New CD4(+) Cell Line Susceptible to Infection by HIV-1

Michel Tremblay, Arthur K. Sullivan, Ronald Rooke, Romas Geleziunas, Christos Tsoukas, Gene Shematek, Norbert Gilmore, and Mark A. Wainberg

Lady Davis Institute for Medical Research, Sir Mortimer B. Davis—Jewish General Hospital (M.T., R.R., R.G., M.A.W.) and Departments of Medicine (M.T., A.K.S., C.T., N.G., M.A.W.) and Microbiology (R.R., R.G., M.A.W.), and McGill Cancer Center (A.K.S., G.S.) McGill University, Montreal, Quebec, Canada

Infection of a newly described human T lymphoid cell line, CEM-CL10, with three different variants of HIV-1 resulted in cytopathic effects followed by cell lysis. Following primary lytic infection, proviral DNA could not be detected by Southern blot analysis in the outgrowth of the surviving CEM-CL10 cells 60 days after initial exposure to HIV-1. These surviving cells could be further grown as a separate line, derived from CEM-CL10, and were found to be resistant to subsequent infection by HIV-1. A marked decrease in CD4 antigen expression was observed in these latter cells but not of the CD3 and transferrin receptor antigens. This decline in cell surface CD4 expression was correlated with both an absence of specific CD4 mRNA and with changes in structure of the CD4 gene. Both the HIV-1-sensitive CEM-CL10 cell line and its CD4(-), HIV-1-resistant derivative line, will be made available to interested investigators.

KEY WORDS: HIV-1-resistant cells, CD4 antigen, genomic rearrangement

INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is characterized by a progressive depletion in numbers of circulating CD4(+) T lymphocytes [Gottlieb et al., 1981; Bowen et al., 1985]. The human immunodeficiency virus (HIV-1), the etiologic agent of AIDS, displays strong tropism for such CD4(+) T cells [Barré-Sinoussi et al., 1983; Gallo et al., 1984; Klatzmann et al., 1984a,b; Levy et al., 1984; Popovic et al., 1984], based on the finding that CD4 is itself the cellular receptor for HIV-1 [Dalgleish et al., 1984; McDougal et al., 1986]. Indeed, viral infection of CD4 (+) cells can be blocked by monoclonal antibodies directed against specific CD4(+) epitopes [Barré-Sinoussi et al., 1983; Klatzmann et al., 1984a,b; McDougal et al., 1985], and the CD4 antigen can co-precipitate with the major HIV-1 envelope glycoprotein, gp120 [McDougal et al., 1986]. In addition, a surface expression of CD4 antigen is decreased in infected cells, which can be shown to contain intracellular complexes of CD4 and gp120 [Hoxie et al., 1986]. Finally, transfection of the CD4 gene into HeLa cells, which do not normally express this antigen, both leads to CD4 expression and confers susceptibility to HIV-1 infection [Maddon et al., 1986].

Persistent non-cytopathic HIV-1 infections have been observed in some T cell lines [Folks et al., 1986; Casareale et al., 1987; Stevenson et al., 1987]. In these instances, the absence of cytopathicity has been ascribed to a diminution in expression of cell surface CD4 antigen. It has been proposed that a decrease in CD4 receptor expression in such chronically infected lines might prevent superinfection and hence lead to the emergence of cytolysis-resistant cells [Stevenson et al., 1987]. Such cells have also been reported to have diminished surface expression of the CD2, CD3, and CD8 antigenic markers.

These decreased levels of CD4 have been explained, in some cases, by correspondingly reduced in CD4 mRNA [Hoxie et al., 1986; Casareale et al., 1987]. It has also been reported that posttranscriptional mechanisms may underlie the down-modulation of CD4 antigen following HIV-1 infection [Stevenson et al., 1987].

We now describe a new CD4(+) T cell line (CEM-CL10), highly susceptible to infection by both laboratory and clinical strains of HIV-1. Those cells, which survive a primary infection by HIV-1, are resistant to further inoculation by this agent. Such resistant cells produce no detectable CD4 mRNA and show evidence of genetic rearrangement within the CD4 gene.

This new HIV-1-susceptible cell line is adherent and

© 1989 ALAN R. LISS, INC.
may be more advantageous for numerous types of studies than other cell types.

**MATERIALS AND METHODS**

**Cell Line**

CEM cells were initially described as belonging to a human leukemic CD4(+) cell line, isolated from a child with acute lymphocytic leukemia [Foley et al., 1965]. This T-lymphoblastoid cell line was obtained courtesy of Dr. J. Menezes (Ste-Justine Hospital, Montreal) and was subcloned by Dr. A.K. Sullivan. A variant spontaneously arose in which the cells were adherent and of the CD4(-) phenotype.

Polyethylene glycol was used to fuse these latter cells, which were hypoxanthine-aminopterin-thymidine (HAT)-sensitive, (HAT)-sensitive, with peripheral blood mononuclear cells from a healthy individual in order to restore the CD4 receptor. These fused products were cloned and subcloned by growth in methylcellulose-containing agar, and an adherent CD4(-) cell line termed CEM-CL10 was established. This line has now been maintained for 2 years at 37°C under 5% CO2 in RPMI 1640 medium, supplemented with L-glutamine (2 mM), streptomycin (250 pg/ml), penicillin (250 U/ml), and 10% (v/v) fetal bovine serum.

**Infection by HIV-1 of CEM-CL10 Cells**

The HTLV-IIIb strain of HIV-1 was harvested from culture fluids of the chronically infected H-9 T leukemic line, kindly supplied by Dr. R.C. Gallo (National Institutes of Health, Bethesda, MD) [Gallo et al., 1984]. In addition, two clinical isolates of HIV-1 (V 27-03 and V 27-06), isolated in our laboratory by co-culture of cord blood lymphocytes with peripheral blood mononuclear cells of different donors, were used in this study. Virus-containing culture fluids were kept at -70°C until use, and the titer of each viral preparation was determined by plaque assay using the MT-4 cell line (chronically infected by the human T lymphotropic virus type 1) [Harada et al., 1985].

For purposes of infection, 2.5 × 10^6 CEM-CL10 cells were harvested during exponential growth, washed once by centrifugation, and were incubated in virus-containing medium (multiplicity of infection = 0.002) at 37°C for 3 hr with gentle shaking. Unbound virus was then removed by centrifugation and the cells were suspended in fresh medium which was changed thereafter at 3-4-day intervals. Samples of culture fluid were collected regularly for determination of HIV-1 antigen expression and reverse transcriptase activity.

**Indirect Immunofluorescence**

The percentage of HIV-1-infected cells was determined using a fixed cell indirect immunofluorescence assay. For this purpose, infected cells were washed in phosphate-buffered saline (PBS), plated on glass slides, air-dried, and fixed in an acetone-methanol mixture (1:1) at room temperature for 30 min. Such fixed cells were reactive with a mixture of mouse monoclonal antibodies directed against the viral proteins p17 and p24 (kindly supplied by Dr. R.C. Gallo, NIH). The cells were finally reacted with a fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin and visualized under a fluorescence microscope.

**Flow Cytometry Analysis**

Fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated monoclonal antibodies Leu-2a, Leu-3a, Leu-4, Leu-12, Leu-M3, and antibodies to transferrin receptor were purchased from Becton-Dickinson (Mountain View, CA). Live cells were incubated for 30 min on ice with the appropriate antibodies, and were then washed by centrifugation and finally fixed in 0.5% paraformaldehyde. These cells were analyzed using a fluorescence-activated cell sorter (FACTStar, Becton-Dickinson Immunocytometry Systems, Mountain View, CA). FITC and PE emissions were measured at 515/545 nm, using a 530/30 bandpass filter, and at 564/606 nm using a 585/42 longpass filter, respectively. Ten thousand cells were analyzed in each case; debris and/or dead cells were excluded by a scatter setting system. Data were collected in list mode by a Consort 30 system (Becton-Dickinson) and analyzed on a Hewlett-Packard (HP) 900 series 500 computer with customized software. Non-specific staining was determined using non-binding isotype-matched monoclonal antibodies. Results are expressed as a percentage of the total population analyzed.

**Reverse Transcriptase Assay**

Poly (rA)-dependent DNA polymerase activity was measured by a modification of a previously reported procedure [Hoffman et al., 1985]. Cell-free supernatants were ultracentrifuged at 100,000 rpm for 30 min at 4°C and pellets were resuspended in 10 μl of serum-free RPMI. Samples (10 μl) were incubated with 50 μl of cocktail containing 50 mM Tris HCl; 5 mM DTT; 5 mM MgCl2; 150 mM KC1; 0.05% Triton X-100; 0.3 mM GSH; 0.5 mM EGTA; 50 μg/ml poly (rA) p (dT)12-15; 10 μCi thymidine triphosphate (40-70 Ci/mM), and 17.25 μl distilled water for 1 hr at 37°C. The reaction was stopped by placing the tubes on ice and adding 2 ml of sodium pyrophosphate 0.01 M (in IN HCl) and 2 ml of ice-cold 10% trichloroacetic acid (TCA). Precipitates were collected on Whatman GF/C fiber filters, which were washed several times with ice-cold 5% TCA, rinsed once with 70% ethanol, and air-dried, prior to being counted in Omnifluor scintillation fluid in a liquid scintillation spectrometer.

**Southern Blot Analysis**

High molecular weight DNA was isolated by standard methods [Maniatis et al., 1982]. Restriction endonuclease-digested DNA (20 μg) was subjected to electrophoresis through 0.8 cm thick, 0.8% agarose slab gels. Gels were blotted in 10 × SSC onto 0.45 μm nitrocellulose filters (Schleicher and Schuell). Hybridization was performed at 42°C for 48 hr in 5 × SSC, 50%
formamide, 10% dextran sulfate, 1 mg/ml of bovine serum albumin, polyvinyl pyrrolidine (200 μg/ml), Ficoll (200 μg/ml), and 250 μg/ml salmon sperm DNA. Filters were washed four times for 5 min each with 0.1 × SSC containing 0.1% SDS at 65°C. The probes to detect HIV-1 provirus (kindly supplied by Dr. F. Wong-Staal, NIH) and the CD4 receptor gene (courtesy of Dr. R. Axel, Columbia University, New York) were nick-translated products of pBH-10 [Shaw et al., 1984] and of pT4.B [Maddon et al., 1985], respectively (specific activity = 2 × 10⁸ cpm/pg DNA for each probe). Blots were exposed to Cronex film with intensifying screens for at least 24 hr.

**RESULTS**

CEM cells have previously been reported to support replication of HIV-1 and to be susceptible to HIV-1-mediated cell lysis [Montagnier, 1985; Folks et al., 1985]. We have developed a CEM variant cell line by subcloning and fusing these cells with normal T lymphocytes. This adherent cell line, termed CEM-CL10, has now been studied to determine its suitability as a target for HIV-1 infection.

Cytopathic effects, such as syncytium formation and lysis, were observed following infection by HIV-1 of CEM-CL10 cells (Fig. 1). The data of Table I indicate the results of infecting CEM-CL10 cells with each of three different HIV-1 variants, two clinical isolates termed V 27-03 and V 27-06, and the HTLV-IIIB laboratory strain. In each instance, maximum expression of

Fig. 1. A: Mock-infected CEM-CL10 cells. B: Arrows indicate multinucleated cells in HIV-1-infected cultures. × 100.
TABLE I. Expression of HIV-1 Antigens and Production of Viral Reverse Transcriptase Activity by HIV-1-Infected CEM-CL10 Cells

<table>
<thead>
<tr>
<th>Days</th>
<th>Viability (%) viable cells</th>
<th>HIV-1 antigen expression (%) positive cells</th>
<th>Reverse transcriptase activity (cpm x 10^-5/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>isolate (V 27-03)</td>
<td>8</td>
<td>85</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>69</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>42</td>
<td>16.5</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>15</td>
<td>2.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>74</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>85</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>85</td>
<td>22.9</td>
</tr>
<tr>
<td>Clinical</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>isolate (V 27-06)</td>
<td>8</td>
<td>86</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>63</td>
<td>6.7</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>47</td>
<td>15.3</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>20</td>
<td>11.4</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>75</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>84</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Laboratory</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>strain (HTLV-IIIb)</td>
<td>8</td>
<td>88</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>62</td>
<td>10.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>46</td>
<td>11.7</td>
</tr>
<tr>
<td></td>
<td>27</td>
<td>25</td>
<td>3.9</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>77</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>89</td>
<td>&lt;1.0</td>
</tr>
</tbody>
</table>

*aCell viability was determined by trypan blue exclusion.
*bCells were stained for indirect immunofluorescence using monoclonal antibodies against viral p17 and p24 antigens.

viral p17 and/or p24 antigen was observed between 12 and 20 days after infection, as determined by indirect immunofluorescence. Peaks in production of progeny HIV-1, as determined by reverse transcriptase assay, were detected at 12, 27, and 27 days following infection with clinical isolates V 27-03 and V 27-06, and HTLV-IIIb, respectively. Cell viability decreased dramatically at times of production of progeny virus, demonstrating the highly cytolytic effect of each variant on the CEM-CL10 cells. High levels of cell viability were attained at 40 days after viral infection, by which time outgrowths of apparently HIV-1-resistant cells had become predominant in the cultures.

After 40 days, we were unable to detect significant levels of p17/p24 antigen expression. We next tried, without success, to reinfect these cells with the same variant of HIV-1 to which they had initially been exposed. To try to explain the resistance of these cells to reinfection, we determined and compared the cell surface markers of mock-infected and HIV-1-infected CEM-CL10 cells after 60 days (Table II). Other investigators have previously reported that HIV-1 infection of susceptible cells leads to a loss in expression of cell surface proteins [McDougal et al., 1985; Folks et al., 1986; Hoxie et al., 1985]. The data of Table II show that cell surface expression of Leu-2a, Leu-4, and of transferrin receptor remained unchanged, whereas a marked decrease in levels of the Leu-3a (CD4 antigen) marker was observed in HIV-1-infected CEM-CL10 cells. Whereas 94% of mock-infected CEM-CL10 cells were positive for the Leu-3a marker, the number of positive cells at 60 days after infection by any of V 27-03, V 27-06, or HTLV-IIIb had decreased to 1%, 4%, and 2%, respectively (Fig. 2).

The status of the HIV-1 genome in these CEM-CL10 cells was ascertained by Southern blot on DNA samples obtained at each of 20 and 60 days after infection, at which times viral replication and antigen expression were present and absent, respectively (Table I). A characteristic pattern of HIV-1 DNA fragments was observed in CEM-CL10 cells at 20 days after infection with either clinical isolate V 27-03 or HTLV-IIIb (Fig. 3A). Characteristic proviral DNA was also seen in cells infected with clinical isolate V 27-06 (data not shown). By 60 days after infection, no viral nucleic acid was detected, indicating that viral infection was apparently not present in these cells.

In order to relate this work to studies of other investigators, we next hybridized with a probe to detect the CD4 gene (Fig. 3B). Restriction enzyme digestion revealed a variant CD4 band pattern in DNA samples from the cells that had survived exposure to HIV-1 and which were now HIV-1-resistant. This variant CD4 pattern was nearly identical in both CEM-CL10 cells that had survived infection by either the HTLV-IIIb strain of HIV-1 or clinical isolate V 27-03. This indicates that in both instances the surviving cells were probably derived from a common progenitor.

We next carried out northern blot analysis of RNA from mock-infected CEM-CL10 cells to reveal a 3.0 kb mRNA CD4 band. No such bands were observed in CEM-CL10 cells at 60 days after infection with either of clinical isolates V 27-03 and V 27-06 or the HTLV-IIIb strain of HIV-1 (Fig. 4).

DISCUSSION

The adherent T-lymphoblastoid cell line CEM-CL10 described in this work was derived from the well-known CEM cell line by fusion with peripheral blood mononuclear cells and repeated subcloning in soft
TABLE II. Cell Surface Markers of Mock-Infected and HIV-1-Infected CEM-CL10 Cells

<table>
<thead>
<tr>
<th>Viral strain</th>
<th>Leu-2a</th>
<th>Leu-3a</th>
<th>Leu-4</th>
<th>Leu-12</th>
<th>Leu-M3</th>
<th>Transferrin receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical isolate V 27-03</td>
<td>1</td>
<td></td>
<td>75</td>
<td>N.T.</td>
<td>N.T.</td>
<td>99</td>
</tr>
<tr>
<td>Clinical isolate V 27-06</td>
<td>1</td>
<td>4</td>
<td>72</td>
<td>N.T.</td>
<td>N.T.</td>
<td>99</td>
</tr>
<tr>
<td>HTLV-IIIb</td>
<td>1</td>
<td>2</td>
<td>74</td>
<td>N.T.</td>
<td>N.T.</td>
<td>94</td>
</tr>
<tr>
<td>Mock-infected</td>
<td>1</td>
<td>94</td>
<td>73</td>
<td>1</td>
<td>1</td>
<td>94</td>
</tr>
</tbody>
</table>

*Cell surface markers were determined 60 days after infection by HIV-1.

A. Flow cytofluorometry analysis of mock-infected or HIV-1-infected CEM-CL10 cells, after 60 days, stained with fluorescein-conjugated anti-Leu-3a. A: Mock-infected cultures, showing 94% CD4(+) cells. B–D: CEM-CL10 cells infected by either clinical isolates V 27-03 (B), V 27-06 (C), or by the HTLV-IIIb strain of HIV-1 (D) revealing greatly diminished numbers of CD4(+) cells.

These CEM-CL10 cells may ultimately be suitable for plaque assays of both laboratory and clinical strains of HIV-1, and may be advantageous for this purpose over other methods which require the use of poly-L-lysine [Harada et al., 1985].

This new cell line did not differ from parental CEM cells in expression of surface CD4 antigen [Dalgleish et al., 1984; Uppenkemp et al., 1988]; 95% of CEM-CL10 cells were positive for this marker. In contrast, CD3 and CD8 antigens were detected in about 10% and 24–53%
Fig. 4. Analysis of CD4 mRNA expression in mock-infected and HIV-1-infected CEM-CL10 cells, 60 days after viral inoculation, as determined by northern blot analysis. Labeled probes used in hybridization included pT4.B, specific for CD4 mRNA, and pRA H3-2, which contains mouse H3 histone coding sequences. A: Mock-infected cells displayed characteristic 3 kb CD4 mRNA, not seen in cells infected with either clinical isolate V 27-03 (B), clinical isolate V 27-06 (C), or the HTLV-IIIB strain of HIV-1 (D).

Fig. 3. Southern blot analysis of DNA (20 pg) of CEM-CL10 cells, 20 and 60 days after infection with either clinical isolate V 27-03 or the HTLV-IIIB strain of HIV-1. A: Use of probe pBH-10 to detect HIV-1 provirus. B: Use of probe pT4.B to detect CD4 sequences. Relative sizes are indicated in the left margin in kilobases (kb).

of parental CEM cells [Uppenkamp et al., 1988] as opposed to 73% and 0.8% of CEM-CL10 cells, respectively.

The major finding of this paper is that the CEM-CL10 line is highly susceptible to lytic infection by both laboratory and clinical isolates of HIV-1. In addition, we have shown that primary lytic infection by HIV-1 of CEM-CL10 cells yielded a subpopulation which could not be infected by this virus. The emergence of CEM subclones with different properties regarding HIV-1 replication and cytopathology have previously been reported. One such clone consisted almost entirely of cells susceptible to HIV-1 infection and to the lytic effect of this virus [Folks et al., 1985]. A second CD4(-) clone which survived primary infection by HIV-1 contained proviral DNA that could be induced by a halogenated pyrimidine, 5-iodo-2'-deoxyuridine, to yield progeny virus [Folks et al., 1986]. A third clone was susceptible to HIV-1 infection but resistant to viral cytopathic effects [Stevenson et al., 1987].

In our study, those CEM-CL10 cells, resistant to HIV-1 infection, were devoid of CD4 antigen expression, as determined by flow cytofluorometry. In other instances, such a decline was associated with either reductions in levels of CD4 mRNA [Hoxie et al., 1986] or with post-transcriptional changes in cellular localization of CD4 protein [Stevenson et al., 1987].

In the case of CEM-CL10 cells, we found that characteristic proviral DNA bands were detected at 20 days but not at 60 days after infection, indicating that a latent infection was not present in those cells which survived primary exposure to HIV-1. Further study revealed that the basis for disappearance of CD4 antigen in such cells was both an absence of relevant mRNA and changes in structure of the CD4 gene. Differences were observed in multiple fragments of this gene, on the basis of restriction digestion with a number of endonucleases, i.e., Eco RI, Hind III, Kpn I, and Xba I.

In summary, the HIV-1-resistant cells obtained following primary lytic infection showed a marked decline in CD4 protein expression due to an absence of specific mRNA. These quantitative differences in levels of mRNA were associated with rearrangements in the intrinsic structure of the CD4 gene. These changes might be explained by an outgrowth of cells which were ini-
tially of the CD4-negative phenotype. Infection by HIV-1 probably eliminated the CD4 (+) cell population, giving rise to the resistant receptor-negative cells described.

Thus, we conclude that the CEM-CL10 cells which survived infection by HIV-1 must have been derived from CD4 (-) cells which were present in these cultures, at least from the time of viral infection. The fact that identical variant restriction patterns of the CD4 gene were observed in each of two separate experiments suggests that the surviving cells, in each instance, were derived from a common progenitor. Given the fact that the CEM-CL10 line was only established after repeated subcloning, this suggests that a rearrangement of CD4 gene must have occurred in our cell line at some time over the past 2 years. The presence of nearly identical variant restriction patterns, on each of two occasions, further suggests that such rearrangements occur only rarely.

The CEM-CL10 cell line will be made available to other investigators upon request. In addition, the availability of a CD4(-) cell line, resistant to HIV-1, may be of significant utility for studies on CD4 gene rearrangement and interactions between HIV-1 gene products and segments of CD4 nucleic acid.

ACKNOWLEDGMENTS

Most aspects of this work were performed by Michel Tremblay in partial fulfillment of the degree of Doctor of Philosophy, Faculty of Graduate Studies and Research, McGill University. We thank James Vatistas for performing flow cytometry analysis. We are grateful to Ms. F. Busschaert for the preparation of the manuscript. This research was supported by grants to Mark A. Wainberg from the Medical Research Council of Canada and from Health and Welfare Canada.

REFERENCES


