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DEFECTIVE HUMAN IMMUNODEFICIENCY VIRUS (HIV) PARTICLES PRODUCED BY CLONED CELLS OF HTLV-I-CARRYING MT-4 CELLS PERSISTENTLY INFECTED WITH HIV

Kazuyoshi IKUTA,[*1] Chizuko MORITA,[*1, *2] Masuyo NAKAI,[*2] Naoki YAMAMOTO[*3] and Shiro KATO[*1]

[*1]*Department of Pathology, Research Institute for Microbial Diseases, Osaka University, Suita, Osaka 565,* [*2]*Department of Microbiology, Osaka Medical College, Takatsuki, Osaka 569 and* [*3]*Department of Virology and Parasitology, Yamaguchi University School of Medicine, Ube, Yamaguchi 755*

Persistently HIV-infected cell lines were isolated from surviving and proliferating cells after infection of HTLV-I-carrying MT-4 cells with cell-free human immunodeficiency virus (HIV); HTLV-IIIB and LAV. The media of the cloned cell cultures did not cause HIV infection of MT-4, MOLT-4, TALL-1, or HL-60 cells. Most of the constituents of the virus in the media were *env* proteins and many defective doughnut-shaped particles released from the cells were identified by electron microscopy.

Key words: MT-4 — HTLV-I-HIV — Defective particle — Persistent infection

The etiological agent of the acquired immunodeficiency syndrome (AIDS) is a retrovirus designated as human immunodeficiency virus (HIV), also known as LAV,[1] HTLV-III,[2] or ARV.[3] OKT-4$^+$ cells were shown to be susceptible to HIV infection.[4] MT-4 cell line[5] carrying HTLV-I, which is recognized as the etiological agent of adult T-cell leukemia (ATL)[6] can be infected efficiently with HIV and infection causes drastic cytopathic effects and cytolysis.[7, 8] Because of this cytolysis, the biologically active virus can be titrated in terms of median tissue culture infectious doses ($TCID_{50}$) or by plaque-forming assay.[7] During studies on the relationship between several HIV strains and host cells, we isolated proliferating cells from cultures of MT-4 after their drastic cytolysis by HIV infection. The cloned cells obtained from these isolates were found to produce defective HIV particles.

The culture fluids of MOLT-4/HTLV-IIIB and TALL-1/LAV were filtered through a 450 nm Millipore membrane and used as HIV inocula. MT-4 cells at 1×10^6 cells/ml were infected with HTLV-IIIB and LAV at about 0.1 and 0.01 multiplicity of infection (m.o.i.), respectively. The HIV-infected MT-4 cells showed drastic cytotoxic effects with the appearance of multinucleated giant cells. Control MOLT-4 cells infected with HTLV-IIIB also showed drastic cytopathic changes with the formation of more multinucleated giant cells. However, a marked difference between these two cell lines was that whereas most HIV-infected MT-4 cells died, about half the HIV-infected MOLT-4 cells survived. Moreover, the surviving HIV-infected MT-4 cells grew very slowly at first, and then increasingly rapidly, whereas the surviving HIV-infected MOLT-4 cells grew at a steady high rate. Proliferating MT-4 cells have been easily isolated twice after infection with HTLV-IIIB and once after infection with LAV, and the lines obtained have so far grown for 5 months and accordingly been designated as M4HB1, M4HB2, and M4LB1, respectively.

The viral expressions and phenotypic markers of these newly isolated cells were characterized by the immunofluorescence (IF) test (Table I). All these cell lines were found to express HIV-associated antigens, by using the serum of an AIDS patient, and V17 and V10 mouse monoclonal antibodies against HIV *gag*, p18 and p24 proteins. Details of these monoclonal antibodies will be reported elsewhere (Ikuta *et al.*, in preparation). In IF tests almost all M4HB1, M4HB2, and M4LB1 cells gave a positive reaction with the serum of the AIDS patient, while only small numbers of the M4HB1 and M4HB2 cells gave a positive reaction with V17 or V10 monoclonal

Table I. Expression of Several Antigens in Persistently HIV-infected MT-4 Cells Detected by IF Tests

Cells	IF test[a] with antibodies[b] to						
	HIV	HIV-p18	HIV-p24	HTLV-I	OKT-4	OKT-8	IL-2R
MT-4	0[c]	0	0	>90	>90	0	>90
M4HB1	>90	5	5	>90	0	0	>90
M4HB2	>90	15	20	>90	0	0	>90
M4HB2-cl	>90	0	0	>90	0	0	>90
M4LB1	>90	>90	>90	>90	0	0	>90

a) Cells were fixed with cold acetone for 5 min before tests for HIV- and HTLV-I-associated antigens. Unfixed cells were used in tests on OKT-4, OKT-8, and IL-2R.
b) The following antibodies were used: HIV, the serum of an AIDS patient; HIV-p18, mouse monoclonal antibody (V17) against HIV *gag* p18; HIV-p24, mouse monoclonal antibody (V10) against HIV *gag* p24; HTLV-I, the serum of an ATL patient; OKT-4, mouse monoclonal antibody against OKT-4 (Ortho-mune); OKT-8, mouse monoclonal antibody against OKT-8 (Ortho-mune); IL-2R, mouse monoclonal antibody against IL-2R (Amersham).
c) Percent of IF-positive cells.

antibodies, although almost all the M4LB1 cells reacted with V17 or V10 monoclonal antibodies. In the IF test, the cloned cells isolated from M4HB2 (M4HB2-cl) did not react with V17 or V10 monoclonal antibodies, although almost all the cells reacted with the serum of the AIDS patient. The intensity of staining of HIV-associated antigens in these cells was much stronger than that in MOLT-4/HTLV-IIIB cells. On the other hand, the intensity of staining of ATL-associated antigens in these cell lines was similar to that in uninfected MT-4 cells. Cell surface OKT-4 was undetectable on these cell lines, as observed previously with other cell systems.[9] On the other hand, the amount of surface interleukin-2 receptor (IL-2R) on these cell lines was similar to that on uninfected MT-4 cells, as observed previously with MT-4 cells acutely infected with HIV.[8] Thus, the newly isolated cell lines, M4HB1, M4HB2, and M4LB1, were clearly persistently infected with HIV.

The infectivity of HIV in the culture fluids of these persistently HIV-infected MT-4 cell lines was titrated on MT-4, MOLT-4, TALL-1, and HL-60 cells by the IF test with the same serum of an AIDS patient as for Table I, as described previously.[10] The culture fluids were obtained after seeding 5×10^5 cells/ml and culturing them for 3 days. The culture fluids from M4HB1, M4HB2, and M4LB1 cells had lower infectivities on MOLT-4, TALL-1, and HL-60 cells than on MT-4 cells, as did the culture fluid from MOLT-4/HTLV-IIIB cells. The infectivities on MT-4 cells of the culture fluids from M4HB1, M4HB2, and M4LB1 cells were $10^{3.8}$, $10^{4.6}$, and $10^{4.6}$ $TCID_{50}$/ml, respectively, whereas that of the culture fluid from MOLT-4/HTLV-IIIB cells was $10^{7.8}$ $TCID_{50}$/ml. However, infectivity assayed by the same method in the culture fluid from M4HB2-cl cells was undetectable ($<10^{0.8}$ $TCID_{50}$/ml).

Next, we analyzed the HIV-related proteins in the persistently HIV-infected MT-4 cell lines by immunoprecipitation followed by SDS - polyacrylamide gel electrophoresis (SDS-PAGE) as described previously.[11] As a control, we also analyzed MOLT-4/HTLV-IIIB cells in the same way. The cells were labeled with [^{35}S]cysteine for 12 hr, and the labeled cells were solubilized in lysis buffer by sonication for 20 sec. The cell lysates were treated with the same serum of an AIDS patient as that used for the results in Table I. Then the immune complexes were precipitated with protein A-Sepharose CL-4B (Pharmacia). The results are shown in Fig. 1. The *env*- and *gag*-precursor proteins, gp160 and p53, respectively, were identified in MOLT-4/HTLV-IIIB cells. In addition, their cleaved products, such as gp120 and p18, were also identified in this cell line. On the other hand, truncated heterogeneous *env* proteins were found in persistently HIV-infected

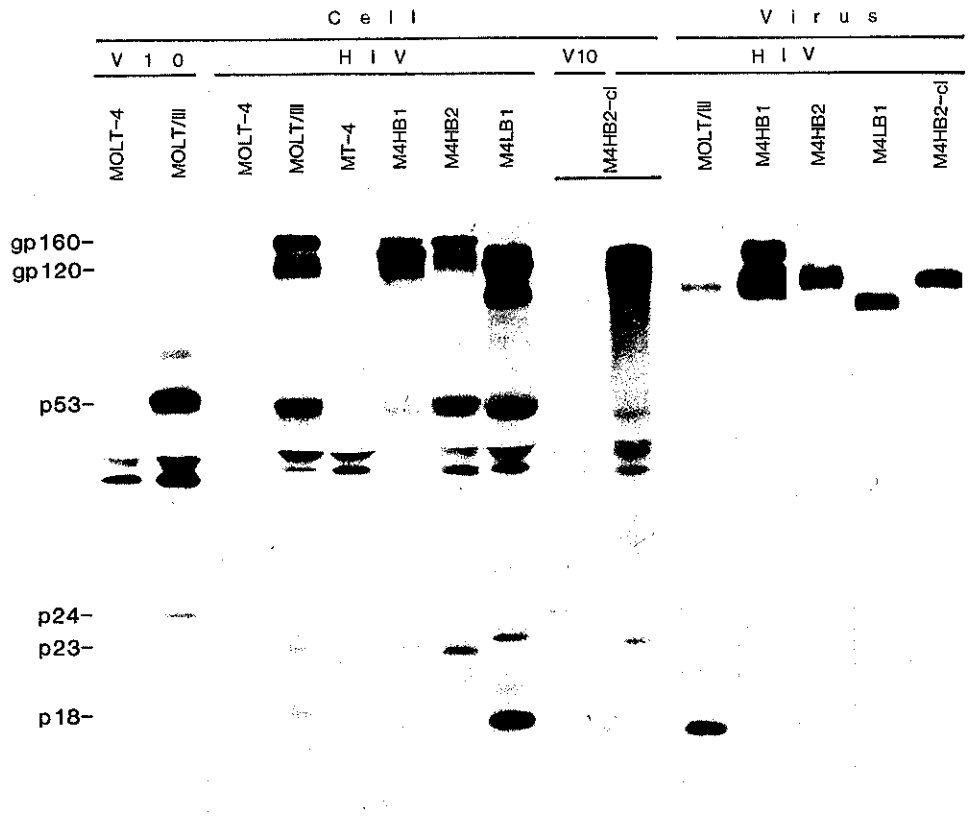

Fig. 1. Immunoprecipitation of the cell and virus fractions of persistently HIV-infected MT-4 cells. Samples of 2×10^6 cells of MOLT-4, MOLT-4/HTLV-IIIB (MOLT/III), MT-4, M4HB1, M4HB2, M4LB1, and M4HB2-cl were labeled for 12 hr with [^{35}S]cysteine (Amersham, 1481 Ci/mmol) in 1 ml of RPMI-1640 medium containing one-tenth the normal concentration of cystine and 5% fetal bovine serum. The cell (Cell) and virus (Virus) lysates in lysis buffer [0.5% NP-40 and 0.5% sodium deoxycholate in NTE buffer (0.05*M* Tris-HCl, pH 7.2, 0.1*M* NaCl, 1m*M* EDTA, 1m*M* phenylmethylsulfonyl fluoride)] were immunoprecipitated with the serum of an AIDS patient serum (HIV) or with V10 monoclonal antibody (V10). The immunoprecipitates were analyzed by SDS-PAGE (separation gel, 10 to 15% linear polyacrylamide gel; spacer gel, 4% gel). The MWs of the polypeptides were estimated by comparison of the mobilities with those of standard proteins (Pharmacia).

MT-4 cells. SDS-PAGE patterns of these truncated *env* proteins were different in each cell line: at least three protein bands in M4HB1, a major band with an MW of about 140K, a minor band of 160K, and heterogeneous bands of 140K to 115K; at least two protein bands in M4HB2, a major band with an MW of about 160K, and heterogeneous bands of 130K to 120K; and at least three protein bands in M4LB1, a major band with an MW of about 120K and two minor bands with MW of about 135K and 98K. The *gag* precursor p53, and the cleaved p18 protein bands were identified in the MT-4 cells persistently infected with HIV as well as MOLT-4/HTLV-IIIB. In M4HB2-cl cells, truncated heterogeneous *env* protein bands were found. However, the *gag* precursor p53 protein band was only faint or not detectable, and the cleaved p24 and p18 protein bands were not detectable. A polypeptide band with an MW of about 23K was identified in M4HB2-cl cells as well as in uncloned M4HB1, M4HB2, and M4LB1 cells by immunoprecipitation with the serum of the AIDS patient. This polypeptide band was also detected in MOLT-4/HTLV-

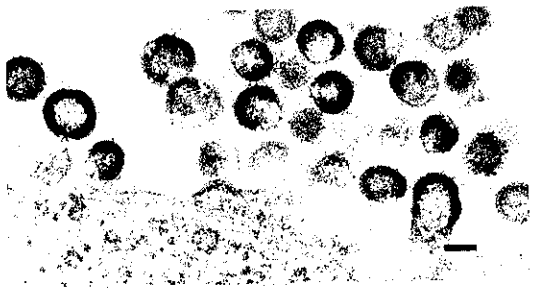

Fig. 2. Defective HIV particles produced by M4HB2-cl cells. Cells were prefixed for 1 hr in 2.0% glutaraldehyde in 0.1*M* phosphate buffer, pH 7.2, thoroughly washed with PBS, post-fixed with 1% osmium tetroxide in 0.1*M* phosphate buffer, dehydrated and embedded in epoxy resin. Sections were stained with uranyl acetate and lead citrate and observed with a Hitachi H-300 electron microscope. Bars = 100 nm.

IIIB cells with the same serum. The MWs of p18 and 23K polypeptides in M4LB1 were slightly different from those in the other cell lines infected with HTLV-IIIB.

The virus fractions prepared from the culture fluids of labeled cells by centrifugation at 46,000 rpm in an SW50.1 rotor for 1 hr at 4° were also analyzed similarly. The virus lysates in lysis buffer were immunoprecipitated in the same way as cell fractions (Fig. 1). The *env* product, gp120, and *gag* product, p18, were identified in the virus fractions prepared from the fluids of MOLT-4/HTLV-IIIB cells. On the other hand, the *env* products were identified in the virus fractions prepared from M4HB1, M4HB2, M4LB1, and M4HB2-cl. The *gag* products were detected weakly in the virus fractions prepared from M4HB1, M4HB2, and M4LB1, but not in that from M4HB2-cl. These *env* products were slightly different from those in the cell fractions and consisted of heterogeneous material of about 160K and 140K to 110K in M4HB1, 160K and 145K to 125K in uncloned and cloned M4HB2, and 98K and 135K in M4LB1.

Next, we examined the defective particles by electron microscopy. Most particles released from the M4HB2-cl cells did not contain an intact core structure, and doughnut-shaped particles were mainly observed as shown in Fig. 2.

Thus, the intact infectious HIV particles in the fluid of MOLT-4/HTLV-IIIB cells consisted of *env* and *gag* proteins, whereas the particles in the fluid of M4HB2-cl cells were defective doughnut-shaped particles and mainly consisted of *env* proteins.

Most of the HTLV-I-carrying cell lines were sensitive to the cytotoxic effect of HIV, but some of the HTLV-I-carrying cell lines that appeared not to express OKT-4 were resistant.[12, 13] Surviving cells were also resistant to HIV replication,[12] or were persistently infected with HIV and produced infectious HIV particles.[13] Similarly, most cells of a variant of the CEM cell line, A3.01, were killed by HIV infection, but a few cells without OKT-4 survived.[14] These surviving cells did not produce HIV, but they could be induced to produce HIV by treatment with 5-iodo-2′-deoxyuridine (IUdR). Thereafter, the clone 8E5 was isolated from HIV-infected A3.01 cells treated with IUdR.[15] This clone contains a single provirus that expresses intact *gag* and *env* constitutively, but is defective in the *pol* region, and releases particles without a condensed rod-shaped nucleoid.[15, 16] Thus, the HIV-host cell interaction in these lines reported previously is different from that in our MT-4 lines of continuously growing OKT-4$^+$ and OKT-8$^-$ cells, which carry HTLV-I, and are persistently infected with HIV and produce defective, noninfectious HIV particles mainly consisting of *env* proteins. Our results with cloned and uncloned MT-4 cells persistently infected with HIV indicated that most surviving MT-4 cells after drastic cell lysis by HIV infection produce defective particles while a few cells produce infectious HIV particles. There are two possible reasons why a few MT-4 cells survived after drastic cytolysis by HIV infection. One possibility is that a small population of MT-4 cells may be different from the predominant population; i.e., the major population of MT-4 cells was killed by HIV infection, but the minor population survived. This minor population of MT-4 may be defective in an enzyme(s) involved in the maturation of HIV *env* proteins, since the cleavage of *env* precursor protein of murine leukemia virus into glycoprotein and transmembrane protein was reported to be accomplished by cellular trypsin-like enzymes.[17] However, in our

studies on several clones of MT-4 cells, all survived HIV infection similarly, indicating that this possibility is unlikely. The other possibility is that the HIV inocula used for infection of MT-4 cells may have contained a small amount of defective virus. The intact HIV kills MT-4 cells, whereas the HIV proteins or particles synthesized from the defective provirus may not, and so a minor population of MT-4 cells could survive HIV infection and proliferate as cell lines. At present, it is unknown whether the HTLV-I gene and gene products are involved in the proliferation of MT-4 cells and the expression of HIV antigens. The expression of HTLV-I-associated antigens in persistently HIV-infected MT-4 cells was similar to that in uninfected MT-4 cells (Table I). In addition, no HTLV-I-related protein reactive with the serum of an ATL patient was identified in the virus fractions from persistently HIV-infected MT-4 cells (data not shown). The polypeptide with an MW of about 23K detected in cell fractions of the cloned and uncloned M4HB2 cells seems to be a *sor* product[18] in terms of similarity in MW. Further characterization of this protein in M4HB2-cl cells should clarify its function.

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