A Peripheral Blood-Derived Monolayer Supports Long-Term Cultures of Human CD4+ and CD8+ T Lymphocytes

By Natalie Sutkowski, Ming-Ling Kuo, Peter S. Amena, Joseph P. Dougherty, and Yacov Ron

An in vitro culture system has been developed for the long-term maintenance of primary, human peripheral blood and umbilical cord blood T lymphocytes, which does not rely on the use of stimulatory cytokines, antigen, or mitogens. In these cultures, a monolayer of adherent cells, some spindle-shaped and some resembling macrophages, developed within a week. All adherent cells were positive for the extracellular matrix proteins laminin and fibronectin, the intermediate filament vimentin, and for the surface markers major histocompatibility complex class II, platelet-endothelial cell adhesion molecule I (CD31), and E-Selectin (ELAM-1; CD62E). They were negative for the leukocyte common antigen (CD45), the macrophage marker MO-2 (CD14), muscle-specific actin, and Factor VIII-related antigen. These monolayers supported the maintenance of nonadherent, resting, mature T cells for up to 3 months, and these cells retained their ability to respond to mitogens and allogeneic cells. Both CD4+ and CD8+ cells were supported. The proportion of CD4+ and CD8+ cells remained unchanged after 3 months in culture. We have also used T cells from 2-month-old cultures as target cells for retroviral vector-mediated gene transfer. Up to 30% of the long-term T cells expressed the transferred lacZ gene after infection with a retroviral vector. The infection efficiency was similar to that obtained for fresh peripheral blood T cells, indicating that the long-term-cultured cells might be suitable for certain gene therapy applications.

MATERIALS AND METHODS

Preparation of long-term T-cell cultures. Mononuclear cells were prepared from human PB or umbilical CB by density separation on Histopaque ficoll (Sigma, St Louis, MO). The entire mononuclear cell fraction was resuspended at a cell density of 0.5 × 10^6 cells/mL either in Dulbecco’s modified Eagle’s medium supplemented with 20% horse serum (GIBCO, Grand Island, NY) and 50 μg/mL 2-mercaptoethanol (Sigma; Dexter “like” cultures without IL-3) or in RPMI media supplemented with 10% fetal calf serum (GIBCO), 50 μg/mL L-2-mercaptoethanol (Whitlock-Witte culture conditions), and 20 ng/mL basic fibroblast growth factor (bFGF; PeproTech, Rocky Hill, NJ). Cells were plated in T25 tissue culture flasks (5 mL/flask) and then incubated at 37°C with 5% CO2. The media was changed once a week by pipetting and replacing one-half of the media with fresh media, being careful not to disturb the nonadherent cells.

We have developed a culture system for long-term maintenance of resting, human peripheral blood (PB) and umbilical cord blood (CB) T lymphocytes. T-cell growth was supported by a PB-derived adherent cell monolayer, which was composed of endothelial-like cells. These cells have a phenotype closely resembling that of activated endothelial cells including the expression of E-selectin and HLA-DR. These cells presumably secrete growth factor(s) that can support the maintenance of mature T cells. The T cells appeared to remain in a quiescent phase. They could be harvested from the cultures and stimulated with mitogens or allogeneic cells at any point, thus remaining immunocompetent. Both CD4+ and CD8+ cells persisted in culture, and the proportion of each cell type remained the same for the duration of the culture. We have harvested T cells from 2-month-old cultures for use as target cells for retroviral vector-mediated gene transfer. Forty-eight hours after cocultivation with helper cells producing a retroviral vector containing the lacZ gene, 20% to 30% of the long-term–cultured T cells expressed the exogenous gene. This efficiency of infection was similar to that obtained for fresh PB T cells (21% to 28%), suggesting that the long-term–cultured cells might also be useful, in certain cases, for somatic cell gene therapy protocols.

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Flow cytometric analysis. Human PB lymphocytes (PBLs) and umbilical CB lymphocytes (CBLs) were analyzed at the time of culture initiation, and nonadherent cells from the long-term cultures were harvested for flow cytometry 1 month and 3 months later. T cells were stained with fluorescein-labeled monoclonal antibodies (MoAbs) specific for human CD3 (OKT3), CD4 (OKT4), and CD8 (OKT8; Ortho, Raritan, NJ), phycoerythrin-labeled B1, (anti-CD20; Coulter, Hialeah, FL), and fluorescein-labeled mouse anti-human Ig (Jackson ImmunoResearch, West Grove, PA) were used to stain B cells. A total of 5,000 cells were analyzed on an Epics Profile (Coulter).

Flow cytometric analyses were performed at various time points on lymphocytes harvested from 4 different cultures with very similar results.

Flow cytometric analysis of newly synthesized DNA. Nonadherent T cells were removed from long-term cultures every 2 weeks for 10 weeks and were analyzed for newly synthesized DNA as a measure for cell proliferation. Cells were incubated for 30 minutes in medium containing 10 μmol/L of BrdU (Sigma), washed, and fixed with 70% ice-cold ethanol. One hour later (cells can be stored for weeks at this stage), cells were resuspended for 30 minutes at room temperature in 2 N HCl containing 0.5% Triton X-100. The cells were then washed 3 times with borate buffer, pH 8.2, and 20 μL of fluorescein isothiocyanate (FITC)-mouse anti-BrdU antibodies (Becton Dickinson, San Jose, CA). Propidium iodide (10 μmol/L) is added just before analysis on the fluorescence-activated cell sorter.

Newly synthesized DNA is brightly stained with the anti-BrdU antibodies.

Mitomycin C-treated cells (25 μg/mL) were used as a marker for no DNA synthesis, and concanavalin A (Con A; Pharmacia, Uppsala, Sweden)–pulsed cells were used as positive controls. To test the sensitivity of this system, we pulsed long-term T cells with 4 μg/mL Con A for 2 hours only to induce only a marginal stimulation of cells.

Immunoperoxidase staining. Adherent cells from the long-term cultures were fixed in 8-well chamber slides with 4% paraformaldehyde in 0.2 mol/L NaPO4 buffer for 15 min at 4°C and were washed once with phosphate-buffered saline (PBS) containing 0.1% Triton X-100. Fixed cells were reacted with periodic acid (0.03 mol/L) to remove intrinsic peroxidase activity; then, aldehyde groups were reduced with NaBH4 in PBS (0.5% wt/vol) for 1 hour. Cells were blocked with 5% bovine serum albumin and then were reacted sequentially, each for 30 minutes, with the respective primary antibody, followed by biotinylated rabbit antimouse or swine antirabbit antibody (Dakopatts, Carpinteria, CA) and then streptavidin-peroxidase (Dakopatts). Subsequently, cells were reacted with diaminobenzidine-HCl (Dakopatts) in 3% H2O2 in TRIS buffer (0.05 mol/L, pH 7.72) for 20 minutes. Optimal antibody-staining dilutions were determined by serial titration. Antibodies used were leukocyte common antigen, Factor VIII-related antigen, anti-CD31, muscle-specific actin, and vimentin (all from Dakopatts); laminin (courtesy of Dr

![Flow cytometric analysis of human umbilical CB cultures.](image-url)
LONG-TERM BLOOD-DERIVED T-LYMPHOCYTE CULTURES

Fig 2. Flow cytometric analysis of human PB cultures. Fresh human PBLs from healthy donors were isolated by density gradient separation on Hypaque ficoll. Long-term mononuclear cells were established as described in the Materials and Methods. An aliquot of fresh cells was stained with FITC-labeled MoAbs specific for human CD4, CD8, and Ig. Long-term cultured cells were harvested for staining after 1 month. A total of 5,000 cells/sample were analyzed on an Epics Profile, depicted in each histogram. The Y-axis corresponds to the relative number of cells. The X-axis represents log of the fluorescence.

Table 1. ³[H]-Thymidine Incorporation by Resting Long-Term T Cells (cpm)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Weeks in Culture</th>
</tr>
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<tbody>
<tr>
<td>Medium</td>
<td>0 575 514 604 585 470</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>471 419 470 480 448</td>
</tr>
<tr>
<td>Con A</td>
<td>4,531 ND ND 3,930 ND</td>
</tr>
<tr>
<td>Mitomycin C/Con A</td>
<td>549 ND ND 484 ND</td>
</tr>
</tbody>
</table>

A total of 7 x 10⁶ cells per half-area well were plated for 12 hours in medium containing 1.0 μCi ³[H]-thyidine. Con A and mitomycin C treatments are described in Materials and Methods. Results are expressed as mean of 3 to 6 cultures; standard deviation was always within 5% to 15% of the mean and has been omitted for simplicity. Abbreviation: ND, not determined.

Retrovirus vector and virus-producing cells. The MFG-NB retrovirus vector is a Moloney murine leukemia virus (MLV)-based vector containing the lacZ gene fused to an simian virus-40 nuclear localization signal sequence, expressed from the viral long terminal repeat. The vector was packaged using a cell line producing virus comprised mostly of MLV proteins except for the envelope glycoproteins that were derived from gibbon ape leukemia virus (GaLV). This cell line, MMG, was made by transfecting the canine osteosarcoma D17 cell line with each of the following plasmid DNAs, (1) pMFG-NB, (2) pgag-pol, (3) pGal.Veny, followed by selection with G418 (0.35 mg/mL), GPT (0.25 mg/mL xanthine, 25 μg/mL mycophenolic acid, and 15 μg/mL hypoxanthine), and hygromycin (1.0 μg/mL). Cells were subcloned, and an individual line yielding vector virus stocks of 2.0 x 10⁹ focus-forming units/mL was used for all experiments. The titer was quantitated by inocu-
lalation of D17 cells with serial dilutions of helper cell supernatant. Inoculated cells were stained with X-gal 2d later, as previously described, and foci consisting of 4 to 16 blue cells were counted. The producer cells were periodically tested and always found to be free of replication-competent virus. Screening for replication-competent virus was performed by assaying for reverse transcriptase activity after a 2-week D17 amplification step. The helper rescue assay was also performed after D17 amplification, using an indicator cell line consisting of D17 cells that were transfected with the defective vector BAG, containing the lacZ gene and the neo gene, and maintained under selection with G418. The BAG-transfected cell line cannot produce vector virus because it lacks the viral trans sequences. If replication-competent virus were present, it would be detected by X-gal staining or G418 selection.

Exogenous gene transfer. Fresh PB mononuclear cells or T cells harvested from 2-month-old cultures were stimulated overnight with PHA (4 μg/mL) in Dulbecco’s modified Eagle’s medium containing 20% horse serum and 2-mercaptoethanol (50 μmol/L). Stimulated cells were washed with PBS and resuspended at a density of 1 × 10^6 cells/mL in medium containing recombinant IL-2 (10 ng/mL; PeproTech) and polybrene (6 μg/mL; Sigma), and were then cocultivated on confluent lawns of irradiated (16 Gy) MFG-NB vector virus–producing cells for 24 hours at 37°C. Forty-eight hours after cocultivation, the target cells and mock-infected cells (cells cocultivated with helper cells not secreting the vector) were stained with X-gal as described, and blue cells were scored under light microscopy. Retroviral-mediated transfer of the lacZ gene was performed twice.

RESULTS

Long-term culture of PB and umbilical CB mononuclear cells results in the formation of an adherent cell layer of stromal-like cells and a nonadherent cell population composed exclusively of T cells. Both human PB and umbilical

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Staining Profile of the PB-Derived Monolayer</th>
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<tr>
<td>HLA-ABC (MHC class I)</td>
<td>All cell types (+)</td>
</tr>
<tr>
<td>HLA-DR (MHC class II)</td>
<td>Macrophages, B cells, dendritic, endothelial cells (+)</td>
</tr>
<tr>
<td>CD45 (leukocyte common antigen)</td>
<td>Pan-leukocyte (-)</td>
</tr>
<tr>
<td>CD14 (MO-2)</td>
<td>Macrophages, monocytes (-)</td>
</tr>
<tr>
<td>CD31 (PECAM-1)</td>
<td>Platelets, endothelial cells (+)</td>
</tr>
<tr>
<td>CD34</td>
<td>Stem cells, endothelial cells (+/-)</td>
</tr>
<tr>
<td>Factor VIII</td>
<td>Endothelial cells (-)</td>
</tr>
<tr>
<td>Actin</td>
<td>Muscle-specific (-)</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>Extracellular matrix (+)</td>
</tr>
<tr>
<td>Laminin</td>
<td>Extracellular matrix (+)</td>
</tr>
<tr>
<td>Vimentin</td>
<td>Intermediate filament (-)</td>
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</tbody>
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PB-derived monolayer cells were analyzed by immunohistochemical staining of various cell surface markers. Cells were fixed, then stained with MoAbs specific for each marker using immunoperoxidase staining. Cells were scored +/- under light microscopy.

Abbreviations: MHC, major histocompatibility complex; PECAM-1, platelet-endothelial cell adhesion molecule-1; ELAM-1, endothelial leukocyte adhesion molecule-1.
CB have been reported to contain hematopoietic, pluripotential stem cells. In an attempt to culture these hematopoietic precursor cells, we applied modified Whitlock-Witte and Dexter culture conditions, which have been shown to propagate murine pre-B cells and macrophages, respectively, from long-term bone marrow cultures. Because bFGF has been reported to increase the yield of hematopoietic progenitors isolated from human PB by inducing their response to multilineage hematopoietins, 20 to 50 ng/mL bFGF was added to some of the cultures. When cultured under Dexter-like conditions with or without bFGF and under the Whitlock-Witte-like conditions with bFGF, both PB and CB mononuclear cells began to form, within 2 weeks, a semiconfluent lawn of adherent, stromal-like cells, on which resided nonadherent cells with lymphocyte morphology. Both cell types remained in culture for up to 3 months. On typing, the lymphocyte population was found to consist exclusively of CD3+ T cells.

Further analysis determined that both CD4+ and CD8+ cells were present in culture. No B cells were detected. Figure 1 shows flow cytometric data of a representative CBL culture at the time of culture initiation and after 1 month and 3 months in vitro. CD3+ T cells comprised 60.7% of the CBLs at initiation and comprised virtually all of the cells after 1 and 3 months. Ig+ B cells initially comprised 23% of the CBLs, and, after 1 month, no B cells could be detected.
Normal ratios of CD4+ and CD8+ T cells were found at both time points. Figure 2 shows flow cytometric data of a representative long-term PBL culture at initiation and after 1 month in vitro. The results were very similar to the CBL cultures in that the B cells initially present (27% of the cells) could not be detected after 1 month. Cells were also counted at various time points after initiation of culture to assess the total number of surviving cells. The biggest drop in T-cell counts (32%) occurs within the first week of culture. At 4 weeks, an additional 13% are lost, and, at 10 weeks after culture initiation, 16% more cells are lost. We think that most of the cell loss during the first week represents the death of cells damaged during the purification procedure, because the percentage of cell loss decreases to around 15% per month in the subsequent 8 weeks.

In both CBL and PBL cultures after 1 month or 3 months in vitro, the lymphocyte population consisted entirely of CD3+ T cells (Figs 1 and 2). This was independent of whether modified Dexter conditions or Whitlock-Witte conditions with bFGF were applied. It is noteworthy that modified Whitlock-Witte culture conditions without addition of bFGF did not permit the formation of long-term cultures. B cells did not survive under any of the culture conditions used. What is particularly interesting to note was that the percentage of CD4+T cells did not decrease even after 3 months in culture, as has been described with long-term IL-2 treatment.1 CD8+ T cells were also present at both time points; however, a subpopulation of weakly staining CD8+ cells initially present in both CBL and PBL cultures did not persist in culture (Figs 1 and 2). The differences in the percentages of CD8+ between the adult and the CB cultures at time 0 and after establishment of long-term cultures (Figs 1 and 2) could be caused, in part, by the different proportions of the weakly staining CD8+ population.

Because bFGF was needed for the maintenance of the Whitlock-Witte–type cultures even though it has no direct effect on T cells,10 we inferred that the adherent cell layer was dependent on bFGF, and that the stromal-like cells in some manner sustained T-cell growth. This was supported by the fact that over 80% of T cells removed from the adherent cells and maintained in similar media with bFGF or with conditioned media from the original culture died within 10 days. In contrast, T cells were not needed to sustain the adherent cells, because removal of the T cells did not
Fig 6 (cont'd).
alter the adherent cells' viability. Because Dexter culture conditions require the addition of high amounts of serum (20%) to the media, it was likely that there was enough bFGF (or other growth factors) in the serum to maintain the adherent cells, making the addition of supplementary bFGF unnecessary.

Analysis of DNA synthesis by long-term T cells. Although there was a slow decrease in the number of T cells over a period of 3 months, to assess whether some of these cells do proliferate in culture, we performed two sets of experiments. First, we measured, at 2-week intervals, the incorporation of $[^3H]$-thymidine by unstimulated T cells. As a control for nondividing cells, we used mitomycin C-treated T cells, some of which were also stimulated with Con A (to control for the effect of mitomycin C). As can be seen in Table 1, thymidine incorporation by untreated T cells was very low, although it was slightly higher than that of mitomycin C-treated cells. This could be because of the fact that mitomycin C treatment results in a faster cell death rate; however, we cannot rule out the possibility that some cells in these long-term cultures are dividing. In a second set of experiments, we measured the amount of newly synthesized DNA by measuring the incorporation of BrdU with fluoresceinated anti-BrdU antibodies. Again, we found no evidence of cell division (data not shown).

PB T cells from long-term cultures can be activated with mitogens and respond to allogeneic stimulator cells in mixed lymphocyte reactions. To assess the immune competence of PBL T cells harvested from long-term cultures, we tested their ability to respond to the mitogens PHA and Con A and to allogeneic cells after 6 weeks, 2 months, or 3 months in culture. T-cell proliferation was measured by $[^3H]$-thymidine incorporation. As can be seen in Fig 3, after 6 weeks in culture, the T cells proliferated in response to both mitogens, although the response to PHA was somewhat better. After 3 months in culture, the T-cell response to PHA did not decrease. Allogeneic responses were measured at 2 months against two different stimulator PB cells. As shown in Fig 4, the long-term T cells responded to both donor cells as measured by $[^3H]$-thymidine incorporation.

The adherent cell population from the long-term cultures is composed of endothelial-like cells with varied morphology. The phenotype of the supportive adherent cell layer was characterized by immunohistochemistry using a battery of MoAbs specific for the cell surface markers listed in Table 2. Although all of the adherent cells had an identical antibody-staining profile, morphologically, the cells could be classified into at least two distinct types. Usually, about half the cells had a spindle-shaped morphology, and the other half had a macrophage-like morphology (Fig 5). The cells were large and stained positively for fibronectin, laminin, and vimentin. Because of their morphology and because the cells were derived from PB or umbilical CB, we suspected their origin to be of the macrophage or endothelial cell lineages, although fibroblasts, dendritic cells, smooth muscle cells, etc were also a possibility. Because the adherent cells did not express either the leukocyte common antigen (CD45) or the macrophage marker MO-2 (CD14), and because macrophages are not known to be dependent on bFGF, we concluded it was unlikely that the cells were macrophages. The cells were also negative for muscle-specific actin, indicating that they are not of smooth muscle origin. The fact that the adherent cells were responsive to bFGF and that they expressed platelet-endothelial cell adhesion molecule-1 (CD31) strongly suggested that they are of endothelial origin. Moreover, all adherent cells, independent of their morphology, stained very strongly with anti-E-selectin (ELAM-1, CD62E) antibodies (Table 2). E-selectin is a marker for activated endothelial cells and cannot be detected by staining on resting endothelial cells. All adherent cells also expressed HLA-DR antigens, a trait that is also characteristic of activated endothelial cells. The cells, however, were negative for Factor VIII-related antigen, another common endothelial cell marker. Therefore, we refer to the cells as endothelial-like.

It is noteworthy that these endothelial-like cells have an antibody-staining profile similar to that of certain Kaposi sarcoma (KS) cell lines, which also stain positive for some endothelial markers. The origin of KS cells has been a controversial topic; however, it is now generally accepted that they are of endothelial origin. The KS cell lines also have spindle-like morphology, and they are also very responsive to bFGF. Because it has been reported that these cell lines proliferate in response to PHA-activated T-cell-conditioned media, we added PHA to some of the long-term cultures. Indeed, activation of the T cells by PHA appeared to stimulate growth of the adherent cell layer, most likely by factors secreted by the activated T cells. The adherent cells approximately doubled in number within 5 days of the addition of PHA (data not shown). Interestingly, when the PHA was washed away 24 to 48 hours after addition to the cultures, the stimulated T cells appeared to proliferate for several days and then reverted to a resting morphology. This is in contrast to the characteristic cell death that follows PHA stimulation of purified T cells.

Long-term cultured T cells can be efficiently infected with a retroviral vector and express the exogenous gene. Because the T cells harvested from the long-term cultures were immune competent, they could be of potential use as target cells for certain gene therapy protocols. To test whether the cells could be efficiently transduced with a retroviral vector containing the lacZ gene with a nuclear localization signal as a marker gene, we infected PBL T cells from 2-month-old cultures, using a protocol originally designed for the infection of fresh primary T cells. MMG producer cells (Gu et al, submitted) packaged the MLV-based vector MFG-NB with amphotropic envelope proteins from GaLV (Fig 6A). The vector virus produced by these cells can infect human cells. Long-term T cells were stimulated overnight with PHA and were then washed and cocultivated, in the presence of IL-2 and polybrene for 24 hours, with lethally irradiated MMG cells that produced vector virus at a titer of $2 \times 10^7$ colony-forming units/mL. The target T cells were then harvested and cultured for 48 hours to allow the cells time to express the exogenous lacZ gene. Fresh PB T cells were simultaneously infected as a control. To determine the percentage of cells that expressed the exogenous gene, the target cells were fixed and stained with X-gal, and blue cells
were counted. As can be seen in Fig 6, on average, 20% to 30% of the 2-month-cultured T cells expressed lacZ after cocultivation (Fig 6B), as compared with 21% to 28% of the fresh PB T cells (Fig 6C) and uninfected controls (Fig 6D). This indicates that the long-term-cultured T cells might be suitable for certain gene therapy applications.

DISCUSSION

The data presented here show that bFGF-responsive adherent cells with surface markers closely resembling those of activated endothelial cells can be cultured from the PB and can support the growth of primary T lymphocytes in vitro for several months. The T-cell population retains the original ratio of CD4+ cells to CD8+ cells and do not proliferate at a high rate, if at all. It is probable that the adherent cells secrete an as yet unknown factor(s) that maintains the T cells in a quiescent state. The resting T cells can be activated to proliferate in response to PHA, even 3 months after the start of the cultures (Fig 3).

The phenotype of the endothelial-like adherent cells bears resemblance to that described for KS cells. All of the surface markers found on the stromal-like monolayer have been described for various isolates of KS cells, including the fact that some are negative for Factor VIII-related antigen, a common endothelial cell marker. Although the origin of KS cells is somewhat controversial, most seem to concur that they are of endothelial origin. Although they are known to circulate in PB. Studies using bFGF and PHA-stimulated T-cell-conditioned media (CM) indicated that KS cells proliferate in response to bFGF and other cytokines present in the CM. Similar results were obtained for the stromal-like monolayer cells (data not shown). Therefore, it is tempting to speculate that these stromal-like cells might be the normal counterpart of the KS cells.

The development of a system in which primary CD4+ and CD8+ T lymphocytes can be maintained in vitro for long periods of time without the need for activation suggests a number of applications. The most obvious use would be for studies on T lymphocyte biology. Currently, the majority of studies concerning T-cell activation are performed on either T-cell hybridomas or T-cell lines. This is because activated primary T cells must be maintained with IL-2 to survive in vitro, and the effects of IL-2 might obscure the effects of other signaling molecules. For example, the effects of various signaling molecules on the induction of very late antigens could be studied in these long-term T-cell cultures, independent of the effects of exogenous IL-2. Similarly, the effects of molecules that might induce apoptosis of T lymphocytes at various time points poststimulation can also be readily assessed in such a system. It would also be interesting to explore whether thymocytes or other types of cells can be cultured on the stromal-like monolayer. Evidently, B cells are not supported in these cultures (see Figs 1 and 2).

A second potential application is for studying the interaction of viruses (in particular, HIV) with T lymphocytes. Because, in this system, T cells can be activated with mitogens without induction of massive cell death, the role of various cellular genes such as nuclear factor kappa B, tumor necrosis factor-α, and other cytokines on the viral life cycle can be readily assessed. Because the infected cells can be maintained for long periods of time, this system is also useful for resolving the controversial issue of latency of HIV infection. In addition, HIV infection of nondividing cells can be directly assessed in such a system. Furthermore, various agents that might be required for or, conversely, block infection could be screened using this in vitro system.

A third use of these primary T-cell cultures is in somatic cell gene therapy. Figure 6 shows that the long-term-cultured cells could be efficiently infected with a retroviral vector. Although for most gene therapy applications the use of fresh primary T cells would be preferable to long-term-cultured cells, because fresh cells home better after transplantation to the host, it might not always be possible to obtain enough fresh PBLs, especially from newborns. A particular use might be in HIV gene therapy for neonates, once the technology for intracellular immunization protocols has been developed. The umbilical CB from babies born of HIV-positive mothers could be cultured until it is known for certain whether the babies are also infected with HIV. The T cells that arise in these cultures would be amenable to such gene therapy approaches.

ACKNOWLEDGMENT

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A peripheral blood-derived monolayer supports long-term cultures of human CD4+ and CD8+ T lymphocytes

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