Abnormal In Vitro Proliferation and Differentiation of T Colony-Forming Cells in Patients With Lymphadenopathy Syndrome

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Patients with acquired immunodeficiency syndrome (AIDS) present impaired colony growth and in vitro differentiation capacity of peripheral blood and bone marrow T colony-forming cells (T-CFC). We show that peripheral blood, bone marrow, and lymph node T-CFC from patients with persistent lymphadenopathy syndrome (LAS), a syndrome that can precede AIDS, displayed similar abnormalities. Indeed, peripheral blood T-CFC generated a low number of colonies in seven out of 12 patients, and almost no colonies were obtained from bone marrow cells of all patients. The simultaneous study of T-CFC from peripheral blood and lymph node mononuclear cells seems to provide a reliable indicator for the risk of developing AIDS. The six patients who developed AIDS displayed extremely low numbers of peripheral blood T-CFC (13 ± 17 colonies per 5 × 10⁶ cells), and in two of them, no colonies could be obtained from lymph node T-CFC. The remaining patients who had not developed AIDS displayed a higher number of peripheral blood T-CFC (141 ± 113 per 5 × 10⁶ cells) and lymph node T-CFC, which, in addition, preserved their clonogenic capacity. In some patients, peripheral blood and lymph node, but not bone marrow, T-CFC were capable of generating colonies in the absence of added growth factors or mitogens, whereas in others, colony formation was obtained with purified interleukin 2 (IL 2) alone. Both spontaneous and IL 2-induced colony formation was abrogated by a monoclonal antibody against the IL 2 receptor. Taken together, these findings suggest that at least some T-CFC expressed IL 2 receptors. Colonies generated either in the presence or in the absence of added growth factors were composed of T4⁺, T6⁺, and T8⁺ cells, indicating impaired in vitro T-CFC differentiation. These findings indicate that a dramatic quantitative and qualitative impairment of the proliferation and differentiation of peripheral blood and lymph node T-CFC precedes the clinical evolution from LAS to AIDS.

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with LAS. All but two patients, a Haitian woman (No. 16) and an intravenous drug user (No. 4), were male homosexuals. All patients fulfilled the LAS criteria. One patient (No. 15) presented with general symptoms, two (No. 2 and No. 17) had syphilis, and one (No. 14) had zoster (Table 1). Cytologic studies of lymph node biopsy sections revealed no evidence of malignant disease and were compatible with the diagnosis of LAS. Six patients (14 through 19) developed AIDS within a year (three to 12 months) after T-CFC investigation. All patients were studied on presentation, before any treatment was applied, and displayed serum antibodies against the lymphadenopathy-associated virus (LAV) as detected by enzyme-linked immunosorbent assay and Western blot. Seven clinically healthy male homosexuals and 17 healthy heterosexuals were also studied as controls. All healthy male homosexuals but one, as well as all healthy heterosexuals, were seronegative for LAV antibodies.

Informed consent for these studies was obtained from all patients. Cell separation. Peripheral blood mononuclear cells (PBMCs) and bone marrow mononuclear cells (BMMCs) were obtained by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) density-gradient centrifugation (d = 1.077 g/cm³). Interface cells were thereafter treated with Hanks’ balanced salt solution (HBSS; Inst Pasteur Production, Marnes-la-Coquette, France) and resuspended in 10⁶ cells per milliliter in growth medium (α-modified Eagle’s medium [α-MEM]; Gibco, Grand Island, NY). Lymph node biopsy specimens were mechanically dissociated, cells were suspended in HBSS, and lymph node mononuclear cells (LNMCs) were obtained by density centrifugation as described earlier. Their viability, tested by trypan blue dye exclusion, was always >90%.

In some experiments, cells were further separated on the basis of sheep red blood cell (SRBC)-rosette formation with AET (2-amino ethylisothiouronium hydrobromide; Sigma Chemical Co, St Louis)-treated SRBCs and a second density-gradient centrifugation. Interface cells were thereafter treated with OKT3 monoclonal antibody (MoAb) (Ortho, Raritan, NJ) (50 μL/10⁶ cells in a final dilution 1:400 vol/vol, at 4°C) and pretested low Tox-H rabbit complement (Cedarlane, Ontario; 70 to 90 μL/10⁶ cells in a final dilution 1:8 vol/vol) at 37°C. This cell fraction contained less than 2% E⁺ and no OKT3⁺ cells and will be referred to as E⁻OKT3⁻ cells. E⁻ cells were obtained from the pellet of the second density centrifugation after hypotonic lysis of the SRBCs. Their viability, tested by trypan blue dye exclusion, was >90%.

T cell colony assay. Mononuclear cells (5 × 10⁵ cells per milliliter) or cell fractions were seeded in 0.8% methylcellulose (Fluka, Buchs, Switzerland) in α-MEM supplemented with 20% (vol/vol) heat-inactivated fetal calf serum (FCS; Gibco), 2 mmol/L L-glutamine and antibiotics, in the presence and the absence of 20% (vol/vol) PHA-leukocyte conditioned medium. This conditioned medium (PHA-LCM) was a seven-day supernatant from PHA (PHA-M 1%, vol/vol, DIFCO Lab, Detroit)-stimulated normal PBMCs (10⁶ cells per milliliter in α-MEM supplemented with 10% FCS and 2 mmol/L L-glutamine) and contained 0.2 U/mL of IL 2 (1 U = 50 BRMP U) activity when tested on an IL 2-dependent human T cell line. In addition, cells were seeded in the presence of 0.3 U/mL of PHA-free semipurified IL 2. This was obtained from 48-hour supernatants of PHA-M (1%, vol/vol)-stimulated normal PBMCs, precipitated with ammonium sulfate, filtered on Sephadex G 75 superfine columns (Pharmacia), and represented the pooled 15-kd to 22-kd fractions that contained the IL 2 activity (5 U/mL).

PHA-free IL 2 could not induce cell proliferation or colony formation from normal PBMCs in the absence of PHA (kindly provided by Dr F. Triebel, UER Pitie Salpetriere, Paris). One tenth milliliter of the methylcellulose-containing cell preparation was seeded per well in 96-well flat-bottomed microtest plates (Nunc, Roskilde, Denmark). Cultures were incubated for five to seven days at 37°C in 5% CO₂ in air, and aggregates containing more than 50 cells were counted as colonies under an inverted microscope.

In some experiments, 5 × 10⁵ cells per milliliter were incubated for 45 minutes at 37°C with increasing concentrations (10⁻⁹ to 10⁻⁷) vol/vol of anti-Tac monoclonal antibody, which recognizes the IL 2 receptor (IL 2-R; anti-Tac kindly provided by Dr TA Waldmann).
National Institutes of Health). The cells were washed extensively and seeded in methylcellulose in the absence or presence of IL 2 as described.

Clonogenic capacity of T-CFC. The clonogenic capacity of T-CFC from PBMCs and LNMCs was determined by incubating 10^5 cells per milliliter in growth medium supplemented with 10% FCS and 2 mmol/L L-glutamine for three to five days. Extensively washed cells were seeded in methylcellulose (5 x 10^4 viable cells per milliliter), in either the absence or the presence of added growth factors as described ("delayed plating").

Phenotypic characterization of PBMCs, BMMC, LNMCs, and colony cells. PBMCs, BMMC, LNMCs were phenotyped using a panel of monoclonal antibodies, including the OKT series (Ortho), the T11 (Coultronics, Margency, France), which recognizes SRBC receptors; and an anti-HLA-class II (BM-50; kindly provided by Dr D. Charron, UER Pitié Salpetrière, Paris). Positive cells were counted with an Ortho Spectrum III Analyser, using a minimum sample size of 2,000 cells.

Colonies of the same size and morphology were individually picked, pooled, dissociated, and washed with HBSS. Colony cells were resuspended in phosphate-buffered saline supplemented with 0.01% sodium azide and 2% FCS, and phenotyped by indirect immunofluorescence using the OKT series monoclonal antibodies. Nonspecific immunofluorescence was always <5%. At least 50 to 100 cells were evaluated for each determination.

Statistical analysis. Statistical comparison of the mean values was performed using the Student's t test.

RESULTS

PBMC, BMMC, and LNMC phenotypes. As expected, T4+ cells were decreased (range, 1% to 43%), while T8+ cells ranged from 25% to 73% (Table 1). All patients displayed, to varying degrees, a low T4+ : T8+ ratio (range, 0.02 to 1.16; normal values, 1.5 to 2.3). Patients who subsequently developed AIDS (14 through 19) had a significantly lower T4+ : T8+ ratio than the remaining patients (mean ± SD: 0.27 ± 0.21 vs. 0.23 ± 0.17, respectively; P < .05). In these patients, both the proportion and the absolute number of T4+ cells (13.7% ± 7.4% and 198 ± 102 cells per cm², respectively) were lower than in those who did not develop AIDS (26.1% ± 6.5% and 483 ± 226 cells per cm², respectively). Tac+ cells were always <5%.

Phenotypic characterization of BMMC showed no significant peripheral blood contamination as evaluated by the proportion of T3+ and T11+ cells, except in two patients (No. 3 and No. 4, respectively; Table 1). No T6+ cells were found in any patient, whereas Tac+ cells were ranged between 0% and 9% (Table 1).

Phenotypic characterization of LNMCs showed 9% to 46% T3+ cells (mean ± SD: 32.3% ± 12%; Table 1) and in four of them (1, 4, 6, 9), T4+ cells were less than 10%. T8+ cells were usually <30%, and the T4+ : T8+ ratio ranged from 0 to 1.2. In two patients (No. 5 and No. 9; Table 1), 23% and 28% of the LNMCs were Tac+, respectively.

T cell colony formation. PBMCs from seven out of 12 patients generated low numbers of colonies (less than 50 colonies/5 x 10⁶ cells). The colony formation from patients who did not develop AIDS was lower than in normal heterosexuals (mean ± SD, 141 ± 113 and 370 ± 179 colonies/5 x 10⁶ cells, respectively; P < .001). Patients who subsequently developed AIDS generated a dramatically decreased number of colonies (13 ± 17 colonies/5 x 10⁴ cells) (Table 2). There was no correlation between the absolute number of T4+ cells and the number of colonies. Furthermore, T cell colony growth from seven clinically healthy male homosexuals who were at risk of developing AIDS (described in detail elsewhere) was also significantly decreased (208 ± 46 colonies/5 x 10⁴ cells; P < .01).

Bone marrow T-CFC could not generate colonies, irrespective of the subsequent development of AIDS. Conversely, in six out of eight patients who did not develop AIDS, lymph node T-CFC yielded a relatively high number of colonies (more than 100 colonies/5 x 10⁵ cells). This contrasts with the absence of colony growth from LNMCs of patients No. 18 and No. 19 and the 24 colonies obtained from patient No. 14 (Table 2), who subsequently developed AIDS.

To determine more precisely the clonogenic capacity of presumably "mature" and immature T-CFC, fractionated E+ and E− T3+ PBMCs and LNMCs were also tested for T cell colony growth. As shown in Table 3, peripheral blood E+ and E− T3+ cells (except in one patient) were unable to generate colonies, although colony growth could be obtained from unfraccionated PBMCs, suggesting that cellular cooperation(s) are important for T cell colony growth. Lymph node E+ cells from two patients (No. 8 and No. 18; Table 3)
colonies, whereas E-cells did. Results as expressed as mean number of colonies developed AIDS, unfractionated LNMCs did not generate a high number of colonies, but E-T3 cells did not. It is noteworthy that in patient No. 18, who subsequently developed AIDS, unfractionated LNMCs did not generate colonies, whereas E+ cells did.

Colony formation in the absence of added growth factors and in the presence of purified IL 2. Both unfractionated PBMCs (in four out of 11 patients) and LNMCs (in four out of 11 patients) generated colonies in the absence of added growth factors or mitogenic stimulation (Table 4). To determine whether IL 2-R were involved in this spontaneous colony growth, cultures were performed after incubating cells with anti-Tac MoAb. As shown in Fig 1, spontaneous colony formation could be abrogated with anti-Tac MoAb, irrespective of the source of the cells. Low amounts (10^6 vol/vol) of anti-Tac inhibited the colony formation in all cases.

Biochemically purified IL 2 induced colony growth from PBMCs and LNMCs in two and three patients (1 and 2 through 7; Table 4), respectively, without previous mitogenic stimulation. Similar results were obtained when, in some experiments, genetically recombinant (Biogen, Geneva, Switzerland) IL 2 was substituted for biochemically semipurified IL 2 (not shown). Because the induction of colony formation with purified IL 2 seems to indicate that some T-CFC could express IL 2-R, both PBMCs and LNMCs were incubated with anti-Tac MoAb before seeding in the presence of IL 2. As shown in Fig 2, anti-Tac abrogated the IL 2-induced colony formation. Conversely, a pool of three anti-HLA–Class II MoAbs, used as control, could not modify the plating efficiency. It is worthy to note that semi-purified IL 2 inhibited T cell colony growth from PBMCs of two patients (No. 12 and No. 15; Table 4) and from LNMCs of three patients (1, 4, and 5; Table 4). Interestingly, BMMCs from seven patients (1 through 5 and 14 through 16) did not generate either spontaneous or IL 2-induced colonies, although in two of them (No. 3 and No. 16; Table 1) 7% and 9% of the cells, respectively, were Tac+.

Phenotypic characterization of colonies. Colonies generated from PBMCs and LNMCs in the presence of PHA-

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**Table 3. Colony Formation From Different Cell Fractions of LAS Patients' PBMCs and LNMCs**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Source of Cells</th>
<th>Unfractionated MNC</th>
<th>E+ Cells</th>
<th>E′OKT3+ Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBMC</td>
<td>285 ± 24</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>PBMC</td>
<td>300 ± 22</td>
<td>0</td>
<td>287 ± 17</td>
</tr>
<tr>
<td>3</td>
<td>PBMC</td>
<td>36 ± 7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14*</td>
<td>PBMC</td>
<td>10 ± 2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16*</td>
<td>PBMC</td>
<td>8 ± 1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>LNMC</td>
<td>216 ± 19</td>
<td>392 ± 28</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>LNMC</td>
<td>82 ± 12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10</td>
<td>LNMC</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18*</td>
<td>LNMC</td>
<td>0</td>
<td>330 ± 23</td>
<td>0</td>
</tr>
<tr>
<td>19*</td>
<td>LNMC</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Unfractionated MNC, E+, and E′OKT3/ (5 x 10^6/mL) were seeded in methylcellulose in the presence of PHA-LCM, as described in Materials and Methods. Aggregates containing more than 50 cells were counted as colonies. Results as expressed as mean number of colonies ± SD/5 x 10^6 seeded cells from triplicate cultures. ND, not done.

*Patients who subsequently developed AIDS.

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**Table 4. Spontaneous and IL 2-Induced T Cell Colony Formation**

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Source of Cells</th>
<th>Spontaneous</th>
<th>IL 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBMC</td>
<td>64 ± 9</td>
<td>503 ± 37</td>
</tr>
<tr>
<td>2</td>
<td>PBMC</td>
<td>42 ± 6</td>
<td>156 ± 12</td>
</tr>
<tr>
<td>3</td>
<td>PBMC</td>
<td>0</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>PBMC</td>
<td>4 ± 2</td>
<td>4 ± 3</td>
</tr>
<tr>
<td>11</td>
<td>PBMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>PBMC</td>
<td>142 ± 12</td>
<td>0</td>
</tr>
<tr>
<td>13</td>
<td>PBMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14*</td>
<td>PBMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>15*</td>
<td>PBMC</td>
<td>82 ± 9</td>
<td>0</td>
</tr>
<tr>
<td>16*</td>
<td>PBMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>17*</td>
<td>PBMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>LNMC</td>
<td>278 ± 19</td>
<td>160 ± 13</td>
</tr>
<tr>
<td>4</td>
<td>LNMC</td>
<td>122 ± 17</td>
<td>26 ± 8</td>
</tr>
<tr>
<td>5</td>
<td>LNMC</td>
<td>320 ± 21</td>
<td>20 ± 4</td>
</tr>
<tr>
<td>6</td>
<td>LNMC</td>
<td>0</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>7</td>
<td>LNMC</td>
<td>26 ± 3</td>
<td>146 ± 17</td>
</tr>
<tr>
<td>8</td>
<td>LNMC</td>
<td>0</td>
<td>31 ± 5</td>
</tr>
<tr>
<td>9</td>
<td>LNMC</td>
<td>0</td>
<td>20 ± 3</td>
</tr>
<tr>
<td>10</td>
<td>LNMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>14*</td>
<td>LNMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18*</td>
<td>LNMC</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>19*</td>
<td>LNMC</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

PBMCs and LNMCs (5 x 10^6/mL) were seeded in methylcellulose in the absence or presence of added IL 2 without mitogenic stimulation and incubated as described in Materials and Methods. Results are expressed as mean ± SD of colonies per 5 x 10^6 seeded cells from triplicate cultures.

*Patients who subsequently developed AIDS.
T CELL COLONY FORMATION IN LAS PATIENTS

**Fig 2.** Inhibition of spontaneous T cell colony formation with anti-Tac MoAb. PBMCs or LNMCs (5 x 10^6 cells per milliliter) were incubated with increasing concentrations of either anti-Tac or a pool of three anti-HLA class II MoAbs at 37°C for one hour. Extensively washed cells were seeded in methylcellulose in the absence of added growth factors and mitogenic stimulation as described in Materials and Methods. Each sign represents a different patient: ○—◊, No. 1 (PBMC); △—Δ, No. 2 (PBMC); Δ—Δ, No. 5 (LNMC); ■—■, No. 15 (PBMC); □——□, No. 4 (LNMC).

LCM were composed of lymphoblastoid cells bearing T cell surface markers (Table 5). Forty-eight percent to 83% of the colony cells were T3⁺, whereas 46% to 94% were T4⁺ and 58% to 95% were T8⁺. In all but one case (No. 5; Table 5), more than 50% of the colony cells were T6⁺, unlike T cell colonies from healthy heterosexuals. Moreover, induced colonies from clinically healthy male homosexuals contained a significantly higher proportion of T6⁺ cells than colonies from normal heterosexuals (P < .001; Table 5).

Spontaneous colonies from PBMCs and LNMCs were smaller than induced colonies (up to 100 cells per colony), and were composed of lymphoblastoid cells bearing T cell surface markers (Table 5). The phenotype of cells from spontaneous colonies was not significantly different from that of induced colonies.

**Clonogenic capacity of T-CFC.** The proliferative capacity of peripheral blood, bone marrow, or lymph node T-CFC was also tested after incubating the cells in liquid culture for three to five days 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.25 As shown in Table 6, “delayed” plating did not, in general, enhance the spontaneous plating efficiency and in two of three patients (No. 1 and No. 2; Table 6), completely abrogated the induced colony growth. However, delayed plating of LNMCs from patient No. 18, who subsequently developed AIDS, significantly enhanced the induced colony formation (Table 6). Colonies obtained by “delayed” plating were also of T cell origin, since they were counted of more than 75% T3⁺ cells.

**DISCUSSION**

The results reported here indicate that LAS patients present important quantitative and qualitative abnormalities of peripheral blood and bone marrow T-CFC. The bone marrow T-CFC from all patients failed to generate colonies and the peripheral blood T-CFC of several patients, especially those who subsequently developed AIDS, gave rise to a particularly low number of colonies. Similarly, other investigators showed impaired T colony formation from PBMCs of hemophiliacs with lymphadenopathy22 and AIDS and of LAS patients.21 However, in these studies,21,22 T cell colony formation was obtained by PHA stimulation of patients’ PBMCs without the addition of IL 2; exogenous IL 2 enhanced the colony growth, suggesting that the observed low plating efficiency could be due to the impaired capacity of patients’ T cells to produce IL 2.20

In our experiments, colony formation was induced with crude conditioned medium containing both IL 2 and low amounts of PHA (0.001%). Contaminant PHA could not yield colony growth, whereas as little as 0.1 U/mL of IL 2 is sufficient for normal T cell colony formation in the presence of PHA.4 Moreover, aggregates containing more than 50 cells were considered as colonies, whereas in the above-mentioned studies,21,22 aggregates with as few as 25 cells

<p>| Table 5. Phenotypic Characterization of Induced and Spontaneous T Cell Colonies From PBMCs, BMMCs, and LNMCs of LAS Patients |
|---|---|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Source of Cells</th>
<th>T3</th>
<th>T4</th>
<th>T6</th>
<th>T8</th>
<th>T3</th>
<th>T4</th>
<th>T6</th>
<th>T8</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PBMC</td>
<td>48</td>
<td>46</td>
<td>56</td>
<td>60</td>
<td>42</td>
<td>40</td>
<td>38</td>
<td>41</td>
</tr>
<tr>
<td>2</td>
<td>PBMC</td>
<td>81</td>
<td>88</td>
<td>92</td>
<td>94</td>
<td>80</td>
<td>82</td>
<td>84</td>
<td>87</td>
</tr>
<tr>
<td>4</td>
<td>LNMC</td>
<td>83</td>
<td>79</td>
<td>65</td>
<td>83</td>
<td>100</td>
<td>88</td>
<td>92</td>
<td>94</td>
</tr>
<tr>
<td>5</td>
<td>LNMC</td>
<td>69</td>
<td>87</td>
<td>34</td>
<td>58</td>
<td>62</td>
<td>50</td>
<td>41</td>
<td>54</td>
</tr>
<tr>
<td>Healthy homosexuals (n = 3)</td>
<td>PBMC</td>
<td>79 ± 9</td>
<td>60 ± 11*</td>
<td>50 ± 14*</td>
<td>48 ± 15*</td>
<td>NCG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy heterosexuals (n = 7)</td>
<td>PBMC</td>
<td>75 ± 5</td>
<td>47 ± 13</td>
<td>17 ± 5</td>
<td>35 ± 4</td>
<td>NCG</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Individually picked colonies were pooled, washed, and dissociated, and colony cells were stained by indirect immunofluorescence with the corresponding MoAb. NCG, no colony growth.

*P < .01 (Student’s t test).
Table 6. Clonogenic Capacity of LAS Patients’ T-CFC

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Source of Cells</th>
<th>Immediate Plating</th>
<th>Delayed Plating*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Spontaneous</td>
<td>Induced</td>
</tr>
<tr>
<td>1</td>
<td>PBMC</td>
<td>64 ± 9</td>
<td>285 ± 24*</td>
</tr>
<tr>
<td>2</td>
<td>PBMC</td>
<td>42 ± 6</td>
<td>300 ± 22</td>
</tr>
<tr>
<td>14*</td>
<td>PBMC</td>
<td>0</td>
<td>10 ± 2</td>
</tr>
<tr>
<td></td>
<td>PBMC</td>
<td>0</td>
<td>4 ± 1</td>
</tr>
<tr>
<td>4</td>
<td>LNMC</td>
<td>122 ± 15</td>
<td>130 ± 12</td>
</tr>
<tr>
<td>18*</td>
<td>LNMC</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*Cells (5 x 10^6/mL) were seeded in methylcellulose after 72 hours of cell incubation in α-MEM in the absence of added growth factors or mitogens. Results are expressed as mean number of colonies per 5 x 10^5 seeded cells from triplicate culture.

*Patients who subsequently developed AIDS.
T CELL COLONY FORMATION IN LAS


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