Infection of human fibroblasts and osteoblast-like cells with HIV-1

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Primary human skin- and lung-derived fibroblast cell cultures and continuous human osteoblast-like and fibroblast-like cell lines were infected with different strains of HIV-1. Infection was measured at the single-cell level using the immunoperoxidase staining method to detect viral proteins. No cytopathic effects were observed in HIV-1-infected cell cultures. One continuous cell line (LC5), derived from embryonic lung, was readily infectable with HIV-1 and showed continuous production of infectious virus. Infection of LC5 cells could be blocked with anti-CD4 monoclonal antibodies. These findings indicate that fibroblasts of skin and lung, and osteogenic cells may be considered as potential target cells for HIV-1, thereby possibly contributing to the establishment of local HIV reservoirs.

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Introduction

Cells expressing the CD4 receptor are considered to be the primary target for HIV. HIV has been shown preferentially to infect CD4+ T cells, a cell population which supports virus replication *in vivo* and *in vitro* and is considered to be a major source of replicating virus [1,2]. Cells of the monocytic/macrophage lineage also express CD4; they have been shown to be infectable *in vitro* and to harbour the virus *in vivo*. The primary virus reservoir in the infected individual is considered to be macrophages and monocytic cells [3,4]. Stimulated by clinical reports of disease apart from immune suppression for HIV-1-infected individuals, we performed *in vitro* studies to define other cell types besides CD4+ T cells susceptible to HIV. One such cell type is the glial cell [5–8], which seems to be infected in a CD4-independent manner [9]. However, confirmation that glial cells are actually infected by HIV-1 in AIDS patients is still awaited. Colorectal carcinoma cell lines are further targets for HIV *in vitro* [10] and fibroblastoid cells have been shown to be susceptible to HIV infection [9,11,12] .

In the present paper we describe the susceptibility of various primary and continuous fibroblast and osteogenic cell lines to different strains of HIV-1. The data show productive infection of human skin, lung and bone-derived cells with HIV-1. None of the infected cells exhibited overt cytopathogenic effects, indicating that mesenchymal cells in the lung, skin and skeleton may serve as reservoirs for HIV-1.

Materials and methods

Primary human cell cultures

Skin biopsies were obtained from patients at the Medizinische Poliklinik, University of Munich. The specimens were freed from adherent fatty tissue, washed with phosphate-buffered saline (PBS) and minced with scissors. Tissue fragments approximately 1 mm in diameter were placed into 35 mm Petri dishes, covered with glass coverslips and incubated with Dulbecco's Modified Eagle's Medium (DMEM) containing 15% heat-inactivated fetal calf serum (FCS), 100 IE/ml penicillin and 100 µg/ml streptomycin in a humidified incubator at 37°C and 5% CO_2/95% air. After the outgrowth of fibroblast-like cells from the tissue fragments, the primary cultures (176-S3,

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178-H1, and 178-H4) were subcultured at 7-day intervals. 176-S3 cells were also immortalized by infection with SV40 virus (kindly provided by H. Fischer, Heidelberg, FRG). Four cell cultures, M5nHF, M6nHF, M7nHF, and M8nHF, were established from skin biopsies taken from HIV-positive individuals and were maintained in the same manner. Two lung fibroblast cell cultures, CCD25-LU and HEL229, were obtained from the American Type Culture Collection (ATCC, Rockville, Maryland, USA) at the 6th and 11th passage level.

Continuous human cell lines

The human osteogenic sarcoma cell lines TE-85 and SAOS-2, and the embryonic lung-derived cell line L132 were obtained from the ATCC and cultured in DMEM supplemented with 10% FCS. L132 cells were cloned three times by the end-point dilution technique. One clonal line exhibiting fibroblast-like morphology was named LC5 and used in these experiments. The cells were cultured in RPMI 1640 medium (Gibco, Karlsruhe, FRG) supplemented with 10% FCS.

Immunohistochemical characterization

Primary and continuous cell lines were characterized by expression of cell matrix and intermediate filament proteins detected by immunofluorescence as described [13]. The following primary and secondary antibodies were used: rabbit anti-fibronectin, rabbit anti-laminin (BRL, Cambridge, UK), rabbit anti-vimentin (Labsystems, Helsinki, Finland), rabbit anti-collagen type I (a gift of K. von der Mark, University of Erlangen, FRG), mouse anti-cytokeratin (Labsystems), and fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit immunoglobulin (Ig) G and goat anti-mouse IgG (Nordic, Tilbury, The Netherlands).

Cell morphology and DNA distribution

To determine the morphology of the target cells subconfluent cultures were fixed with methanol/acetone (1 : 1, −20°C, 5 min) and photographed under differential interference (DIC) and phase contrast (PC). The diploid DNA content of the primary cell cultures and the aneuploid DNA content of the continuous cell lines were determined by performing DNA histograms as described [14]. Fresh human peripheral lymphocytes were used as diploid control cells.

Virus infection

For virus production and infectivity studies based on cocultivation techniques we used the KE37-1 T-lymphoma cell line chronically infected with HIV-1 isolates HTLV-III_B, HTLV-III_{RF} and HTLV-III_{Rutz}, and uninfected KE37-1 control cells (kindly provided by M. Popovic, National Institutes of Health, Bethesda, Maryland, USA). Virus activity in the supernatant is indicated as infectious dose (ID)/ml (determined with KE37-1 cells) or reverse transcriptase (RT) values (c.p.m./ml); for example, supernatant of KE37-1 cells infected with HTLV-III_B showed an RT value of 5–10 × 10^5 c.p.m./ml and an infectious dose of 10^4 units/ml. The supernatant was either used immediately or concentrated by polyethylene glycol (PEG) precipitation. Briefly, 100 ml supernatant harvested 4 days after the last change of medium was added to 50 ml PEG (30%)/NaCl (2%) solution, incubated for 1 h at 4°C and pelleted (700 **g**). The pellet was suspended in 1 ml PBS and the virus suspension was used for infection. Primary and continuous cells were infected with HIV-1 by different procedures.

(1) Incubation of subconfluent cell cultures with cell-free supernatant from HIV-1-infected KE37-1 cells: 5 × 10^4 cells/well were seeded in six-well plates and were incubated on the following day with 2 ml HIV-1-containing supernatant (ID: 10^4 units/ml) for 2 h at 37°C. The supernatant was removed, cells were washed twice with PBS and 3 ml RPMI/10% FCS was added. For infection of primary cells this procedure was repeated twice. Three to 10 days later viral anitgen was detected by immunoperoxidase staining.

(2) Treatment of trypsinized target cells with 100 µl concentrated cell-free supernatant from HIV-1-producing lymphoma cells (KE37-1): the target cells were trypsinized, washed twice with PBS and centrifuged (800 r.p.m. for 5 min). The cell pellet (5 × 10^4 cells) was suspended in 100 µl concentrated supernatant from infected cells, incubated for 60 min and seeded into six-well plates for further cultivation (5 × 10^4 cells/2 ml RPMI per well.

(3) Cocultivation of target cells with HIV-1-producing lymphoma cells: 5 × 10^4 cells were seeded into the cavities of six-well plates and cultures in RPMI. After 24 h the supernatant was removed and 10^5 HIV-infected KE37-1 cells/cavity were added. Cells were cocultivated for 48 h at 37°C. Thereafter the lymphoma cells were then removed by extensive washing with PBS, and the cells were cultured further for 3–7 days in RPMI/10% FCS before immunoperoxidase staining. Permanent cell lines were subcultured on day 4 after HIV infection by cocultivation.

Indirect immunoperoxidase staining

Indirect immunoperoxidase staining (IPS) of the target cells for detection of HIV-1 antigens was carried out as described, using a human anti-HIV-1-positive serum [15]. Briefly, cells were fixed with methanol/acetone (1 : 1, −20°C, 5 min) and dried at room temperature. Human anti-HIV-1-positive serum (diluted 1 : 150 in PBS) was added and incubated for 1 h at room temperature. After washing five times with PBS, a second antibody (horseradish peroxidase-conjugated rabbit anti-human IgG, diluted 1 : 150 with PBS/5% FCS) was added and incubated for 1 h at room temperature. Cells were washed again five times with PBS and peroxidase-substrate (3-amino-9-ethylcarbazole/H_2O_2) was added and incubated for 10 min. The number of IPS-positive cells was evaluated using an inverted light microscope. Non-infected and HIV-1-infected cells incubated with normal human serum or PBS served as negative controls. Endogenous peroxidase activity was not detectable in the cell lines used here.

Reverse transcriptase assay and p24 antigen enzyme-linked immunosorbent assay

RT activity of supernatants from HIV-1-infected KE37-1 and LC5 cells cultures was measured as described [16]. HIV p24 antigen was assayed in the supernatant using the Abbott antigen capture assay (Abbott Laboratories, North Chicago, Illinois, USA).

To compare the sensivity of the different antigen assays, LC5 cells (10^5 cells/cavity of six-well plates, seeded on the previous day) were infected with serial twofold dilutions of HIV-containing supernatant (RT activity: 10^6 c.p.m./ml), washed three times with PBS and cultured further. Five days later RT activity and p24 antigen levels were determined in the supernatant, and the number of HIV-1 antigen-positive colonies was determined by immunoperoxidase staining.

Virus recovery

Subconfluent HIV-1-infected LC5 and 178-H1 cells were cocultivated with KE37-1 lymphoma cells for 4 days. The lymphoma cells were then harvested, washed twice with PBS, cultivated further, and checked weekly for infection by imunoperoxidase staining.

Western blot analysis

Approximately 10^7 HIV-1-infected cells were pelleted, lysed with low salt extraction buffer and used for Western blotting [17]. The presence of HIV-1-specific proteins in the blots was examined using an HIV-1-positive reference serum.

Inhibition of HIV-1 infection

To investigate the requirement of CD4 receptor for virus infection, inhibition experiments were performed using an anti-CD4 monoclonal antibody (MT310). The cells (2×10^3) were treated with 50 µl MT310 for 60 min as described [18]. The antibody concentrations ranged from 0.5 to 50 µg/ml. Following antibody treatment, the target cells were infected with 50 µl cell-free supernatant from HIV-1-producing cells exhibiting RT activity of 5×10^5 c.p.m./ml, incubated for 60 min, washed with PBS, and cultured further in 96-well cluster plates (Nunc, Roskilde, Denmark). Five days after infection, the cells were fixed and examined for virus infection by IPS using an HIV-1-positive human reference serum as described above.

CD4 messenger RNA analysis

Total RNA was isolated from cells by homogenization in 6 mol/l guanidinium isothiocyanate followed by ultracentrifugation through a 5.7 mol/l caesium chloride cushion. RNA was denatured by treatment with glyoxal and dimethylsulphoxide (DMSO) and electrophoresed through a 1.2% agarose gel [19]. Transfer to Zeta-probe-membrane (Biorad, Richmond, California, USA) and Northern blot hybridization was performed according to the manufacturer's instructions. The CD4-specific hybridization probe was obtained by isolating the 3 kb CD4-complementary DNA (cDNA) insert of plasmid T4-pMV7 [20], and labelling by nick translation in the presence of ^{32}P-labelled cytidine triphosphate (CTP) to a specific activity of 3×10^8 c.p.m./µg DNA.

Results

Characterization of primary and continuous cells

For this study we selected three different primary skin fibroblast cell cultures at passage 3 to 4 and two lung fibroblast cell cultures as well as continuous cell lines of osteogenic and embryonic lung origin. The properties of these cell cultures are summarized in Table 1.

Cells of the primary skin and lung cell cultures exhibited characteristic fibroblast morphology. At subconfluency, the cells were elongated and showed distinct cytoplasmic extensions; after confluency, both skin and lung fibroblast cultures consisted of long, bipolar, spindle-like cells. Immunohistochemical analysis revealed the expression of laminin and fibronectin in all five skin and lung primary cultures whereas collagen type I was detected

Table 1. Characterization of primary and continuous human mesenchymal cells.

Cells	DNA distribution	Vimentin	Laminin	Fibronectin	Collagen type I	Cytokeratin
Primary skin cell cultures						
176-S3*	Diploid	−	+	+	+ +	−
178-H1	Diploid	+ +	+	+ +	+	−
178-H4	Diploid	+	+	+ +	+ +	−
Primary lung cell cultures						
CCD25-LU	Diploid	+	+	+ +	−	−
HEL 299	Diploid	+	+	+ +	−	−
Osteosarcoma cell lines						
SAOS-2	Aneuploid	ND	+ +	−	−	ND
TE-85	Aneuploid	ND	+ +	+	+	ND
Embryonic lung cell line						
LC5*	Aneuploid	+	+	+	−	+

−, No antigen detected; +, low amounts of antigen detected, + +, strong fluorescence indicative of high amounts of antigen; ND, not investigated. *The immunohistochemical data of the primary skin cell culture 176-S3 and continuous LC5 cells were confirmed by Western blot analysis (data not shown here).

in skin cell cultures only. Vimentin was found to be expressed in 178-H1 and 178-H4 cells. Lung fibroblast cell cultures showed laminin, vimentin and fibronectin expression. The DNA distribution of all five primary cultures revealed the presence of distinct G0/G1 stem line peaks at 2c, indicating the characteristic diploid DNA content of primary cells in culture (Fig. 1).

Cells of the continuous osteosarcoma cell lines TE-85 and SAOS-2 showed a more epithelioid morphology, particularly in the confluent state. The cells exhibited high alkaline phosphatase activity (data not shown), indicative of osteogenic cells, and high laminin expression. TE-85 cells also expressed fibronectin and collagen type I. LC5 cells exhibited elongated, fibroblast-like morphology with multipolar cytoplasmic extensions. The cells showed laminin, vimentin, fibronectin, and low cytokeratin expression (Table 1). The DNA histograms of the three cell lines showed aneuploidy with a maximum DNA content between 3c and 3.8c (Fig. 1).

Infection with HIV-1

Infection of non-lymphoid cell types may be a rare event; hence we decided to use the IPS method to detect single HIV-infected cells. To determine the sensitivity of this method, LC5 cells were infected with serial twofold dilutions of HIV-1 and the detection of HIV-infected cells by IPS was compared with measurements of RT activity and determinations of HIV p24 antigen levels in the supernatant. As shown in Fig. 2, RT activity and p24 antigen levels in the supernatants of the cell lines infected with virus dilutions between 1 : 64 and 1 : 128 could not be distinguished from background levels. Nevertheless, single infected cell colonies were readily observed in LC5 cells infected with virus dilutions of 1 : 128, indicating that IPS is as sensitive as p24 antigen determinations, and even superior to RT activity analysis in the detection of low rates of HIV infection.

Primary human skin and lung fibroblasts were infected with HIV-1 strains HTLV-III_B and HTLV-III_{RF} either by cocultivation with HIV-1-infected lymphoma cells, or by treatment of the target cells with virus (cell-free supernatant). The RT activity in the virus-containing supernatants used for infection was 5–10 × 10^5 c.p.m./ml. The results of these experiments are summarized in Table 2. Ten days after infection with cell-free HTLV-III_B suspension up to 1% virus-positive cells were found in 176-S3 (Fig. 1a), 178-H1 and 178-H4 skin fibroblast cell cultures. Immortalization of 176-S3 skin fibroblasts by infection with SV40 enhanced the susceptibility to cell-free HTLV-III_B considerably. After infection of 178-H1 cells or SV40-transformed and non-transformed 176-S3 cells by 2-day cocultivation with HTLV-III_B-producing lymphoma cells, 0.5–1% of the cells were HIV-positive. HIV-1-infected cells were also detected in 176-S3 and 178-H4 cell cultures after cocultivation with HTLV-III_{RF}-producing lymphoma cells. Moreover, 178-H4 cell cultures were infected by treatment with cell-free HTLV-III_{RF}-containing supernatant.

Four skin fibroblast cell cultures established from biopsies taken from HIV-positive individuals were negative for HIV-antigen expression when tested by IPS. After cocultivation with HTLV-III_B and HTLV-III_{RF}-producing lymphoma cells, 0.1–0.5% of the cells were infected in these cell cultures.

Infection of the two lung fibroblast cell cultures with cell-free supernatant from HTLV-III_B-producing lymphoma cells resulted in the appearance of a few single HIV-antigen positive cells (Fig. 1c).

After treatment of the osteogenic sarcoma cell lines TE-85 and SAOS-2 with three different HIV-1 strains, virus-positive cells growing in distinct colonies were observed (Fig. 1e) resulting in up to 1% infected cells. After exposure of the osteogenic sarcoma cell lines to each of the three virus strains by cocultivation with HIV-producing lymphoma lines, up to 5% of the cells were infected (Table 2).

Following infection of the LC5 cell line, 10% of the cells were positive for HIV-1. IPS carried out 5 days after infection revealed distinct colonies existing of three to five HIV-1-antigen-expressing cells (Fig. 1g). These findings indicate that the continuous cell lines, particularly the LC5 cell line, were significantly more susceptible to HIV-1 infection than the primary cell cultures.

The susceptibility of the fibroblast-like cells to HIV-1 was dose-dependent. LC5 cells were readily infected with an HIV suspension showing RT activity of 5 × 10^5 c.p.m./ml. The osteosarcoma cell line required virus suspensions of at least 10^6 c.p.m./ml RT activity for infection; the primary cultures were infected with virus suspensions containing 2.5 × 10^6 c.p.m./ml RT activity or by repeated infections at 2–3 day intervals. The different infection procedures described above (Virus infection) gave generally similar results; repeated infection of primary cell cultures resulted in slightly increased numbers of infected cells. Virus infection did not induce overt cytopathogenic effects within an observation period of 3 weeks. In particular, immunohistochemically labelled virus-positive target cells did not show altered morphology or multinucleation (Fig. 1).

Expression of CD4 and inhibition of HIV-1 infection

Total cellular RNAs prepared from HIV-1-producing T-lymphoma cells and HIV-infected and uninfected embryonic lung and osteosarcoma cell lines were examined for CD4 messenger RNA (mRNA) expression. Northern blot analysis with a CD4-specific cDNA probe revealed two signals, one of 3.0 kb and a stronger signal of approximately 1.8 kb in the KE37-1 lymphoma cell line and in uninfected as well as in HIV-infected LC5 embryonic lung cells. Low levels of CD4 expression were found in TE-85 cells whereas CD4 mRNA expression was not observed in SAOS-2 cells (Fig. 3).

Immunofluorescence analysis revealed CD4 receptor molecules on the surface of approximately 90% of KE37-1 lymphoma cells but not on LC5 cells (data not shown). Treatment of LC5 cells with a monoclonal antibody against CD4 (MT310) inhibited HIV-1 infection in a dose-dependent manner. Fifty micrograms per millilitre of anti-CD4 monoclonal antibody reduced the number of HIV-

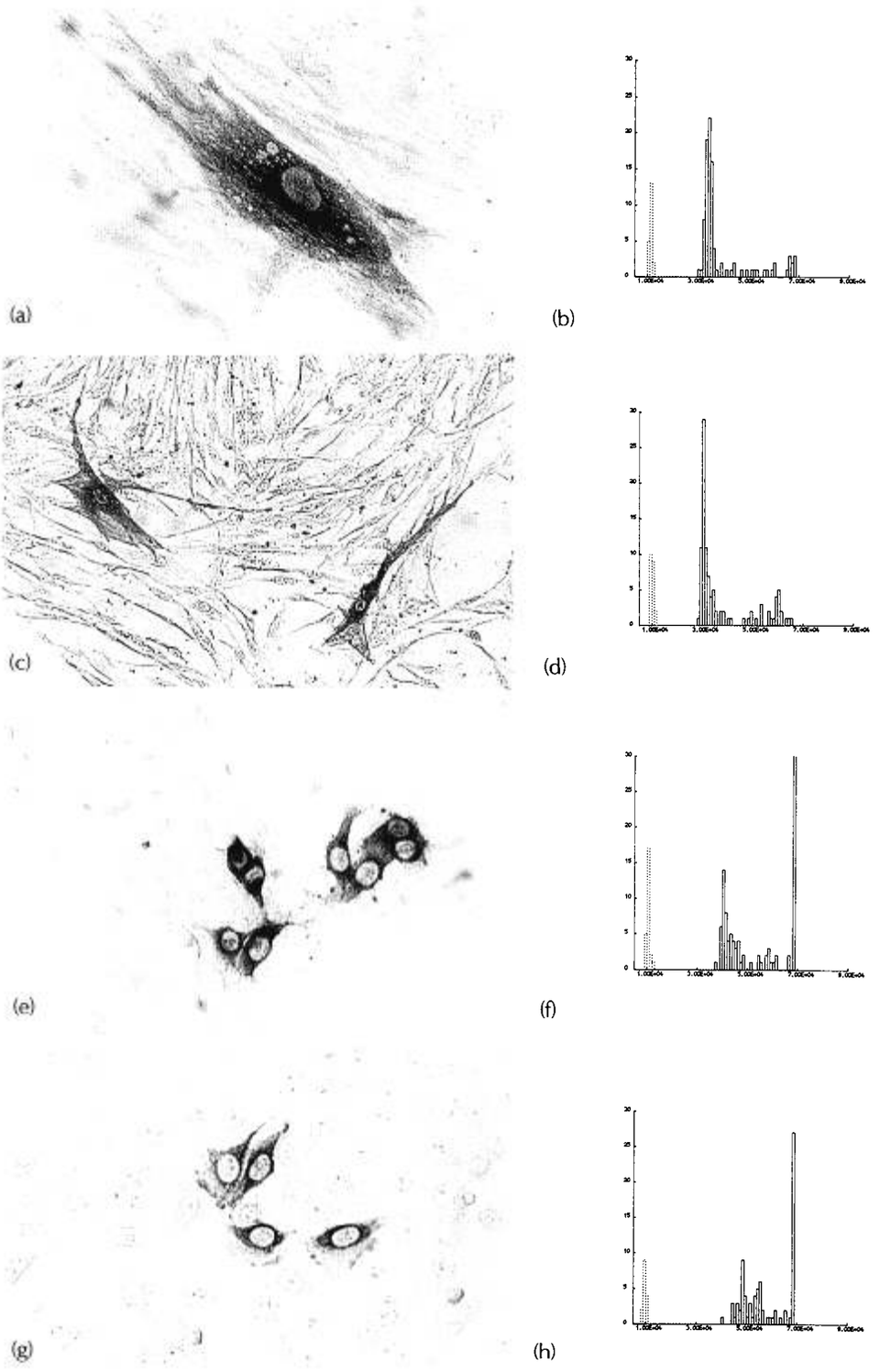

Fig. 1. Morphology and DNA distribution of HIV-1-infected mesenchymal cells. HIV-1-infected cells were visualized by immunoperoxidase staining using HIV-1-positive human serum. Micrographs were prepared under differential interference (DIC) or phase contrast (PC). DNA distributions were performed as described [13]. Ordinate units, absolute frequency of cells; abscissa, DNA content, arbitrary units. (a) HIV-infected cell in the primary skin fibroblast cell culture 176-S3 (DIC, × 532); (b) DNA distribution of the cell culture shown in (a) exhibiting diploid DNA content; (c) HIV-infected cells in the primary lung fibroblast cell culture CCD-25Lu (PC, × 266); (d) DNA distribution of the cell culture shown in (c) exhibiting diploid DNA content; (e) HIV-infected cells in the continuous osteogenic osteosarcoma cell line TE-85 (DIC, × 266); (f) DNA distribution of the cell line shown in (e) exhibiting a stem line peak at approximately 2.7c; (g) HIV-infected cells in the fibroblast-like LC5 cell line (PC, × 266); (h) DNA distribution of the cell line shown in (g) exhibiting aneuploidy.

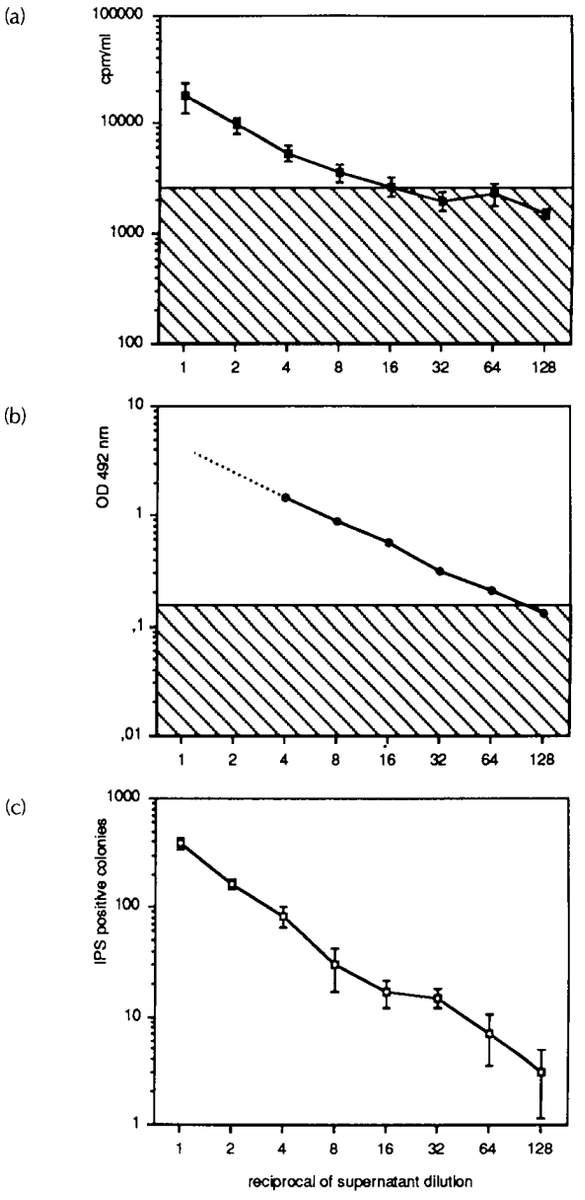

Fig. 2. Detection of HIV-1 (HTLV-III_B) in LC5 cells. LC5 cells were infected with serial twofold dilutions of HIV-1. Infection was determined by (a) reverse transcriptase activity in the supernatant, (b) p24 antigen enzyme-linked immunosorbent assay in the supernatant, and (c) immunoperoxidase staining of HIV protein-expressing LC5 cells. The shaded areas indicate background levels of the different virus detection assays.

positive colonies of LC5 cells by 93%, 5 μg/ml by 73% and 0.5 μg/ml by 66%. Control treatments carried out in parallel using a monoclonal antibody against CD8 (MT 811; 50 μg/ml) inhibited virus infection by only 15%.

Productive infection of LC5 cells and of primary 178-H1 cells

LC5 cells were infected with HTLV-III_B and an HIV-infected cell clone (LC5-HIV) was obtained by the end-point dilution technique after screening single cell clones for HIV protein production. The presence of viral genomes integrated in LC5-HIV DNA was demonstrated by Southern blot analysis using an HIV-specific probe (data not shown). Western blot analysis of a lysate obtained from approximately 10^7 LC5-HIV cells revealed the presence of HIV-1-specific proteins: p18, p24, p32, gp41 and p65 (Fig. 4). Electron microscopy of LC5-HIV cells revealed budding of virus particles from the cell membrane and large numbers of mature virus particles in the intercellular spaces. RT activity in the supernatant of cell cultures containing approximately 2×10^5 cells/ml ranged between 74 840 and 121 500 c.p.m./ml. In comparison, the RT activity in the supernatant of KE37-1-HIV cell cultures containing $2–3 \times 10^5$ cells/ml and determined at the same time intervals after infection ranged from 65 600 to 350 000 c.p.m..

Treatment of KE37-1 cells with LC5-HIV supernatant resulted in productive infection of the T-lymphoma cell line. RT activity increased until 16 days after infection from 2×10^3 to 7.5×10^4 c.p.m./ml and decreased thereafter to levels of 5.5×10^4 c.p.m./ml until the end of the observation period, 32 days after infection.

178-H1, primary skin fibroblasts, seemed to be less susceptible to HIV-1 infection than the continuous cell lines. However, repeated treatment of 178-H1 cells at 2-day intervals with HIV-1-containing supernatant increased the number of infected cells. Virus infection was found in single cells rather than in colonies because of slow cell growth of primary 178-H1 cells.

Neither the RT assay nor the p24 antigen enzyme-linked immunosorbent assay were sufficiently sensitive to demonstrate virus infection. Therefore, 178-H1-derived virus was recovered by cocultivation with susceptible KE37-1 cells. Furthermore, KE37-1 cells could be infected with supernatant from IPS-positive 178-H1 cells by a 2-day treatment. Infection was shown by immunoperoxidase staining and RT assay (Table 3).

Discussion

This report describes the susceptibility of human primary fibroblasts and continuous fibroblast- and osteoblast-like cells to HIV-1 infection. Morphology and immunohistochemical analysis of cell matrix proteins and intermediate filaments of the described cells support the fibroblastic characteristics of the primary cell cultures, the origin of the two osteoblast-like cell lines from osteogenic tissue as well as the fibroblast-like characteristics of the LC5 cell line. The presence of small amounts of cytokeratin in the continuous LC5 cell line indicates possible loss of control of cytokeratin expression, a feature which has been observed in diverse continuous cell lines of non-epithelial origin [21]. Furthermore, the absence of desmosomes in LC5 cells (unpublished data) strongly suggests the fibroblastic origin of the LC5 cell line.

The data presented in this paper confirm recent reports of infectability of continuous fibroblastoid cell lines with HIV-1 [11,12]. In addition, we present evidence for pro-

Table 2. Infection of primary and continuous human mesenchymal cells with HTLV-III_B, HTLV-III_{RF} and HTLV-III_{Rutz} strains of HIV-1.

	HTLV-III_B		HTLV-III_{RF}		HTLV-III_{Rutz}	
Origin of cells	Cocultivation	Cell-free	Cocultivation	Cell-free	Cocultivation	Cell-free
Primary cell cultures						
Skin biopsies from healthy individuals						
176-S3	+	+	+	−	ND	ND
176-S3-SV40	+	+ +	ND	ND	ND	ND
178-H1	+	+	ND	−	ND	ND
178-H4	−	+	+	+	ND	ND
Skin biopsies from HIV-positive individuals						
M5nHF	+	ND	+	ND	ND	ND
M6nHF	+	ND	+	ND	ND	ND
M7nHF	+	ND	+	ND	ND	ND
M8nHF	+	ND	+	ND	ND	ND
Embryonic lung						
CCD25-Lu	ND	+	ND	ND	ND	ND
HEL 299	ND	+	ND	ND	ND	ND
Continuous cell lines						
Osteogenic sarcoma						
TE85	+ +	+	+ +	+	+ +	+
SAOS-2	+ +	+	+ +	+	+ +	+
Embryonic lung						
LC5	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +

+, Up to 1% virus-positive cells; + +, 1–5% virus-positive cells; + + +, more than 5% virus-positive cells; ND, not investigated; Cocultivation, cocultivation of target cells with HIV-1-producing lymphoma cells; cell-free, treatment of target cells with cell-free supernatant from HIV-1-producing lymphoma cells.

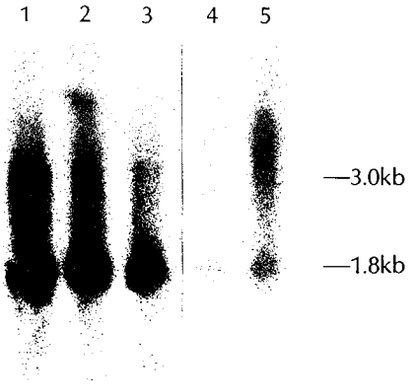

Fig. 3. Northern blot analysis of CD4 messenger RNA (mRNA) expression in permanent human mesenchymal cell lines. A 3.0 kb and a stronger 1.8 kb band was noted in the KE37-1 lymphoma cell line and in the LC5 cell lines. The osteosarcoma cell lines showed very little CD4 expression. Lane 1, KE37-1 T-lymphoma cell line, infected with HIV-1 (HTLV-III_B); lane 2, LC5 cell line; lane 3, LC 5 cell line, infected with HIV-1 (HTLV-III_B); lane 4, SAOS-2 cell line; lane 5, TE-85 cell line.

ductive infection of primary cells derived from human skin and lung. The diploid DNA content of these primary cells shows that they have retained basic properties of cells growing *in vivo*, raising the possibility that these cells may also be infectable *in vivo*. Attempts to identify HIV-1-infected fibroblasts in cultures derived from skin biopsies from seropositive individuals were negative in all four cases. However, infection of fibroblasts in skin may be too infrequent to be readily detected in skin biopsies.

Infection of primary cells *in vitro* seems to be a rare event, since 10-fold larger quantities of virus used to infect osteoblast-like and fibroblast-like cell lines were required for infection of primary cells. Infection of primary and continuous cells does not seem to induce cytopathic effects and the infected cells are able to replicate as indicated by colony formation in the continuous cell lines (Fig. 1). This has also been observed for other continuous fibroblastoid cell lines [11,12]. HIV-1 infection of all cell cultures under study was productive with respect to direct expression of HIV-1 antigen, as detected by IPS, and release of infectious virus particles, as shown for LC5 and 178-H1 cells. Cell-free supernatants of these cultures showed relatively high infectivity when incubated with HIV-1-susceptible KE37-1 cells. In addition, LC5 showed similar amounts of RT activity and p24 antigen in the culture supernatant compared with lymphoma cells. In contrast to the results obtained with LC5 cells, productive infection of three human fibroblastoid cell lines with various isolates of HIV-1 [11] was only detected in indicator cell lines following cocultivation. This suggests that various fibroblastoid cells may differ in their susceptibility to HIV-1 infection, as well as in their capacity to support virus production.

The high susceptibility of LC5 cells to HIV-1 suggests that they express CD4 molecules, a fact which has been described predominantly for cells of the immune system

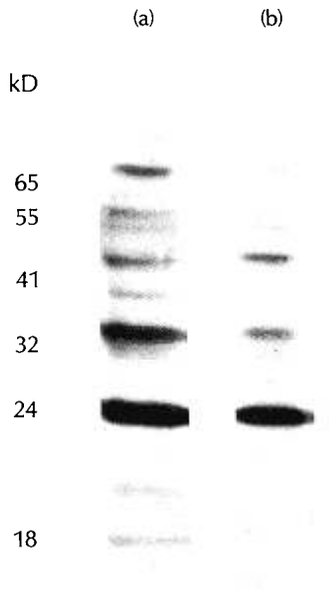

Fig. 4. Western blot of cell lysates from (a) HIV-1-infected KE37-1 and (b) LC5 cells. Note the pattern of HIV-1-specific proteins in both cell lines.

Table 3. Evidence of infection of a primary human skin cell line (178-H1) and a permanent human embryonic fibroblast cell line (LC5).

Assay	178-H1	LC5
Immunoperoxidase staining	+	+
Reverse transcriptase assay	−	+
p24 antigen ELISA	−	+
Recovery of infectious virus	+	+

+, Evidence and −, lack of evidence for HIV-1 infection as shown by the indicated methods. ELISA, enzyme-linked immunosorbent assay.

[2,9]. Although assays for the detection of CD4 molecules on LC5 cells by immunohistochemistry were negative, the presence of CD4-specific mRNA was detected by Northern blot analysis (Fig. 3).

The presence of a 3.0 kb transcript together with a 1.8 kb transcript is in agreement with data obtained from brain tissue [23, 24] and may be due to the use of alternative polyadenylation or splicing sites for generation of the CD4 mRNA [20]. Although the surface of LC5 cells may contain only minute quantities of CD4 receptor, undetectable by immunhistochemistry, HIV-1 infection of LC5 cells could be inhibited by a monoclonal antibody against CD4 in a dose-dependent manner. This suggests that *in vitro* infection of LC5 cells is mediated by the CD4 molecule. In addition to CD4, the relative high susceptibility of LC5 cells to HIV-1 could also be due to different non-CD4 cell surface molecules, as shown by Chesebro *et al.* [25] for human brain and skin cells. Although the CD4 molecule is the major receptor for HIV on T cells and some cells of the monocyte-macrophage lineage [24,26], the infection of various non-lymphoid and monocyte cells with HIV-1 was shown to be CD4-independent. This has been reported for human glioblastoma cells, medulloblastoma and primary fetal neural cells [27], for human fetal dorsal root ganglion cells [28], for brain and muscle cells [9] and for CD4-negative human fibroblast cells [11]. Furthermore, human brain and skin cells in which CD4 expression has been elevated do not appear to be more easily infected with HIV [25].

The fact that fibroblast-like cells are infectable with HIV suggests that these cells may serve as virus reservoirs *in vivo*, although initially only few fibroblasts may be infected and susceptibility of these cells for HIV may be quite low. The observation that a certain type of fibroblast especially, such as the LC5 cell, is capable of producing amounts of virus roughly equivalent to that of infected lymphoid cells strongly implicates the involvement of fibroblast cells in persistence and distribution of the virus *in vivo*. However, the different susceptibilities reported for fibroblastoid cells [11,12] indicate that not all fibroblast-like cells may equally support virus production. Moreover, the regulation of HIV-1 replication in infected fibroblasts may depend on the nature of the mesenchymal tissue and on the microenvironment in which these cells are located. In conclusion, the unimpaired cell division observed for certain HIV-1-infected fibroblasts suggests that they could represent local, continuously growing virus reservoirs, leading to disease development in organs such as the lung [22].

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