Characterization of 17 Human Immunodeficiency Virus-1 Carrier Cell Lines with T cell, Myelomonocyte, or Megakaryocyte Lineages (43535)

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Abstract. **Of 29 hematopoietic cell lines tested for susceptibility to human immunodeficiency virus (HIV)-$1_{HTLV\text{-}IIIB}$ infection, all $CD4^+$ cell lines became infected. Continuous culturing of infected cell lines resulted in nine HIV-1 carrier cell lines, including, for the first time, an HIV-1 carrier megakaryoblastic cell line, MEG-01/HIV. The immunophenotypic profiles of a total of 17 HIV-1 carrier cell lines (nine newly and eight previously established cell lines) were compared with their respective parental noninfected cell lines. Except for total absence of CD4 expression, the expression of other antigens was variable among the 17 HIV-1 carrier cell lines. Persistent and consistent replication of infectious HIV-1 was detected in all of them in varying quantities. The great variability observed in both the altered marker expression, with respect to that of the noninfected parental cell lines, and in the quantities of persistently produced infectious HIV-1 was, nevertheless, specific to the individual cell lines. Furthermore, the present study demonstrates that there is no apparent correlation in the quantity of HIV-1 produced to either T cell, myelomonocytic cell, or megakaryocytic cell types. Instead, the results suggest that a particular interaction between HIV-1 and individual clonal cell lines may provide insight into the extremely complex immune dysregulation associated with the pathogenesis of acquired immune deficiency syndrome. Thus, the 17 HIV-1 carrier cell lines of diverse origin presented here provide valuable and unique models for further understanding acquired immune deficiency syndrome pathogenesis at the cellular and molecular levels.** [P.S.E.B.M. 1993, Vol 202]

Human immunodeficiency virus type-1 (HIV-1) is known as the causative agent for acquired immune deficiency syndrome (AIDS) (1, 2). Since the virus has a tropism to the CD4 human leukocyte differentiation antigen, a $CD4^+$ inducer/helper T cell subset has been recognized as a prime target cell type in AIDS (3, 4). CD4 is, however, not only a specific membrane marker for the inducer/helper T cell subset, but also a membrane marker for some subsets of monocytes, macrophages, B cells, and megakaryocytes (5). It is reported that HIV-1 infection occurs in B cells (6, 7), monocytes/macrophages (8–11), and megakaryocytes (12, 13), and that these non-T cell types are relatively resistant to HIV-1-induced cytopathic effects. Therefore, B cells, monocytes/macrophages, and megakaryocytes can be considered reservoirs responsible for HIV-1 dissemination in the body.

We studied the susceptibility of various human hematopoietic cell lines to HIV-1 infection and established nine new HIV-1 carrier cell lines, including, for the first time, an HIV-1 carrier human megakaryoblastic cell line, MEG-01/HIV. In specific, the immunophenotypic profiles between the virus carrier and control parental cell lines and the persistent production of infectious HIV-1 virus were studied.

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Materials and Methods

Cells. All human hematopoietic cell lines listed in Table I were cultured and maintained in RPMI-1640 medium (Nissui Pharmaceutical, Tokyo, Japan) supplemented with 5% heat-inactivated fetal bovine serum (Gibco Oriental, Tokyo, Japan) containing 100 units/ml of penicillin and 50 μg/ml of streptomycin at 37°C in a 5% CO_2 incubator. The 29 hematopoietic cell lines (14) used include 17 T cell lines (eight TcR-$\alpha\beta$, two TcR-$\gamma\delta$ [15], one TcR-$\beta\delta$ [16], and six TcR^--T cell lines), three B cell lines, one non-T non-B cell line, six myelomonocytic cell lines, one megakaryoblastoid cell line (17), and one erythroblastoid cell line. Eight of the HIV-1 (a strain of $HIV_{HTLV\text{-}IIIB}$) carrier cell lines established previously (2, 18–20) were cultured and maintained in the same medium under the same conditions. They were H9/HTLV-III, Molt-4/HTLV-III, Molt-4/LAV, Molt-4/ARV, Jurkat/HTLV-III, SKW-3/HTLV-III, CCRF-CEM/HTLV-III, and MT-2/HTLV-III.

Virus Preparation and Inoculation. One of the HIV-1 carrier cell lines, Molt-4/HTLV-III (20), was used in the logarithmic growth phase to prepare a strain of $HIV\text{-}1_{HTLV\text{-}IIIB}$ inoculum. Cell-free culture supernatant from the cell line was filtered through a 0.2-μm pore size filter (MILLEX-GV; Japan Millipore Ltd., Tokyo, Japan). After centrifugation, cell pellets of the test cell lines (1×10^7 cells) were inoculated with 1.0 ml of the HIV-1 preparation, giving multiplicity of infection of 0.05–0.01 tissue culture infectious dose 50% ($TCID_{50}$) for 1 hr at 37°C in a 5% CO_2 incubator. The inoculated cell suspension was then diluted to 10 ml (approximately 1×10^6 cells/ml) with RPMI-1640 medium containing 10% fetal bovine serum in a 25-cm^2 flask (Corning 25100FK 25N; Iwaki Glass Co., Tokyo, Japan). The cell cultures thus set up were fed with appropriate amounts of the same medium at desired intervals. Infection of the cells with HIV-1 was monitored by detecting either HIV-1 p24 antigen expression using cytoplasmic indirect immunofluorescence or infectious progeny virus using the $TCID_{50}$ assay as described below.

Establishment of Additional HIV-1 Carrier Cell Lines. The HIV-1-infected cell cultures were maintained at 37°C in a 5% CO_2 humidified atmosphere and the cell cultures were fed two to three times/week with fresh culture media until sustained and continuous growth was attained. The growth and viability of the cell cultures were determined at desirable intervals by trypan blue dye exclusion test.

Immunophenotypic Analysis Using a Panel of Antibodies. The indirect immunofluorescence analysis for cell surface and intracellular antigens was performed by flow cytometry (EPICS-PROFILE II; Coulter Electronics, Hialeah, FL) or fluorescent microscopy as described previously (21). Antigens for terminal deoxynucleotidyl transferase (TdT) were detected using rabbit anti-calf TdT serum (P-L Biochemicals, Milwaukee, WI) as a primary reagent and fluorescein isothiocyanate-conjugated goat anti-rabbit globulin (Cappel Laboratories, West Chester, PA) as a secondary reagent in methanol-fixed smear preparations. The panel of monoclonal antibodies used included the following: NU-T2(CD1b), NU-TER(CD2), NU-T3(CD3), NU-TH/I(CD4), NU-Tpan(CD5), NU-TS/C(CD8), NU-N1(CD10), SPV-L7(CD11a, LFA-1), MCS-2(CD13), KOLT-2(CD28), and NU-Ia(HLA-DR) (all from Nichirei, Tokyo, Japan); 3A1(CD7) and UCHL-1(CD45RO) (both from Sera-Lab, Crawley Down, Sussex, UK); MY-4(CD14), 4B4(CD29), MY-9(CD33), MY-10(CD34), and 2H4(CD45RA) (all from Coulter Immunology); OKT-10(CD38) (from Ortho Diagnostic Systems, Raritan, NJ); Leu-7(CD57) (from Becton Dickinson, Mountain View, CA); anti-Tac(CD25) (provided by T. Waldmann, NCI, Bethesda, MD); SV90-800(CD54, ICAM-1) and SV90-805(CD58, LFA-3) (provided by S. Ferrone, New York Medical College, Vallhala, NY); VIM-D5(CD15), VIPL-I(CD41), and VIPL-II(CD61) (from Dr. Walter Knapp, University of Vienna, Austria); B3/25(CD71) and A3/10 (HLA class I) (provided by I. Trowbridge, The Salk Institute, La Jolla, CA); GRP19 (anti-HTLV-1 p19 core protein) (provided by R. C. Gallo, NCI); VAK-5 (anti-HIV p24 core protein) (provided by K. Sagawa, Kurume University, Fukuoka, Japan) (22); TcR δ-1 (anti-TcR δ-chain) (provided by M. B. Brenner, Dana-Farber Cancer Institute, Boston, MA) (23); and WT-31 (anti-TCR $\alpha\beta$ heterodimer) (provided by W. Tax, St. Radboud Hospital, University of Nijmegen, The Netherlands) (24). Fluorescein isothiocyanate-conjugated goat anti-mouse IgG (Cappel Laboratories) was used as a secondary reagent. Except for the nuclear TdT and cytoplasmic HIV p24 antigens, which were both detected by intracellular staining, all other antigens were detected on living cell surface membranes.

HIV-1 Infectivity Assay Using the $TCID_{50}$ Method. Aliquots of infected cultures taken at the indicated times were separated into cell-free virus and cell-associated virus by centrifugation at 1500 rpm for 10 min at 4°C. Both cell-free and cell-associated virus samples were frozen at −80°C until used.

The infectious HIV-1 titer was determined using the method described by Pauwels *et al.* (25). In brief, 50 μl of cell suspension containing 1×10^4 MT-4 cells (19) were seeded into each well of a 96-well flat-bottomed plate (Corning 25860MP 96F; Iwaki Glass Co.). Fifty microliters each of serially diluted HIV-1 samples were then added to triplicate wells. After 5 days of incubation at 37°C in a 5% CO_2 humidified atmosphere, 20 μl of 7.5-mg 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide (Sigma Chemical Co., St. Louis, MO) solution were added to each well. The plate was further incubated at 37°C for 3 hr. Then the formazan crystals that formed were solubilized by

the addition of 5% (v/v) Triton X-100 in acidified (0.1 *N* HCl) isopropanol (Wako Pure Chemical Co., Osaka, Japan) with vigorous pipetting using a Titertek multichannel pipetter (Flow Laboratories, Helsinki, Finland). The plates were read on a Titerteck Multiskan MCC (Flow Laboratories) for the absorbance of the substrate in each well at 540 nm. The $TCID_{50}$ titer was defined as the reciprocal of the highest dilution of the sample that gives 50% or less absorbance of the noninfected control well.

Electron Microscopic Analysis. For transmission electron microscopy, cultured cells were centrifuged at low speed. The resulting pellets were prefixed for 1 hr with 1% glutaraldehyde in Sörensen's phosphate buffer, washed well, postfixed for 1 hr with 1% osmium tetroxide in the same buffer, dehydrated, and embedded in epoxy resin. Sections were stained with uranyl acetate and lead citrate and observed under a Hitachi H-700H electron microscope (Hitachi, Mito, Japan). For the purpose of observation by scanning electron microscopy, suspensions of cultured cells were mounted on poly-L-lysine-coated coverslips. After 15 min, the coverslips were prefixed for 1 hr with 2% glutaraldehyde in phosphate-buffered saline, rinsed in phosphate-buffered saline, postfixed for 1 hr with 1% osmium tetroxide in phosphate-buffered saline, dehydrated, and critical-point dried. Before observation with a Hitachi S-900 scanning electron microscope, the specimens were coated with platinum in an ion coater.

Results

Susceptibility to HIV-1 and Establishment of HIV-1 Carrier Cell Lines. Twenty-nine human leukemia/lymphoma cell lines, as listed in Table I, were tested for HIV-1 susceptibility. As expected, all cell lines expressing CD4 were susceptible to HIV-1 infection. In contrast, none of the $CD4^-$ cell lines were susceptible to HIV-1 infection. The typical sequence of events observed in the infected cultures was that the cell growth and viability of the cultures inoculated with HIV-1 decreased considerably during the first 2 weeks after infection, and then gradually a slow but steady increase of cell growth and viability was observed for the next 2 weeks in nine of the 19 infected $CD4^+$ cell lines. As judged by immunofluorescence and the infectivity assay for HIV-1, all nine HIV-1-infected lines eventually established themselves as virus carriers. They included the following T cell lines: two $CD3^+TcR\ \alpha/\beta^+$ (HPB-ALL/HIV and MKB-1/HIV), one $CD3^+TcR\ \beta\delta^+$ (DND-41/HIV), one $CD3^+TcR\gamma\delta^+$ (PEER/HIV), and one $CD3^-TcR^-$ (C91/PL/HIV); three myelomonocytic lines; HL-60/HIV, MOBS-1/HIV, and U-937/HIV; and one megakaryoblastic cell line MEG-01/HIV. Two of the five $CD3^-TcR^-$ lines (C91/PL/HIV and the previously established MT-2/HTLV-III) thus presented a dual-virus (HTLV-I and HIV-1) carrier state. In addition, a novel megakaryoblastic cell line, MEG-01, was susceptible to HIV-1 infection, and for the first time with this type of hematopoietic cell, this culture became an established HIV-1 carrier cell line (MEG-01/HIV) (Table III). A total of 17 HIV-1 carrier cell lines (the eight previously established HIV-1 carrier cell lines together with the nine newly established HIV-1 carrier cell lines) were compared with their parental noninfected cell lines for the following studies.

Table I. Human Hematopoietic Cell Lines Used

Cell line	Age	Sex	Clinical diagnosis[a]
T cell lines			
CCRF-CEM[b]	3	F	ALL
DND-41[b]	13	M	ALL
HPB-ALL[b]	14	M	ALL
JM[b]	14	M	ALL
TALL-1[b]	28	M	ALL
Molt-4[b]	19	M	ALL
Molt-14	2	F	ALL
Molt-16	5	F	ALL
MKB-1[b]	17	F	AML
Jurkat[b]	14	M	ALL
PEER[b]	4	F	ALL
SKW-3[b]	51	F	CLL
H9[b]	53	M	SS
HUT-102[b]	26	M	MF
C5/MJ[b]	50	M	NT
MT-2[b]	0		NT
C91/PL[b]	29	M	NT
B cell lines			
NALM-6	18	M	ALL
U-698-M	7	M	LS
BALL-1	75	M	ALL
Non-T non-B lymphoid			
KM-3	12	M	ALL
Myelomonocytoid			
KG-1	59	M	AML
HL-60[b]	36	F	APL
THP-1	1	M	AMOL
KCL-22	28	F	CML
U937[b]	27	M	LY
MOBS-1[b]	57	M	AMOL
Megakaryoblastoid			
MEG-01[b]	55	M	CML
Erythroblastoid			
SPI-801	9	F	ALL

[a] Diagnosis abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CLL, chronic lymphocytic leukemia; SS, Sézary syndrome; MF, mycosis fungoides; NT, normal HTLV-I-transformed T cell; LS, lymphosarcoma; APL, acute promyelocytic leukemia; AMOL, acute monoblastic leukemia; CML, chronic myelogenous leukemia; LY, lymphoma.
[b] $CD4^+$ cell line.

Antigen Expression. All HIV-1 carrier lines were found to be positive for cytoplasmic expression of the p24 HIV-1 antigen at 60–100% at any given time as detected by the VAK-5 monoclonal antibody. The expression of other cell surface antigens is summarized in Tables II and III. Expression of the CD4 antigen was uniformly negative in all HIV-1 carrier cell lines. Interestingly, when three T cell lines (HPB-ALL, Molt-4,

Table II. Marker Profiles of Parental and HIV-1 Carrier Cell Lines (Percent Positive as Determined by Fluorescent Microscopy)

Cell line	Stage[a]	Age	Sex	Diagnosis[b]	TdT	HLA-DR	CD1b	CD2	CD3	Cy[c]	TCR$\alpha\beta$	TCRδ	CD4	CD8	CD10	CD13	CD28	CD29	CD45RA	CD45RO	CD71	P19[d]	P24[e]
CCRF-CEM	T-II	3	F	ALL	100	0	90	0	100	(+)	100	0	100	0	100	0	100	100	20	90	100	0	0
CCRF-CEM/HTLV-III					0	0	0	0	0	(+)	0	0	0	0	10	0	100	80	0	90	100	0	100
HPB-ALL	T-II	14	M	ALL	100	0	100	100	100	(+)	100	0	100	100	100	0	100	50	10	0	100	0	0
HPB-ALL/HIV					100	0	100	100	100	(+)	100	0	0	100	100	0	100	90	50	0	100	0	100
DND-41	T-II	13	M	ALL	100	0	100	100	100	(+)	0	100	100	0	100	0	80	100	0	0	100	0	0
DND-41/HIV					100	0	100	100	100	(+)	0	100	0	0	100	0	50	100	0	30	100	0	100
Molt-4	T-III	14	M	ALL	100	0	100	100	0	(+)	0	0	100	100	0	0	90	90	90	80	100	0	0
Molt-4/LAV					0	0	100	100	0	(+)	0	0	0	100	0	0	0	90	90		50	0	100
Molt-4/ARV					0	0	100	100	0	(+)	0	0	0	100	0	0	0	90	90		50	0	100
Molt-4/HTLV-III					0	0	100	100	0	(+)	0	0	0	100	0	0	0	90	90	90	90	0	100
Jurkat	T-III	14	M	ALL	60	0	100	100	100	(+)	100	0	100	0	0	0	100	90	90	50	100	0	0
Jurkat/HTLV-III					20	0	100	100	100	(+)	100	0	0	0	0	0	70	90	50	90	100	0	100
MKB-1	T-III	17	F	AML	50	0	0	0	50	(+)	50	0	100	0	100	0	0	90	0	10	100	0	0
MKB-1/HIV					50	0	0	0	80	(+)	80	0	0	0	100	0	0	80	0	90	100	0	100
SKW-3	T-IV	51	F	CLL	0	0	100	100	0	(+)	0	0	100	100	0	0	100	100	0	90	100	0	0
SKW-3/HTLV-III					0	0	100	100	0	(+)	0	0	0	100	0	0	70	80	0	90	100	0	60
PEER	T-IV	4	F	ALL	0	0	0	0	100	(+)	0	100	100	0	0	0	100	100	0	100	100	0	0
PEER/HIV					0	0	0	0	100	(+)	0	100	0	0	0	0	100	80	90	90	100	0	60
H9	T-V	53	M	SS	0	100	0	0	100	(+)	100	0	100	0	0	0	0	100	80	80	100	0	0
H9/HTLV-III					0	100	0	0	100	(+)	100	0	0	0	0	0	0	50	80	30	100	0	80
MT-2	T-V	0		NT	0	100	0	0	0	(+)	0	0	100	0	0	0	0	100	0	0	100	100	0
MT-2/HTLV-III					0	100	0	0	0	(+)	0	0	0	0	0	0	0	100	0	0	100	100	100
C91/PL	T-V	29	M	NT	0	100	0	0	0	(−)	0	0	100	0	0	0	0	90	0	100	100	100	0
C91/PL/HIV					0	100	0	0	0	nt	0	0	0	0	0	0	0	90	0	0	100	100	100

[a] Stage of differentiation referred to in Ref. 14. T-II, T-blast II; T-III, T-blast III; T-IV, T-blast IV; T-V, T-blast V.
[b] Diagnosis abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloblastic leukemia; CLL, chronic lymphocytic leukemia; SS, Sézary syndrome; NT, normal HTLV-I transformed T cell.
[c] Cytoplasmic expression; nt, not tested.
[d] P19 HTLV-I antigen.
[e] P24 HIV-1 cytoplasmic antigen.

Table III. Marker Profiles of Parental and HIV-1 Carrier Cell Lines (Percent Positive as Determined by Fluorescent Microscopy)

Cell line	Stage[a]	Age	Sex	Diagnosis[b]	TdT	HLA-DR	CD3	CD4	CD8	CD13	CD11a	CD15	CD33	CD34	CD41	CD45RA	CD45RO	CD61	CD71	P19[c]	P24[d]
HL-60	Pr my	36	F	APL	0	0	0	100	0	100	80	100	100	0	0	20	nt	0	100	0	0
HL-60/HIV					0	0	0	0	0	100	90	100	100	0	0	0	0	0	100	0	100
U-937	Mobl	37	M	LY	0	0	0	100	0	100	100	100	100	0	0	100	nt	0	100	0	0
U-937/HIV					0	0	0	0	0	100	100	100	100	0	0	100	90	0	100	0	100
MOBS-1	Mobl	55	M	AML	0	0	0	100	0	100	100	100	100	70	0	100	nt	0	100	0	0
MOBS-1/HIV					0	0	0	0	0	100	100	50	100	80	0	100	90	0	100	0	100
MEG-01	Megbl	55	M	CML	0	30	0	50	0	100	50	50	100	80	80	0	nt	80	100	0	0
MEG-01/HIV					0	0	0	0	0	100	0	50	100	70	100	0	20	100	100	0	100

[a] Stage of differentiation referred to in Ref. 12. Pr my, promyelocyte; Mobl, myeloblast; Megbl, megakaryoblast.
[b] Diagnosis abbreviations: APL, acute promyelocytic leukemia; LY, lymphoma; AML, acute myeloblastic leukemia; CML, chronic myelogenous leukemia.
[c] P19 HTLV-I antigen.
[d] P24 HIV-1 cytoplasmic antigen.

and SKW-3) with double positive ($CD4^+$, $CD8^+$) phenotypes became HIV-1 carrier cell lines, they were no longer positive for CD4; instead, they exhibited an enhanced expression of CD8. For the expression of the other antigens tested, the first group (CD3, CD38, CD71, TCR, and HLA class I) showed a higher expression in the HIV-1 carrier cell lines than in the parental noninfected cell lines. The second group of antigens (CD1b, CD2, CD5, CD10, and CD58) showed a lower expression in the HIV-1 carrier cell lines. The third group of antigens (CD7, CD11a, CD28, CD29, CD45RA, CD45RO, CD54, and CD57) showed no change in expression in either the infected or the noninfected cell lines. Although the observed difference in the expression of individual antigens within the same set of HIV-1 carrier and respective parental noninfected cell lines was consistent and reproducible, the differences observed among the 17 sets of cell lines were by no means uniform with respect to the individual antigens involved (Tables II and III). As an example, the results of flow cytometric analysis of the relevant antigens of the HPB-ALL and HPB-ALL/HIV cell lines can be seen in Figure 1.

Virus Production. Persistent replication of infectious HIV-1 in all HIV-1 carrier cell lines was detected by $TCID_{50}$ assay. As shown in Figure 2, there was a wide range in the quantity of infectious HIV-1 production among the 17 HIV-1 carrier cell lines. Particularly, MT-2/HTLV-III and C91/PL/HIV lines produced 10 to 100 times more infectious HIV-1 as compared with some of the other cell lines. Both of them were, incidentally, derived from *in vitro*-immortalized, normal, mature T lymphocytes with HTLV-I infection. Thus, after HIV-1 infection, both MT-2/HTLV-III and C91/PL/HIV represent a dual virus-carrier state with HTLV-I and HIV-1. At the same time, two of the three HIV-1 carrier myelomonocytic cell lines, HL-60 /HIV and U-937/HIV, and two of the 13 HIV-1 carrier T cell lines, CCRF-CEM/HTLV-III and HPB-ALL/HIV, produced three to 10 times less infectious HIV-1 than the average of the other HIV-1 carrier cell lines. The HIV-1 carrier megakaryoblastic cell line, MEG-01/HIV, produced an average of 250 $TCID_{50}$ infectious HIV-1, which suggests that the megakaryocyte can be as good a virus producer as some T cells. Similarly, the present study demonstrated that as far as the strain of HIV-1 is concerned, there are no preferential cell types for the amount of infectious HIV-1 produced by T cells, myelomonocytic cells, or megakaryocytes.

Electron Microscopic Observation. Two HIV-1 carrier cell lines, Molt-4/HTLV-III and U937/HIV, were examined under an electron microscope. Scanning electron microscopic examinations showed numerous spherical particles on the surfaces of and occasionally in association with filopodias extended from the cells of both cell lines. Transmission electron microscopic surveys, however, showed different ultrastructural fea-

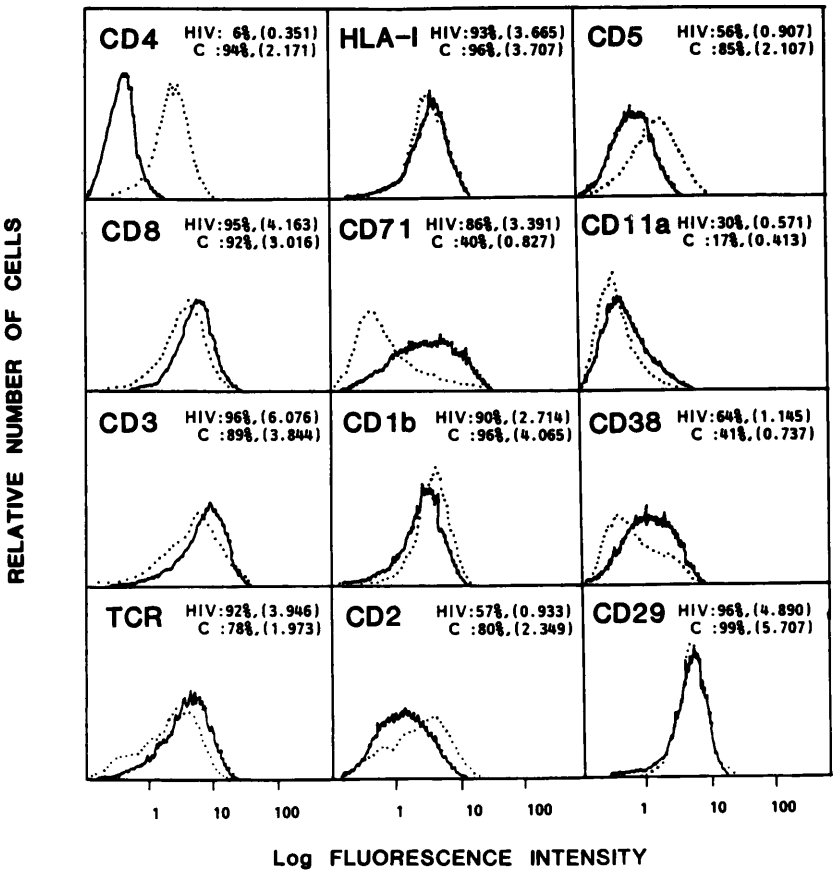

Figure 1. Comparative flow cytometric analysis of 12 relevant antigens of HPB-ALL (dotted line) and HPB-ALL/HIV (solid line). Inserts: HIV, HPB-ALL/HIV cell line cells, percentage positive values. The values in parentheses are the mean fluorescence intensity. C, control noninfected parental HPB-ALL cells. Log fluorescence intensity of the horizontal axis is an arbitrary unit. The results shown here are representative of three similar experiments.

tures of extracellular viral particles between the two cell lines; i.e., numerous mature particles with cores sectioned at various orientations were observed in Molt-4/HTLV-III cells, whereas many doughnut-shaped immature particles were found in U937/HIV cells. Although the extent of the electron microscopic observation was indeed limited, the observation was in agreement with previous reports (2, 9). These transmission electron microscopic observations are compatible with the results of the infectivity assays of the viral samples recovered from both cell lines (Fig. 2).

Discussion

A total of 29 human hematopoietic cell lines of various lineage types were studied for susceptibility to Molt-4/HTLV-III single-isolate HIV-1 infection. As expected, all $CD4^+$ cell lines, including T cells, myelomonocytic cells, and megakaryoblastic cells, were infected with HIV-$1_{HTLV\text{-}IIIB}$, even though this virus isolate is known to possess a very limited ability to infect cells of monocyte lineage (9). None of the $CD4^-$ cell lines tested was susceptible to HIV-1 infection. However, the present finding does not exclude the possibility that some cell lines might be susceptible to HIV-1 rather latently (26). Continuous culturing of the infected cells resulted in the establishment of nine HIV-1 carrier cell lines. Together with the eight HIV-1 carrier cell lines established previously (2, 18–20), a total of 17 HIV-1 carrier cell lines were further characterized. As shown in Tables II and III and Figure 1, the complete and uniform absence of CD4 expression was noted in all HIV-1 carrier cell lines. According to previous reports, in early HIV infection of either interleukin-2-dependent $CD4^+$ T cell clones or freshly isolated peripheral blood $CD4^+$ cells, their membrane phenotype, including CD4 and CD3/Ti complexes, is not significantly affected due, in part, to a 10- to 50-fold lower multiplicity of HIV-1 infection than that in the present study. However, as observed in this study, their CD4 expression was eventually down-modulated as the infection subsequently progressed (27, 28).

In addition, persistent HIV-1 infection caused variable changes in the expression of other antigens as compared with the antigen profiles of the respective parental cell lines. These changes in antigen expression, except that of CD4, appear to vary according to the HIV-1 carrier state of the individual cell lines. In view of the monoclonal nature of the individual cell lines (14) and the extremely complex immunodysfunction associated with AIDS (29, 30), the present observation

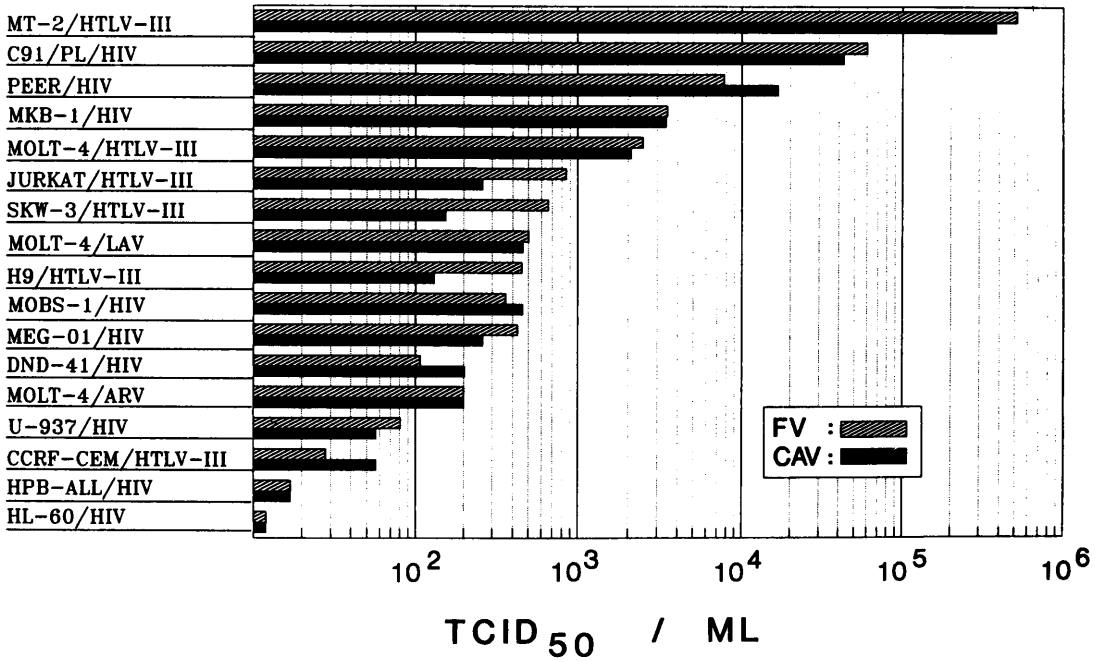

Figure 2. Quantity of infectious HIV-1 virus produced from continuous culture of 17 HIV-1 carrier cell lines. FV is the cell-free virus titer released from the cells into culture medium. CAV is the cell-associated virus titer extracted from $2.0 \pm 0.1 \times 10^6$ cells by freeze-thawing five times and reconstituting it in 1.0 ml of fresh nutrient medium. The titers shown are the mean from three to five independent samplings and measurements.

may provide significant insight into the interaction between the virus and the particular host cell clones. For example, three "double positive" cell lines (HPB-ALL, Molt-4, and SKW-3), with respect to CD4 and CD8 expression, resulted in the establishment of three "single" $CD8^+$ HIV-1 carrier cell lines with persistent shedding of infectious HIV-1 (Figs. 1 and 2). The significance of CD8 T cells in AIDS is, however, conflicting. While in one report CD8 T cells play a suppressive role against the progression of AIDS pathogenesis (31), in another report it was reported that they may facilitate spreading of and latency in HIV-1 infection *in vivo* in the presence of activated CD4 cells (32).

Expression of CD8 is thought to be a marker of suppressor/cytotoxic T cells. However, the results of some T cell lines used in this study at immature differentiation stages (14) suggested that the occurrence of HIV-1 infection in such immature T cells is itself significant. Even more significant is the fact that the infected cells are CD8 single positive and are harboring infectious virus. The present study also demonstrated susceptibility to and establishment of the HIV-1 carrier state in the T cells of the TcR$\alpha\beta$ type as well as the TcR$\gamma\delta$ or TcR$\beta\delta$ types and in megakaryoblasts. The results suggest that all intrathymic and extrathymic immature T cells, and immature megakaryoblasts in the bone marrow may also participate in HIV pathogenesis. This finding may account, in part, for the selective loss of CD4 T cells, for the transient increase of CD8 cells, characteristic of the early phase of HIV-1 infection (29), and for the inverse CD4 to CD8 ratio in the peripheral blood from the very early asymptomatic period of the disease (33, 34), one of the hallmarks of AIDS patients.

In addition, the present finding may account for the severe skin lesions and progressive thrombocytopenia commonly associated with AIDS (13, 29, 30, 33). It is, in fact, reported that HIV-1 infection of megakaryocytes (12, 13), myeloid progenitors (35), or bone marrow stromal fibroblasts (36) might lead to severe dysregulation in hematopoiesis. Furthermore, HIV-1 infection of HTLV-I-bearing cells that had been transformed *in vitro* by HTLV-I infection led to very high levels of HIV-1 production. It can be explained that some HTLV-I gene products, such as *tax*, enhance long terminal repeat of not only HTLV-I but also of HIV-1 (37, 38).

We reported here various types of HIV-1 carrier human hematopoietic cell lines that are useful and valuable to study in order to further our understanding of the mechanism of HIV-1 infection at the cellular and molecular levels.

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