by incubating them at 56 °C for one hour. After this treatment, which inhibits their replicative ability, viral particles were mixed with T cells. Under these conditions, the number and the size of colonies obtained after incubation with heat-treated virus were found to be the same as that observed with heat-untreated virus (Table 2). So, abolishing the replicative potential of the virus did not impair IL2-independent T colony proliferation.

Experiments were then performed to prevent the binding of viral particles to T cells. HTLV-I virions were incubated with sera from two ATL patients. The sera contained specific antibodies against HTLV-I (as determined by enzyme-linked immunosorbent assay [ELISA] and competition assays), and probably reacted with envelope and core polypeptides of the virus, as demonstrated by previous studies. As shown in Table 2, colony formation in the absence of IL2 was inhibited in a dilution-dependent manner by serum A. A significant inhibition was observed in serum B only when it was used undiluted. No such inhibition was observed with an HTLV-I seronegative serum from one individual living in the same endemic area. These results suggest that antibodies present in the sera from ATL patients mask viral antigenic determinants on the envelope of the particles and prevent their binding to T colony-forming cells. The results of these experiments indicate that events leading to IL2-independent T colony formation appear to follow the attachment of HTLV-I to T cells.

![Table 2. Effect of Heat-Inactivated HTLV-I and Neutralized HTLV-I on the Number of Colonies Formed by E Cells, in the Absence of IL2](image.png)

<table>
<thead>
<tr>
<th>Treatment Prior Infection</th>
<th>Number of Colonies /Plate</th>
<th>Percentage of Inhibition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2212 ± 138</td>
<td>-</td>
</tr>
<tr>
<td>Heat-inactivated</td>
<td>2074 ± 513</td>
<td>-</td>
</tr>
<tr>
<td>Incubated with</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTLV-I--negative serum, undiluted</td>
<td>2081 ± 181</td>
<td>-</td>
</tr>
<tr>
<td>HTLV-I--positive serum A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>undiluted</td>
<td>31 ± 19</td>
<td>98.5</td>
</tr>
<tr>
<td>diluted 1:2</td>
<td>381 ± 6</td>
<td>81.7</td>
</tr>
<tr>
<td>diluted 1:10</td>
<td>1756 ± 69</td>
<td>15.7</td>
</tr>
<tr>
<td>HTLV-I--positive serum B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>undiluted</td>
<td>1356 ± 169</td>
<td>34.9</td>
</tr>
<tr>
<td>diluted 1:2</td>
<td>2143 ± 56</td>
<td>-</td>
</tr>
<tr>
<td>diluted 1:10</td>
<td>2525 ± 50</td>
<td>-</td>
</tr>
</tbody>
</table>

HTLV-I was heat-inactivated at 56 °C for one hour just before infection of E cells. Incubation of HTLV-I with serum was carried out at room temperature for one hour just before infection of E cells. $2 \times 10^8$ HTLV-I--infected cells were seeded per plate. Data expressed as mean ± SD.
DISCUSSION

Identification and characterization of IL2 has allowed the development of specific and well-controlled assays to study the in vitro proliferation and differentiation of T lymphocytes.1-4 Among these assays, the T colony assay consisting of culturing either PBL or purified T cells in agar or methylcellulose has permitted the identification of a T cell population endowed with the capacity to form colonies.5-6 Two prerequisites for a high efficiency of colony formation by T cells are the addition of exogenous IL2 and the presence of accessory cells.

Results reported in this paper indicate that a subset of T colony-forming cells, present in either E+ or monocyte-depleted T cells, T4 lymphocytes, or T8 lymphocytes, is able to proliferate in semisolid agar independently of IL2 and accessory cells after contact with HTLV-I. Heat-inactivated virus is also able to induce colony growth independently of IL2. Furthermore, the inhibition of colony formation by T cells with HTLV-I and pretreated with ATL patients’ sera containing antibodies to HTLV-I underlines the direct involvement of viral particles in promoting colony formation. As expected, colony formation was not observed with virus-uninfected T cells, unless IL2 and accessory cells (or soluble factors produced by these cells) were added to the culture colony medium. These results therefore indicate that inactivated HTLV-I particles or viral glycoproteins are sufficient to promote T colony growth in the absence of any added growth factor. Previous studies5-6,17 on the transforming effects of HTLV-I have shown that T lymphocytes from cord blood or peripheral blood infected with HTLV-I, after cocultivation with irradiated HTLV-I–producing cells, were also able to grow in vitro in the absence of IL2. However, this autonomous growth (transforming effect) takes place only several weeks after initiation of the coculture. These observations are different from those reported in this paper, which demonstrate the ability of T colony–forming cells to proliferate independently of extrinsic IL2 within one week after virus addition.

Further experiments with monoclonal antibodies directed against the viral envelope glycoprotein (gp46) are needed to determine whether the viral neutralizing epitope is involved in the IL2-independent proliferation. Moreover, it will be of interest to define at what level on the T cell membrane HTLV-I is acting to trigger cell proliferation. As already mentioned by McGrath and Weissman24 in a study on the relationship of normal immune receptors to retrovirus lymphomagenesis, it is reasonable to consider that HTLV-I is acting at the level of cell surface receptor complexes leading to the activation of the gene program required for T cell proliferation. Several receptors on the surface of T lymphocytes have been implicated in the activation and proliferation of these cells in vitro. Among these signals transducing receptors, the CD3–T cell receptor complex and the CD2 E-rosette receptor are mitogenic for T cells upon reaction with specific antibodies recognizing these structures.29-31 Whether HTLV-I receptors are identical to those surface receptors or are located close to them remains to be investigated.

The finding that T colony formation is independent of exogenous IL2 suggests that T cell proliferation is induced through one of two mechanisms. The first is related to the direct activation of protein kinase C in the cell membrane, as already described, with PBL treated with the tumor promoter teledocin.32 The second mechanism is linked to the autocrine secretion of IL2 by T cells in response to HTLV-I. In fact, the lack of T lymphocyte proliferation observed when HTLV-I–infected T cells were seeded at low concentrations might be secondary to their inability to produce sufficient endogenous IL2 (Fig 1).

Spontaneous T cell colony-forming ability has already been described in immature T cells derived from the peripheral blood of patients with T acute lymphoblastic leukemia.33 Such cells were able to proliferate in methylcellulose in the absence of added growth factors or of mitogenic stimulation, but were partially dependent on factors released by mature T cells. However, it cannot be ruled out that the ability to form T colonies displayed by the leukemic cells may be the consequence of the leukemic process, which interferes with the mechanisms regulating normal proliferation and differentiation.

As underlined above, HTLV-I isolated from the peripheral blood of ATL patients is able to transform T cells in vitro. The other known human retrovirus, LAV/HTLV-III, was isolated from the peripheral blood of patients with the acquired immunodeficiency syndrome. This virus replicates exclusively in a subset of T cells, i.e., helper/inducer cells with the CD4 phenotype.34-36 In contrast to HTLV-I, LAV/HTLV-III is unable to induce T colony formation in the absence of IL2. It is noteworthy that the CD4 epitope, which may be the receptor for this virus,34,35 is not involved in T cell activation, since perturbation of the CD4 molecules delivers a negative signal to T lymphocytes.33

The growth of T cells in the absence of exogenous IL2 as reported here underlines an early effect of HTLV-I viral particles on target cells. The fact that such an effect occurs within seven days after the addition of HTLV-I is of paramount importance for the understanding of the mechanisms involved in viral-induced proliferation. Thus, the colony formation induced by HTLV-I in the absence of added IL2 may be considered as a preleukemic event favoring viral integration and viral replication in actively dividing T cells.

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