

Development and Utilization of an Human Immunodeficiency Virus Type 1 (HIV-1)-Based Retroviral Vector System to Express HIV-1 Envelope Glycoprotein in CD4⁺ T Cells

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=국문초록=

HIV-1 Retroviral Vector System의 개발 및 CD4⁺ T 세포에서 HIV-1 Envelope 당단백질의 발현

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박진서

본 연구에서는 CD4⁺ T 세포를 일회성으로 감염시킬 수 있고 CD4⁺ T 세포에 HIV-1 envelope 유전자를 전달할 수 있는 바이러스 입자를 생산하는 HIV-1 complementation system을 개발하였고 이 system을 이용하여 CD4⁺ T 세포에 선택적으로 HIV-1 envelope 당단백질을 발현시켰다. 이 system은 Gag/Gag-Pol expressor와 Env expressor로 구성되어 있다. Gag/Gag-Pol expressor는 바이러스 입자 생산에 필요한 구조단백질과 기능단백질을 발현시키지만 packaging signal이 결핍되어 바이러스 입자로 유전자가 들어가지 못하도록 제조되었다. Env expressor는 Tat, Rev와 envelope 당단백질을 발현시키고 packaging signal을 갖고 있어 바이러스 입자로 envelope 유전자가 들어가도록 제조되었다. Gag/Gag-Pol expressor로부터 Gag와 Gag-Pol의 발현은 Rev 단백질을 요구하였고 Env expressor로부터 Rev 단백질이 제공될 때 Gag와 Gag-Pol 단백질은 효율적으로 발현되었다. Gag/Gag-Pol과 Env expressor로 cotransfection된 COS-1 세포에서 CD4⁺ T 세포를 일회성으로 감염시킬 수 있는 바이러스 입자가 생산되었다. 생산된 바이러스 입자는 CD4⁺ T 세포에 HIV-1 envelope 유전자를 전달하여 envelope 당단백질을 발현시켰고 복제 가능한 자손 바이러스의 생성을 유도하지 못하였다. 본 연구에서 개발된 방법은 CD4⁺ T 세포에서 envelope 당단백질의 기능을 분석하고 관심있는 유전자를 CD4⁺ T 세포에 전달하는 바이러스 입자의 생산에 이용할 수 있다.

Key Words: HIV-1, Envelope glycoprotein, CD4⁺ T cells, Packaging, Expression.

INTRODUCTION

The genome of HIV-1 is about 9.5 kilobases (kb) in length (Fig. 1A) [17,24]. In common with other retroviruses, it contains two long terminal repeats (LTR) and three major genes, *gag* (group specific antigen), *pol* (polymerase), and *env* (envelope), which encode structural

and functional proteins that are incorporated into the virus particle [11]. The envelope glycoproteins of HIV-1 are synthesized initially as a 160 KDa precursor through the secretory pathway of the cell. The gp160 precursor is cleaved in the Golgi by a cellular protease to produce a 120 KDa surface envelope protein (gp120) and a 41 KDa transmembrane envelope protein (gp41) which are transported

to the plasma membrane for incorporation into virion particles [7,27]. The gp120 glycoprotein is responsible for tropism of the virus and contains the determinants that interact with the host receptor CD4 [9,13]. The gp120 glycoprotein also determines the ability to form syncytia and to infect cells of the monocyte/macrophage lineage [12,26]. The gp41 glycoprotein contains a stretch of hydrophobic amino acids that serve to anchor the protein in the cell membrane and mediates fusion between the virus membrane and the membrane of the target cell [3,8]. Therefore the envelope glycoproteins of HIV-1 are involved in virus entry, cell-to-cell transmission, and cytopathicity.

Considerable effort has been directed at understanding the structure and function of HIV-1 envelope glycoproteins (reviewed in Ref. 25). HIV-1 has several regulatory genes in addition to structural genes, which enable HIV-1 to have a complicated life cycle in infected cells [11]. Thus in studies with replication competent HIV virions it is often difficult to determine the role of each viral component in the host cell. In order to study the function of HIV-1 envelope glycoprotein in CD4⁺ T cells, we want to establish a system to allow the envelope protein to be expressed in CD4⁺ T cells in the absence of viral replication.

Here, we report the development of an HIV-based retroviral vector system employing a *trans*-complementation for production of recombinant viruses which are able to deliver HIV-1 *env* gene into CD4⁺ T cells. The virus obtained by *trans*-complementation was able to express the envelope glycoprotein after infection of CD4⁺ T cells. Furthermore, recombinant viruses produced by a complementation system were capable of undergoing one round of replication but not multiple cycles of infection in CD4⁺ T cells.

MATERIALS AND METHODS

Materials

All chemicals, unless otherwise noted, were purchased from Sigma Chemical Co. Tissue culture reagents were obtained from GIBCO/BRL. Restriction enzymes and DNA modification enzymes were purchased from New England Biolabs. The [³⁵S]methionine and [³H]TTP were purchased from Amersham. Sera from AIDS patients were obtained through the Dana-Farber Cancer Institute, Boston.

Tissue culture cells

A monkey kidney cell line, COS-1 [4] was maintained in Dulbecco modified Eagle medium (DMEM) supplemented with 5% newborn calf serum-5% fetal calf serum (complete medium). A human T-cell lymphotropic virus type 1-transformed human lymphocyte cell line, C 8166 [20] was maintained in RPMI supplemented with 10% fetal calf serum.

Construction of Gag/Gag-Pol expressor

The majority of the techniques for isolation and manipulation of plasmid DNAs were done by standard protocols [21]. The packaging system and vector utilized in this study are depicted in Fig. 1. All HIV-1 sequences were derived from the molecular clone HXBc2 which is an infectious proviral clone of the human T-cell leukemia virus (HTLV) IIIB isolate [2], and numbered according to Los Alamos database numbering, in which position 1 is the first base of the 5' LTR. To construct the Gag/Gag-Pol expressing plasmid pCMVGP, pKSCMV Δ P1 Δ envA was generated by introducing the *Bam*HI fragment of pCMV Δ P1 Δ envA into pBluescript SK(+) digested with *Bam*HI [16]. The *Nde*I fragment of pKSCMV Δ P1 Δ envA was deleted and religated to give pKSCMV Δ NdeI. The *Nde*I fragment corresponds to a region from 5122 to 6403 of HXBc2 proviral clone. The *Bam*HI fragment of pKSCMV Δ NdeI was subcloned into pCMV Δ P1 Δ envA digested with *Bam*HI. The resulting plasmid was named as pCMVGP.

Construction of Env expressor

An infectious proviral clone (pSVC21) was linearized by digestion with *Cla*I (830) in HIV-1 coding region [19]. The linearized DNA was blunt-ended by using T4 DNA polymerase. A synthetic *Sal*I linker (9mer) was ligated into the blunt-ended linearized DNA, following by digestion with *Sal*I to remove excess linkers and the sequences between *Cla*I (830)-*Sal*I (5786) fragment from pSVC21 and religation to circularize the plasmid. The resulting plasmid, named as pLTRENV, contains the 5' and 3' LTRs, packaging signal, and the first 42 bp of *gag* gene. This plasmid also contains the *tat*, *rev*, and *env* gene. pLTRENV plasmid expresses the HIV-1 *env*, *rev*, and *tat* gene of the HXBc2 strain under the control of the HIV-1 long terminal repeat.

DNA transfections and generation of recombinant HIV-1

Transfection of COS-1 cells was carried out by the DEAE-dextran method as described by Park and Morrow [15]. The COS-1 cells were plated at a seeding concentration of 2×10^6 cells per 100-mm-diameter dish 24 h prior to transfection and were 50% confluent at the time of transfection. Transfections were performed with 5 μ g of CsCl-purified plasmid DNA per ml by using DEAE-dextran (molecular mass, 500,000 Da) at 500 μ g/ml as a facilitator. After 3 h with the DNA-DEAE-dextran mixture, the cells were washed and placed in complete media with chloroquine (20 μ g/ml) for an additional 2 h. Following a 10% dimethyl sulfoxide shock for 2 min, the cells were washed with DMEM and incubated at 37°C for 48 h in complete medium.

For recombinant virus production, culture medium was replaced with the fresh medium 24 h before the virus was harvested. The supernatants from the COS-1 cells mock transfected or transfected with plasmids containing the HIV-1 proviral genomes were col-

lected at 3 days posttransfection. The medium was clarified by centrifugation at $1,000 \times g$ for 5 min, followed by filtration through a 0.2- μ m-pore-size Acrodisc (Gelman Sciences Inc., Ann Arbor, MI) and the reverse transcriptase activity was measured as described below.

For infection, C8166 cells were resuspended at a density of 10^6 cells per ml in supernatant from the transfected COS-1 cells which contains virus equivalent to 5×10^5 cpm of reverse transcriptase activity per ml. Infected cells were passaged daily to maintain cell density in the range of 5×10^5 cells per ml.

Reverse transcriptase assays

For the analysis of virion-associated reverse transcriptase activity, 25 μ l samples from the pelleted particles were placed in a well of a 96-well plate and 75 μ l of a reaction cocktail containing 50 mM Tris-HCl (pH 8.0), 5 mM dithiothreitol, 5 mM MgCl₂, 150 mM KCl, 0.1% Triton X-100, 0.5 mM EGTA [ethylene glycol-bis (β -aminoethyl ether) -N, N, N', N'-tetraacetic acid], 1.25 μ g poly(A) . oligo(dT), 5 μ Ci of [³H]GTP. The reaction was allowed to proceed for 90 min at 37°C, and then the mixture was directly spotted onto ion exchange paper DE 81(Whatman Laboratory Products Inc., Clifton, NJ). The samples were washed once with 0.2 M NaPO₄ (pH 7.5), and air dried, and the radioactivity was quantitated with a liquid scintillation counter [15].

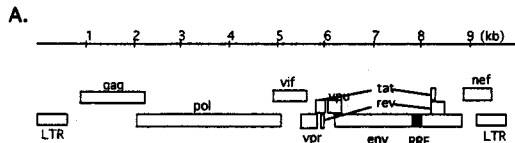
Metabolic labeling of cells and immunoprecipitation

For metabolic labeling, the cells were washed with DMEM and incubated for 1 h in methionine-free DMEM. The medium was then removed and replaced with DMEM minus methionine supplemented with [³⁵S]methionine at 50 μ Ci/ml. After the labeling period, the medium was removed and the cells were washed once with DMEM. The cells were lysed by incubation in radioimmunoprecipitation assay (RIPA) buffer (1% Triton X-100, 1% sodium deoxycholate, 0.1% so-

dium dodecyl sulfate [SDS], 150 mM NaCl, 25 mM Tris-HCl [pH 7.34]) on ice for 5 min. Following removal of high-molecular-weight DNA and cell debris by centrifugation, the specified antibodies were added and allowed to react at 4°C for 12 h. The immunoprecipitates were collected by addition of protein A-Sepharose (20 µl of a 1:10 dilution [wt/vol] in RIPA buffer) for 1 h at room temperature, followed by centrifugation and three washes with RIPA buffer. To release the immunoprecipitates, the beads were boiled in gel sample loading buffer (50 mM Tris-HCl [pH 6.8], 5% SDS, 5% mercaptoethanol and 0.1% bromophenol blue) for 5 min and then briefly centrifuged. The immunoprecipitated proteins were analyzed by SDS-polyacrylamide gel electrophoresis followed by fluorography and subsequent autoradiography using Kodak X-Omat AR film with intensifier at -70°C [15].

FACS analysis

Cell surface viral protein expression on each culture at day 3 after infection was determined by fluorescence-activated cell sorter analysis with sera from AIDS patients and a fluorescein isothiocyanate (FITC)-conjugated mouse anti-human immunoglobulin mono-



B. Gag/Pol and Env Expressors

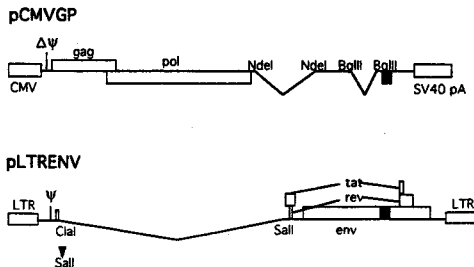


Fig. 1. Construction of HIV-1 vectors. (A) HIV-1 proviral genome. The organization of the HIV-1 proviral genome is shown with the relevant genes. (B) HIV-1 packaging system. pCMVGP and pLTRENV are shown and constructed as described in the text.

clonal antibody (Sigma, St. Louis, Mo). Approximately 5×10^5 cells were pelleted and washed twice in phosphate-buffered saline (PBS). A 1:100 dilution of sera from patients with AIDS was added to the cells in a volume of 100µl of PBS-2% serum. Cells were incubated for 45 min on ice, washed two times with PBS. A FITC-conjugated mouse anti-human immunoglobulin monoclonal antibody diluted 1:50 (10 µg/ml) was added. Cells were

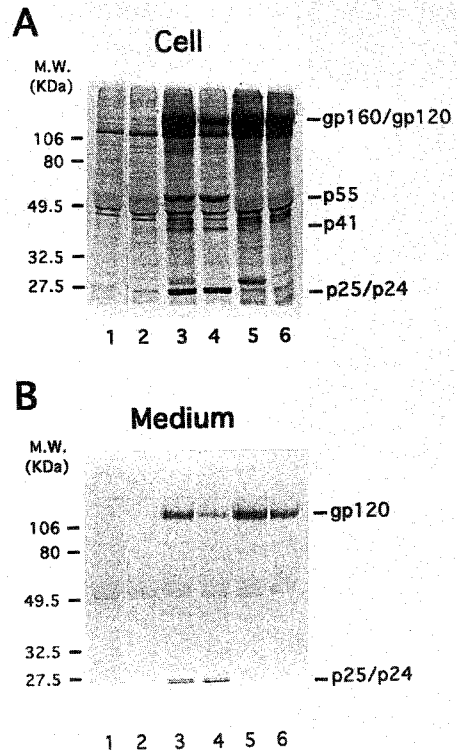


Fig. 2. Expression of viral proteins in COS-1 cells. COS-1 cells were transfected with individual plasmids or cotransfected with the designated plasmids and were metabolically 48 h post-transfection with [³⁵S]methionine. After 6 h, the cell lysates (A) were analyzed by immunoprecipitation with pooled sera from AIDS patients, and SDS-polyacrylamide gel electrophoresis with subsequent autoradiography. The media (B) from COS-1 transfected cells which had been metabolically labeled with [³⁵S]methionine were clarified by a low-speed centrifugation, and analyzed by immunoprecipitation. The molecular weight size standards (left) and the appropriate viral proteins (right) are noted. Lanes: 1, mock transfected; 2, pCMVGP; 3, pCMVGP/pLTRENV; 4, pCMVGP/pCMVENV; 5, pLTRENV; 6, pCMVENV.

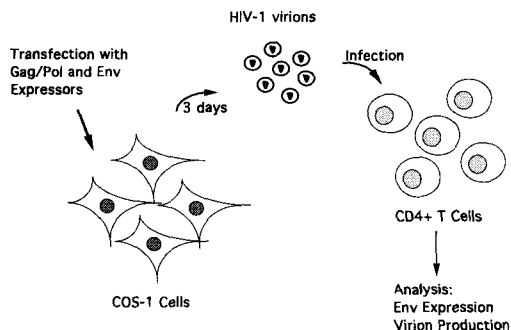


Fig. 3. A single-step infection procedure. Virions recovered from COS-1 cells cotransfected with pCMVGP and pLTRENV or pCMVENV are able to undergo one round of replication and transduce HIV-1 *env* gene into CD4⁺ T cells.

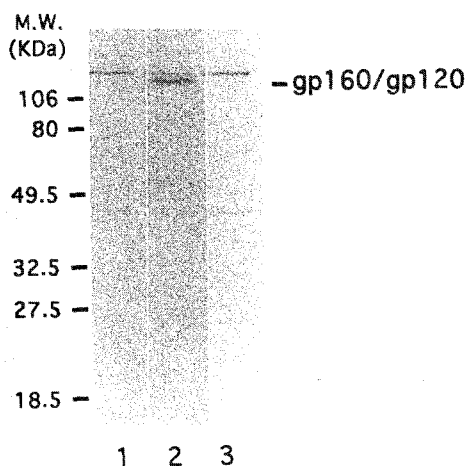


Fig. 4. Analysis of HIV-1 envelope glycoprotein in CD4⁺ T cells. C8166 CD4⁺ T cells were infected with virion particles from COS-1 cells cotransfected with pCMVGP and pLTRENV or pCMVENV and were metabolically labelled with [³⁵S]methionine 72 h postinfection. After 6 h, the cell lysates were analyzed by immunoprecipitation with pooled sera from AIDS patients, and SDS-polyacrylamide gel electrophoresis with subsequent autoradiography. Lanes: 1, mock transfected; 2, pCMVGP/pLTRENV; 3, pCMVGP/pCMVENV.

incubated for 45 min on ice, washed two times with PBS. Cells were fixed with 2% formaldehyde and analyzed on a FACScan flow cytometer (Becton Dickinson, Lincoln Park, NJ) [1].

RESULTS AND DISCUSSIONS

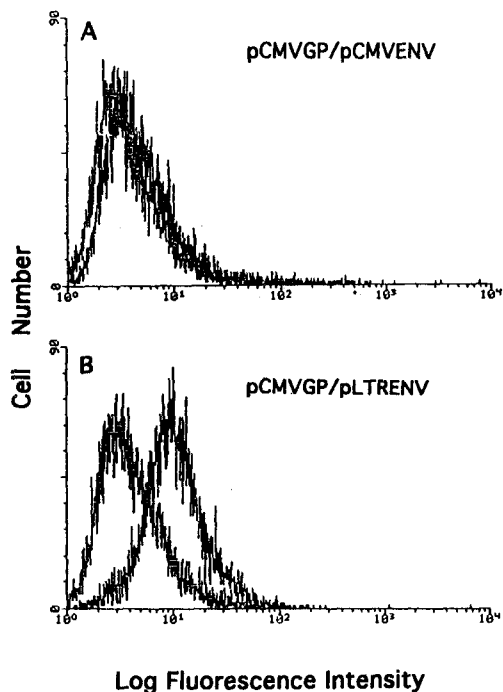


Fig. 5. Cell surface expression of envelope glycoprotein in infected CD4⁺ T cells. CD4⁺ T cells were either mock-infected as control or infected with virus particles from COS-1 cells cotransfected with the designated plasmids. Fluorescence-activated cell sorting was performed on each culture at day 3 after infection by using sera from patients with AIDS and a FITC-conjugated mouse anti-human immunoglobulin monoclonal antibody. Cells were fixed with 2% formaldehyde and analyzed on a FACScan flow cytometer.

Construction of HIV-1-based viral vector system

To generate HIV-1-based viral particles by transient transfection, the expression system was separated into two different plasmids, pCMVGP and pLTRENV (Fig. 1). pCMVGP plasmid was derived from the previously described pCMV Δ P1 Δ envpA [16]. Preliminary experiment with pCMV Δ P1 Δ envpA suggested that replication-competent virus was generated by recombination when *trans*-complemented with envelope expressor. To prevent this recombination event, pCMVGP was constructed from pCMV Δ P1 Δ envpA by dele-

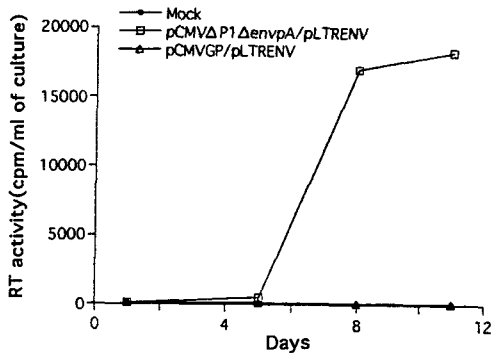


Fig. 6. Reverse transcriptase activity during long-term culture. The production of virus from CD4⁺ T cell cultures was analyzed over a 2-week period by measuring RT activity. The RT activity was measured as described in the text. The value presented is for the 1 ml culture supernatant.

tion of a fragment which contains most of central region including the first exon of *tat* and *rev*, and the beginning part of the *env* gene. pCMVGP has CMV promoter in place of HIV-1 5' LTR and contains a SV 40 poly A signal. And this plasmid contains *gag* and *pol* genes as well as *cis*-acting sequences responsive to Rev protein, so that Gag/Gag-Pol production should be dependent on the presence of HIV-1 Rev protein. Also, in this plasmid, packaging signal has been deleted. This 19 bp deletion has been shown to prevent packaging of viral RNA into virions [10]. Thus this plasmid will provide *gag* and *pol* gene products necessary for virion formation, but their genes will not be packaged into virions due to lack of packaging signal.

pLTRENV plasmid was constructed to provide Tat and Rev functions for *gag* and *pol* gene expression. In addition, this plasmid contains the *env* gene to be transferred, the packaging signal, and the sequences necessary for reverse transcription, viral integration, and the expression of *env* gene. Viral RNA from this plasmid will be packaged into virions. Thus, *env* gene will be transduced into CD4⁺ T cells by infection process. Then, envelope glycoprotein will be expressed in CD4⁺ T cells under the control of Tat and Rev proteins. Rev pro-

tein acts to facilitate the transport of unspliced *env* transcripts from the nucleus to the cytoplasm in the infected cells. Tat protein is required when the HIV LTRs are used to direct transcription [5]. pCMVENV was obtained from Dr. Sodroski. It will provide Tat, Rev and envelope proteins in COS-1 cells but will not be packaged into virions due to lack of packaging signal.

Expression of HIV-1 specific viral proteins

To analyze the expression of HIV-1 proteins, pCMVGP, pLTRENV, or pCMVENV was transfected into COS-1 cells, and the cell extracts were analyzed for the presence of HIV-1-specific proteins 48 h later by immunoprecipitation using pooled sera from AIDS patients (Fig. 2A). In the cells transfected with pCMVGP alone, only small amounts of Gag polyprotein were detected, as demonstrated by the immunoprecipitation of proteins with molecular masses consistent with those of the p55 protein, possibly due to the efficiency of CMV promoter. The HIV-1 envelope proteins immunoprecipitated from the COS-1 cells transfected with pLTRENV consisted of gp160 and gp120. The expression of HIV-1 proteins from COS-1 cells transfected with pCMVENV was similar with that observed for pLTRENV.

To determine whether Gag/Gag-Pol expression can be fully rescued by providing Rev function in *trans*, experiments in which pCMVGP and pLTRENV or pCMVENV were cotransfected into COS-1 cells were performed (Fig. 2A). From the cells cotransfected with pCMVGP and pLTRENV, Gag precursor, p55, as well as the proteolytically processed p41 and p24 products were immunoprecipitated. Significant levels of *env* products, gp160/gp120 were also immunoprecipitated from the cells cotransfected with pCMVGP and pLTRENV. When cotransfected with pCMVENV, similar patterns of protein expression were observed. This result confirms the fact that expression of HIV-1 structural proteins is completely dependent on concomitant Rev protein ex-

pression [22].

To determine whether HIV-1 proteins were released from the transfected cells, we analyzed the media from metabolically labelled cells. The media were clarified by a low-speed centrifugation and the resulting supernatant was analyzed by immunoprecipitation using pooled sera from AIDS patients (Fig. 2B). Even though it was a concern that the low level of Gag polyprotein detected from transfection of pCMVGP might lead to the production of virion particles, no HIV-1-specific proteins were evident in the medium from cells transfected with pCMVGP. However, we readily detected gp120 in the medium from cells transfected with pLTRENV and pCMVENV, due to shedding into the medium. This indicates that correct processing and surface expression of envelope protein had occurred. The proteolytically processed *gag* product p24/25 as well as gp120 envelope glycoprotein were detected in the medium of cells cotransfected with Gag/Gag-Pol expressor DNA and Env expressor DNA.

The release of viral proteins from the COS-1 cells cotransfected with pCMVGP and pLTRENV or pCMVENV suggested that virus-like particles might be released from these cells. To test this possibility, the supernatants from transfected COS-1 cells were fractionated by using ultracentrifugation to pellet any virus-like particles. The levels of reverse transcriptase were quantitated in the pellet after ultracentrifugation for each sample. Approximately, 50,000 cpm of RT activity per ml of culture medium was obtained from the supernatants of each cotransfected cells. Taken together, these results indicate that cotransfection resulted in the release of mature virion particles, as judged by the reverse transcriptase activity and the appearance of p24/p25 in media.

Transduction of HIV-1 specific gene

Recombinant viruses recovered from COS-1

cells cotransfected with Gag/Gag-Pol expressor and Env expressor were analyzed for their abilities to transfer *env* gene into human CD4⁺ T cells. To deliver the envelope gene into the CD4⁺ T cells, recombinant virion particles from COS-1 cells were used to infect CD4⁺ T lymphocyte, C8166 cells which are very efficient targets for HIV-1 infection. Previous study has reported that almost 100% of the cells became infected within 6 h of exposure to high-titer viral stocks [23]. The infection protocol is depicted in Fig. 3. The supernatants from transfected cells were analyzed for reverse transcriptase activity to normalize further infection process. Approximately, 50,000 cpm of RT activity per ml of the supernatants of each cotransfected cells was used for infection of 10⁶ C8166 cells. And the infected cells were cultivated at a density of 5 x 10⁵ cells/ml.

After transduction of *env* gene into CD4⁺ T cells, expression of envelope glycoprotein in CD4⁺ T cells was analyzed 3 days postinfection by immunoprecipitation using sera from AIDS patients (Fig. 4). HIV-1 specific proteins were not detected in the CD4⁺ T cells infected with virus from the COS-1 cells cotransfected with pCMVGP and pCMVENV. However, expression of envelope glycoprotein was evident in the CD4⁺ T cells infected with virus from the cells cotransfected with pCMVGP and pLTRENV. The presence of uncleaved gp160 indicated that processing was incomplete, as is typically observed with the low level expression of envelope glycoprotein [14]. It has been reported that only a small percentage (5% to 15%) of newly synthesized gp160 in HIV-1-infected T lymphocytes is processed into the mature gp120 component, the remainder being transported to lysosomes where it is degraded [27]. Using transfected COS-1 cells, we have shown that the intracellular processing of the envelope glycoprotein in non-infected cells was also very inefficient [18]. Therefore, the failure to detect processed gp120 in CD4⁺ T cells may have been the result of low level

of expression and/or inefficient processing of envelope glycoprotein. Despite the lack of detectable gp120 in CD4⁺ T cells, these cells were able to form syncytia with human CD4⁺ T cells, indicating the functional envelope glycoprotein was expressed (data not shown).

To examine the cell surface viral protein expression in the CD4⁺ T cell cultures, fluorescence-activated cell sorting was performed at day 3 after transduction with virus (Fig. 5). Sera from patients with AIDS were used in this analysis. Flow cytometry indicated envelope glycoprotein was expressed at significant levels at the cell surface in the CD4⁺ T cells infected with viruses recovered from COS-1 cells cotransfected with pCMVGP and pLTRENV. Expression of envelope glycoprotein at the cell surface was not detected in CD4⁺ T cells infected with viruses released from the cells cotransfected with pCMVGP and pCMVENV.

Since the pLTRENV sequences have signals important for HIV-1 packaging into virions [10], the functional envelope glycoproteins are expressed in the target cells. This result confirms the importance of the previously identified packaging signal in the 5' LTR for encapsidation of HIV-1 RNA. Part of the HIV-1 packaging signal positioned between the major splice donor and the *gag* initiation codon as well as *env* sequences encompassing the Rev-responsive element (RRE) have been suggested to contain the packaging signals [16]. However, the failure of the *env* gene sequences in pCMVENV to direct encapsidation of the vector RNA indicates that *env* sequences are not sufficient to direct encapsidation of HIV-1 RNA. Recent studies indicate that the *env* gene sequences alone cannot allow the packaging of HIV-1 RNA [6].

Reverse transcriptase activity assay during long-term culture

It was a concern that replication-competent retroviruses from transduced cells in this study

might be generated. We have investigated this possibility by testing culture supernatants from transduced cells during about two weeks. Replication ability of each virus was analyzed over a 2-week period by measuring RT activity of cultures (Fig. 6). An increase in RT activity was evident from the cultures derived from CD4⁺ T cells transduced with the virus from pCMV Δ P1 Δ envpA and pLTRENV, indicating that replication-competent virus was generated and replicated efficiently. However, on any days postinfection, no increase in RT activity was observed in CD4⁺ T cell cultures transduced with the virus obtained from COS-1 cells cotransfected with pCMVGP and pLTRENV, indicating no infectious viruses were made from the transduced CD4⁺ T cells. The procedure described in this study does not induce the creation of replication-competent viruses. Thus, only a single round of cell-free infection is likely to occur in this system. Under our experimental conditions, we conclude that the virus obtained from cotransfection with pCMVGP and pLTRENV was unable to establish a productive infection in CD4⁺ T cells. As shown in this study, only the virus from pCMV Δ P1 Δ envpA and pLTRENV replicated efficiently, indicating infectious virus was generated probably due to recombination between the *gag/pol* and *env* RNAs. In addition to the physical separation of *gag/pol* and *env* genes, the deletion of the central region from pCMV Δ P1 Δ envpA did remove the possibility of generation of an infectious virus due to recombination events.

In conclusion, a transient *trans*-complementation system was developed to provide a virus that can initiate a single round of infection in CD4⁺ T cells. This virus can transfer a HIV-1 vector containing HIV-1 envelope gene to CD4⁺ T cells. The transferred vector was able to express HIV-1 envelope glycoprotein.

There are still many unanswered questions about the HIV-1 envelope glycoproteins. The structure of the HIV-1 envelope glycoprotein,

its mechanism of cell fusion, and its ability to elicit a neutralizing immune response on vaccination are all under study. In addition, the role of HIV-1 envelope protein in cytopathicity of virus is yet determined. The expression of HIV-1 envelope glycoprotein in the CD4⁺ T cells by the HIV-based retroviral vector system developed in this study will provide a useful means to address these questions. Experiments are under way to determine whether the expression of HIV-1 envelope glycoprotein in CD4⁺ T cells induces syncytia formation and single-cell lysis. Finally, the method described in this study will be useful for preparing viruses that encode target proteins in the CD4⁺ T lymphocytes.

Acknowledgements

This work was supported by research grants from the Korea Science and Engineering Foundation (1995).

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