Effect of Nef-Deleted Pseudotyped HIV Virions Bearing an Enhanced Green Fluorescent Protein (EGFP) Gene in the env on HIV-Sensitive Transformed T Cells

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ABSTRACT

For determining the actual antigenic molecules in human immunodeficiency virus type-1 (HIV-1) recognized by cytotoxic T lymphocytes (CTLs) generated among long term non-progressors (LTNP) who might gain protective immunity against HIV-1 through nef-deleted mutants, we have designed replication-defective recombinant HIV-1 particles pseudotyped with vesicular stomatitis virus glycoprotein (VSV-G), carrying an enhanced green fluorescent protein (EGFP) gene in place of the env. VSV-G pseudotyped virions had significantly augmented infectivity for both dividing and non-dividing cells, and EGFP enables single cell analysis to identify the infected cells producing viral antigen p24. These pseudotyped viral particles could also infect Herpesvirus saimiri-transformed human CD4+ T cells (HVS-T) to produce p24 antigen with or without the nef gene. Although the surface expression of CD4 and class I MHC molecules but not class II MHC, Fas and B7-2 molecules was down-modulated in T cells infected with pseudotyped virions expressing the nef gene, none of the above molecules were down-modulated in the cells infected with nef-deleted pseudotyped virions. VSV-G pseudotyped HIV-1 particles encoding the EGFP gene and HSV-T cells will be useful for analyzing the actual target molecules recognized by CTLs having protective capacity against HIV-1 in vivo and thus, will open new paths for vaccine development.

The human immunodeficiency virus (HIV)-1 nef gene product is known to be one of the major pathogenic modifiers for acquired immunodeficiency syndrome (AIDS) (11). It has been reported that the nef gene alone was sufficient to generate AIDS-like disease in transgenic mice (16). Nef is also required for the development of AIDS-like disease in primates via simian immunodeficiency virus (SIV) infection (20) and inactivation of nef results in viral attenuation in SIV (12) as well as simian human immunodeficiency virus (SHIV) (22, 34). Indeed, pre-injection of rhesus macaques with nef-deleted SIV (SIV-Δnef) (10, 12, 19, 42) or SHIV (SHIV-Δnef) (18, 28, 40) provided protection against infectious virions. In addition, intranasal administration of monkeys with SHIV-Δnef of the NM-3rN strain (1) also induced protective immunity against intravaginal challenge with a pathogenic virus, SHIV-89.6P (14). Moreover, HIV-1 strains with a deletion of the nef gene have been identified in long-term nonpro-
gressors (LTNP) among AIDS patients (13, 21). These in vivo findings strongly indicate a crucial role for Nef in the development of AIDS by promoting viral replication and the deletion of the nef gene alone from those fatal viruses will totally convert them to attenuated forms with which protective immunity can be generated.

We have observed cytotoxic T lymphocytes (CTLs) which might be a key element for protection against challenge with a pathogenic SHIV in rhesus monkeys pretreated with SHIV-Δ nef of the NM3rN strain (40). The target molecules for the CTLs did not appear to be encoded in env but rather in the gag/pol region because they could eliminate targets infected with strain SIVmac239 as well as with pathogenic SHIV-89.6p bearing unrelated envelope protein (manuscript in preparation). Therefore, the immunity established by SHIV-Δ nef inoculation seems to be generated via an env-unrelated portion. This might be compatible with the recent finding that Nef is required for generating the infectivity of the virions in the context of retroviral envelope protein like HIV-1 or amphotropic viral envelope protein but not for the viruses pseudotyped with Ebola virus envelope glycoprotein or vesicular stomatitis virus glycoprotein (VSV-G) (5). The former retroviral or amphotropic viral envelope proteins fuse at the cell surface at neutral pH (37), whereas the latter Ebola glycoprotein or VSV-G use the endocytic low pH route (43). Thus, deletion of the nef gene may reduce not only the infectivity of HIV-1 or SIV through cell fusion mediated by envelope proteins but also the antigenicity of those among various antigenic viral components.

In the present study, based on the findings observed in rhesus monkeys having a protective capacity mediated through CTLs by SHIV-Δ nef immunization, we tried to develop tools for determining the antigenic molecules in HIV-1 virions recognized by CTLs generated in individuals like LTNP who have gained protective immunity against HIV-1 probably through nef-deleted mutants. For such experiments, we have to consider the following obstacles. One is the downregulation of class I MHC molecules that inhibits recognition of the antigenic molecules by specific CTLs (9). Because the downregulation was mediated through Nef protein via HIV-1 infection (8, 26, 35), we have to delete the nef gene from our target virions. However, deletion of the nef gene will usually abolish the infectivity of the virions bearing HIV-1 envelope proteins (5). Thus, we tried to make highly infectious virions without the nef gene in HIV-1. For this purpose, we used pseudotyping with VSV-G as has been demonstrated (2, 23). As described above, in order to study the protective immunity organized by the CTLs generated by nef-mutated virions, env does not seem to be critical for CTL recognition. Also, it should be important to identify the cells expressing the target genes easily. Contamination of uninfected targets will reduce the reliability for determining the specific CTL activity. Moreover, envelope-defective HIV or SIV undergoes only one cycle of replication when pseudotyped with another viral envelope encoded by a distinct plasmid and thus provides a safe way to transduce viral genes (33). Therefore, we inserted an enhanced green fluorescence protein (EGFP) gene as a marker into the env region to make an ideal construct. With the EGFP gene, transduced cells are easily identified without fixing or staining either by flow cytometry or fluorescent microscopy.

Next, we have established a target T cell line expressing both CD4 and co-receptor molecules like CXCR4 or CCR5 for HIV-1 infection from peripheral blood lymphocytes (PBL) by transformation using Herpesvirus saimiri (HVS) as described elsewhere (3, 44). Using those systems, we have confirmed that nef-deleted VSV-G pseudotyped virions have a strong capacity to infect HVS transformed T cells (HVS-T) to express both EGFP and HIV-p24 antigen at almost the same level as the cells infected with VSV-G pseudotyped virions having the nef gene. As expected, class I MHC and CD4 molecules as well as class II MHC, B7-2 and Fast molecules were not downregulated at all when HVS-T were infected with the former nef-deleted virions, whereas both class I MHC and CD4 molecules were strongly downregulated with the latter virions having nef similar to original HIV infection. Thus, downregulation of class I MHC and CD4 molecules in PBL-derived T cells can also be generated via an envelope independent endocytic pathway mediated by HIV-1 Nef protein.

These virions and HVS-T cells shown here will offer useful tools to analyze the actual target molecules recognized by the CTLs having protective capacity in vivo as well as to sensitize non-dividing dendritic cells (DCs) essential for CTL induction and thus, will open the new paths for vaccine development.

MATERIALS AND METHODS

Construction of plasmids. As shown in Fig. 1, the plasmid pNL4-3/EGFPenv expressing the nef gene
of HIV-1 was made by inserting an EGFP gene derived from pEGFP1 (Clontech, Palo Alto, CA, USA) between positions 6159 and 7612 in the env gene of the HIV-1 pNL4-3 strain (1). Then, a frame shift was introduced at the XhoI site within nef by filling the recessed 3'-terminal end with Klenow fragments of DNA polymerase and dNTP for the establishment of a nef-deleted construct, pNL4-3/EGFPenvΔ nef. The expression vectors for either envelope gene of the 4070A amphotropic Moloney murine leukemia virus (MoMLV), p-env (32), or vesicular stomatitis virus glycoprotein (VSV-G), pMDG (31), were described elsewhere.

Recombinant HIV-1 production. Based on the method described by Zhao-Emonet et al. (45), human colon cancer -derived HCT116 cells (ACTT CCL247) are co-transfected with either p-env or pMDG, and one of the following two plasmids, pNL4-3/EGFPenv, and pNL4-3/EGFPenvΔ nef, for the production of pseudotyped HIV-1 virions using polyethylenimine (PEI, 25 kDa, Sigma-Aldrich, St. Louis, MO, USA). At 24 h after the transfection, the culture medium was replaced with fresh medium after being washed with PBS and cells were further incubated for the additional 48 h. After the incubation, culture supernatant was collected, filtrated through a 0.2 µm filter, and measured for the concentration of p24 antigen by the following procedure before being kept frozen at -150°C for further manipulation.

P24 Antigen capture assay by ELISA. Quantification of the HIV-1 p24 gag protein in the culture supernatant was done as described previously (41). Briefly, the bottom of Immulon II plates was coated with anti-HIV-1 p24 monoclonal antibodies (mAbs) (183-H12-SC) (6, 39), and the culture supernatant was added to the plates for 30 min. After being washed five times with PBS, biotinylated human anti-HIV immunoglobulin (NIH AIDS Research and Reference Reagent Program, Catalog No. 3957) was further added to determine the concentration of p24. As a standard, HIV-1 p25/24 Gag protein (NIH AIDS Research and Reference Reagent Program, Catalog No. 382) was used (36).

Establishment of HIV-sensitive CD4+ T clones by transformation with HVS. CD4+ T cell clones were established from PBL by growth transformation with HVS as previously described (3, 44). Briefly, peripheral blood mononuclear cells (PBMC) were isolated from freshly drawn blood of HIV-1-negative donors on a Ficoll-paque gradient (Amersham-Pharmacia, Uppsala, Sweden) and adherent cells were separated by incubation on plastic tissue culture plates for 2 h at 37°C to obtain PBL. Non-adherent PBL were resuspended in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Moregate, Bulimba, QLD, Australia), 2 mM L-glutamine, penicillin (100 units/ml), and streptomycin (100 µg/ml) (all from Invitrogen, Carlsbad, CA, USA) and infected with HVS at a multiplicity of infection (MOI) of 0.1. Infected cells were cultured in bulk for 2–3 weeks until immortalization was evident and then cloned at 0.1–1.0 cells/well in 96-well culture plates with irradiated allogeneic PBMC (105 cells/well) cultured in RPMI complete T-cell medium (CTM) (38) containing 10 U/ml of recombinant IL-2 (rIL-2) (Biosource, Camarillo, CA, USA). After confirmation of the expression of CD4 and co-receptors such as CCR5 and CXCR4 by flow cytometry (FACScan, BD Immunocytometry Systems, San Jose, CA, USA), the cloned T cells were used for the experiments.

Transduction of HCT116 cells. On day 1, HCT116 cells were plated in 6-well plates at 105/well. On day 2, the cells were washed once with PBS and incubated with 500 µl of thawed viral supernatant containing an appropriate amount of p24 antigen with polybrene at 8 µg/ml (Sigma-Aldrich). After 48 h of incubation, the cells were harvested by trypsinization, fixed with 4% paraformaldehyde (PFA) and analyzed by flow cytometry to determine the efficiency of transduction by EGFP expression.

Transduction of HVS-T CD4+ cells. HVS-T cells (1 × 105) were pelleted by centrifugation and resuspended in thawed viral supernatant containing an appropriate amount of p24 antigen with 20 µg/ml of DEAE dextran (Sigma-Aldrich), centrifuged at 1200 g for 2 h at 32°C. Then the cells were washed with PBS and further incubated in CTM containing 10 U/ml of rIL-2.
RESULTS
Generation and infectivity of replication-defective pseudotyped virions
As shown in Fig. 1, in order to detect virus infected cells easily as well as to study the protective immunity organized by the CTLs, firstly we have designed an env mutant HIV-1 expression plasmid, in which the stretch between positions 6159 and 7612 in the env gene of the HIV-1 pNL4-3 strain was replaced by the EGFP gene (pNL4-3/EGFPenv) since env does not seem to be critical for recognition by those CTLs observed among protected individuals sensitized with nef-deleted HIV-1 or SIV as described above. Next, we prepared two distinct plasmids for pseudotyped virus particles covering the env-deleted plasmid in trans to obtain highly infectious virions. One was a plasmid encoding amphotropic envelope (Amph-ENV) from 4070A Moloney murine leukemia virus (MoMLV) termed p-env (32) that would fuse the target cell membrane at neutral pH to transfer internal viral genes. The other was a plasmid encoding vesicular stomatitis virus glycoprotein (VSV-G) termed pMDG (31) that would use the endocytic low pH route to insert viral genes.

HIV-1 Genomic RNA (pNL4-3)

\[ \text{pNL4-3/EGFP}_{\text{env}} \]

\[ \text{pNL4-3/EGFP}_{\text{env} \Delta \text{nef}} \]

Fig. 1 Construction of the plasmids.
pNL4-3/EGFP_{env}; The EGFP gene was inserted between position 6159 and 7612 of pNL4-3. Then, a frameshift was inserted at the XhoI site (8887) in nef (pNL4-3/EGFP_{env}Δnef). Those plasmids were co-transfected to HCT116 cells with VSV-G-expression plasmids (pMDG) to produce pseudotyped recombinant HIV-1 virions.

Based on a previous observation, HCT116 cells, a human cell line isolated from a patient with colonic carcinoma (4), were transiently co-transfected with pNL4-3/EGFPenv and one of the above plasmids, p-env or pMDG for pseudotyping. The pseudotyped viral particles were able to infect target cells without producing progeny and thus we could compare the efficacy of single-cycle infectivity between the virions. Then, collected supernatants from those transfectants were used to infect the HCT116 cells and the extent of transduction was measured by monitoring GFP expression by FACS analysis. As shown in Fig. 2A, when the same amount of virions (corresponding to 2 ng of p24 antigen) was added to HCT116 cells, the percentage of GFP-positive cells infected with recombinant HIV-1 particles encoding pNL4-3/EGFPenv pseudotyped with VSV-G was far higher than that with Amph-ENV pseudotyped virions after 48 h of infection.

Then, using the VSV-G-pseudotyped virions, we titrated the concentration of p24 antigen in the supernatant for HCT116 infection. As represented in Fig. 2B, the percentage of GFP-positive cells was increased in a dose-dependent manner when we added those pseudotyped virions to 10^5 HCT116 cells. We also tried to confirm whether the GFP-positive cells infected with VSV-G pseudotyped viruses could actually produce HIV-1 antigens. Using a cell sorter system (FACS Vantage (BD Immunocytometry Systems)), GFP-positive cells and -negative cells were separated and the p24 antigen concentration was determined in each group. The result indicated that p24 antigen could be measured among GFP-positive cells but not among negative cells (data not shown). Thus, cells detected via GFP by flow cytometry would express HIV-1 gene products.

Since HIV-1 and HIV-1-based vectors can generally infect non-dividing cells (24), our modified HIV-1 viral particles pseudotyped with VSV-G could also transduce non-dividing cells. Therefore, we examined whether the VSV-G pseudotyped recombinant HIV-1 particles could transduce the irradiated (G2/M-arrested) HCT116 cells. First, we analyzed the cell cycle status of the irradiated HCT116 cells by propidium iodide staining as shown in Fig. 2C. Without irradiation, 66.3% of the HCT116 cells were in G1 and only 14.1% were in G2/M state (M1 and M2, left panel of Fig. 2C, respectively). In contrast, when the cells were irradiated with 5000 rad, 72.4% of the cells were arrested in non-dividing condition of G2/M (M2, right panel of Fig. 2C) which MoMLV-based vector pseudotyped with either Amph-ENV or VSV-G could not
Fig. 2 Infectivity of replication-defective pseudotyped virions.
A) Comparison of transduction efficiency in the recombinant pseudotyped HIV-1 virions. The infectivity of the recombinant HIV-1 virus encoding the EGFP gene in env (pNL4-3/EGFPenv) pseudotyped with either Amph-ENV or VSV-G was compared. HCT116 cells (1 × 10⁵) were incubated with the culture supernatant containing 2 ng of HIV-p24 pseudotyped virions for 48 h and analyzed by FACScan.
B) Titration of HIV-p24 antigen concentration on EGFP expression. 10⁵ HCT116 cells (1 × 10⁵) were infected with VSV-G-pseudotyped virions at the indicated dose. Three days after the infection, cells were collected and analyzed with FACS.
C) Effect of 5000 rad-irradiation on the cell cycle of HCT116 cells. Two days after the irradiation, the HCT116 cells were incubated with 50 μg/ml propidium iodide, 0.1% citrate, 0.2% NP-40 solution for 30 min and analyzed with FACS for their DNA content. Without irradiation, 66.3% of the HCT116 cells were in G1 state (M1, left panel), while 72.4% of the HCT116 cells were arrested in G2/M (M2, right panel) and only 13.2% were in G1 (M1, right panel) when the cells were irradiated at 5000 rad.
D) Transduction of irradiated (G2/M arrested) non-dividing HCT 116 cells. HCT 116 cells were irradiated at 5000 rad and distributed in a 12-well plate at 10⁵ cells/well. After 24 h for adhesion, the irradiated cells were further incubated with the supernatant containing 2 ng of HIV-p24 pseudotyped virions. Two days later, the cells were fixed with 4% PFA and analyzed by FACS.
infect (data not shown). However, with VSV-G pseudotype recombinant viral particles derived from pNL4-3/EGFPenv, we observed that 5000 rad-irradiated HCT116 cells could be infected as efficiently as, or even more efficiently than, untreated HCT116 cells, as demonstrated in Fig. 2D. Consequently, we could establish pseudotyped HIV-1 virions having the EGFP marker gene with high infectivity for both dividing and non-dividing cells that were easily identified either by flow cytometry or fluorescent microscopy.

Effect of nef gene deletion from pseudotyped virions on virus infectivity

Using fluorescent microscopy without fixing or staining, we confirmed that supernatant containing 2 ng of p24 antigen was capable of generating GFP-positive cells at around 50% when 10^5 HCT116 cells were infected with the recombinant HIV-1 virus encoding pNL4-3/EGFPenv pseudotyped with VSV-G three days after the infection (left panel of Fig. 3A).

Because those highly infective pseudotyped virions contained the nef gene, the infected cells might lose their capacity to present internal HIV-1 antigens to specific CTLs via class I MHC molecules. Thus, in order to establish recombinant viruses that could present HIV-1 antigens in association with class I MHC molecules, we attempted to make nef-deleted pseudotyped virions by inserting a frame shift at the Xhol site within nef and filling the recessed 3'-terminal end with Klenow fragments of DNA polymerase and dNTP as described in Materials and Methods.

As represented in the right panel of Fig. 3A, more than 60% of the HCT116 cells expressed GFP when 10^5 HCT116 cells were infected with the same amount of nef-deleted recombinant HIV-1 virus encoding pNL4-3/EGFPenvΔnef pseudotyped with VSV-G three days after the infection. We also tried to infect freshly isolated peripheral blood mononuclear cells with those nef-deleted recombinant HIV-1 particles and confirmed that 19.3% of them could be infected, whereas much higher percentage of cells (27.2%) could be infected with the recombinant HIV-1 having intact nef gene (data not shown).

Therefore, although the deletion of the nef gene would usually abolish the infectivity of HIV-1 bearing the authentic envelope proteins, the established pseudotyped virions containing pNL4-3/EGFPenvΔnef showed relatively higher infectivity.

Then, we tried to compare the kinetics of infectivity between those two pseudotyped virus particles. As shown in Fig. 3B, the infectivity of each virion reached a maximal level at around a week after the infection with a nearly equal amount of p24 antigen (2 ng). Again, a slight higher infectivity was observed by flow cytometry when 10^5 HCT116 cells were infected with pseudotyped virions containing the nef-deleted pNL4-3/EGFPenvΔnef (right panel of Fig. 3B). However, almost the same amount of HIV-p24 antigen in the culture supernatant was produced from 3 to 6 days after the infection (Fig. 3C). These results indicate that VSV-G pseudotyped virions containing the nef-deleted pNL4-3/EGFPenvΔnef also have a strong capacity to transduce the target cells with the HIV genome.

Effect of nef gene deletion in pseudotyped virions on surface marker expression of HVS-transformed CD4+ T cells

Next we asked whether those VSV-G pseudotyped virions could infect HIV-sensitive CD4+ T cells to present internal HIV-1 gene product associated with class I MHC molecules which might be recognized by HIV-specific CD8+ CTLs. So far, autologous Epstein-Barr virus (EBV)-transformed B-cell lines infected with recombinant vaccinia viruses expressing the various HIV-1 antigens have been most commonly used as target cells for such CTLs. However, B-cells from humans or monkeys will not usually be infected by HIV or SIV and thus such recombinant vaccinia virus-infected B-cells do not seem to present the actual target antigens for specific CTLs found in individuals with acquired protective immunity.

Based on recent findings, we attempted to generate transformed CD4+ T cells from human PBL taking advantage of HVS. We have successfully induced CD4+, CXCR4+, CCR5+ T cell clones, termed HVS-T, sensitive to a variety of HIV isolates and could measure p24 antigen production in those HIV-infected HVS-T (Fig. 4). However, both class I MHC and CD4 molecules were downmodulated on the HIV-infected HSV-T and thus internal HIV-1 antigens would not be presented.

As shown in Fig. 4, we have confirmed the production of HIV-p24 antigen in GFP-positive cells when the HSV-T were infected with VSV-G pseudotyped virions bearing either pNL4-3/EGFPenv or pNL4-3/EGFPenvΔnef and the level of p24 production for those two distinct pseudotyped virions were almost equal. In addition, as expected, although class I MHC and CD4 molecules as well as class II MHC, B7-2 and Fas molecules were not downmodulated at all when HSV-T were infected with the nef-deleted pseudotyped virions, both class I MHC and
Fig. 3 Infection of HCT116 cells and production of HIV-p24 viral protein by pseudotyped virions with or without the nef gene.

A) Fluorescent microscopic analysis. HCT116 cells (1 X 10⁵) were infected with the supernatant containing 2 ng of HIV-p24 pseudotyped virions derived from either the pNL4-3/EGFPenv construct (left panel) or nef-deleted pNL4-3/EGFPenvΔnef construct (right panel). Photographs indicate the cells after 3 days of infection observed with a fluorescent microscope.

B) Kinetics of GFP-positive cells. Six wells of 10⁵ HCT 116 cells were infected with the supernatant containing 1 ng of HIV-p24 pseudotyped virions derived from either the pNL4-3/EGFPenv construct (left panel) or nef-deleted pNL4-3/EGFPenvΔnef construct (right panel). The cells of each well were collected and analyzed by FACScan at the indicated times (1, 3, 8, 11, and 14 days) after infection.

C) Production of HIV-p24 antigen. HCT116 cells (1 X 10⁵) were infected with the supernatant containing 2 ng of HIV-p24 pseudotyped virions derived from either the pNL4-3/EGFPenv construct or nef-deleted pNL4-3/EGFPenvΔnef construct. Three days after the infection, the cells were washed three times with PBS and fresh medium was added. Six days after the infection, culture supernatant was collected and p24 concentration was measured by ELISA as described.
CD4 molecules were strongly downregulated with the virions having nef when compared with left-sided EGFP-negative uninfected controls (Fig. 5). Thus, downregulation of class I MHC and CD4 molecules in PBMC-derived T cells can also be induced via an envelope-independent endocytic pathway mediated by HIV-Nef protein.

DISCUSSION

In the present study, we have established VSV-G pseudotyped HIV-1 virions having an EGFP marker gene in the env with high infectivity for both dividing and non-dividing cells like DCs or resting T cells that were easily identified either by flow cytometry or by fluorescent microscopy. Those HIV-1-derived particles were able to infect target cells without producing progeny and thus we could compare the efficacy of single-cycle infectivity between the virions. Also, we could confirm the production of HIV-1 p24 antigen among GFP-positive cells infected with the pseudotyped virions. Thus, the established virus particles pseudotyped with VSV-G enabled us to efficiently express both the EGFP marker gene and the HIV-1 viral gene in the infected cells.

In addition, we have found that VSV-G pseudotyping augmented infectivity about 30-fold in comparison with Ampl-ENV pseudotyping when lentiviral HIV-vector was encapsulated (Fig. 2A). However, a MoMLV-based vector pseudotyped with VSV-G showed no augmentation of infectivity.
when compared with Ampl-ENV pseudotyping (E.S. and H.T., unpublished observation), indicating that the actual infectivity appeared to depend on the internal virus gene construct of HIV-1 synergistically. Because the infectivity of HIV-1 was markedly affected by deletion of the nef gene (7, 29), we have examined the infectivity of nef-deleted pseudotyped virions. The result was similar to a previous report (2) even with the construct inserted EGFP gene in env. Thus, pseudotyping by VSV-G would markedly reduce the requirement of nef for the infection.

As far as we know, similar pseudotyped virions expressing EGFP with a deletion of the nef gene have been reported (15, 17, 25, 30). However, the effect of nef among those EGFP-positive cells which actually produced p24 antigen like HIV-1 infected cells can be compared only by using our highly potent pseudotyped virion combinations, since the EGFP gene was inserted either within the gag/pol region (17), nef region (15, 25), or nef absent construct (30) in those reported virus particles. Thus, the pseudotyped virions shown in this paper enabled us to examine the actual difference between cells infected with HIV-1 with or without the nef gene irrespective of whether the cells are dividing or non-dividing like DCs.

Accumulating evidence indicates that HLA class I-restricted CD8+ CTLs play an important role in immune defense against HIV-1 infection (27). Several assay systems are used for evaluation of the HIV-specific CTL response. Among them, EBV-transformed B-cell lines infected with vaccinia viruses expressing the various HIV antigens have been used as targets. However, the principal host target cells for HIV-1 infection are not B cells but CD4+ T lymphocytes. In addition, the majority of antigens expressed on those recombinant vaccinia virus-infected targets must be vaccinial antigens and thus, CTL activity against those targets might catch only a small variant of HIV-1 antigen. To overcome these problems and to analyze the CTL activity against the actual antigens reflecting HIV-1 infection, the HIV-sensitive target cell system described here seems feasible.

Although it has recently been reported that HIV-1 Nef downregulated the surface expression of class I MHC molecules which must be critical for CTL recognition of the HIV-1 antigens, the percentage of infected T cells was not high since they used HIV-1 envelope-coated virions that might fuse with the cell membrane to deliver viral genes (9). If the nef gene was deleted from the virions, the viral particles would have reduced infectivity, and consequently the effect of nef would become hard to examine. As demonstrated here, when we used an alternate endocytic pathway to transduce viral genes into the established HIV-sensitive T cells by VSV-G pseudotyping, expression levels of the viral genes were far higher and the effect of nef in the cells was much clearer. The nef-deleted VSV-G pseudotyped virions did not affect either class I MHC or CD4 expression, but produced HIV-1 p24 antigen in the infected T cells at almost the same amount produced by infection with nef-intact virions. Therefore, those nef-deleted virions would be a useful tool for analyzing the actual target molecules produced by infected T cells recognized by CTLs having protective capacity in vivo and thus, will offer new ways to develop vaccine.

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