ORIGINAL

Generation and characterization of APOBEC3G-positive 293T cells for HIV-1 Vif study

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Abstract : We have established a number of 293T cell lines that express a human anti HIV-1 factor APOBEC3G. Out of seven cell clones examined, four were readily demonstrated to express APOBEC3G by immunoblotting analysis. In particular, two clones (A3G-C1 and -C4) were found to produce a much higher level of functional APOBEC3G relative to that by pooled cell clones. The transfection efficiency of all these cell clones were similar to that of the parental cells, producing a comparable level of virions upon transfection of wild type and *vif*-minus proviral DNA clones. Furthermore, the expression level of APOBEC3G in the best cell line (A3G-C1) was far much higher than those of an APOBEC3G-positive lymphocyte cell line and peripheral blood mononuclear cells. We finally monitored the incorporation of APOBEC3G into virions produced in A3G-C1. APOBEC3G was easily detected in progeny viral particles upon transfection of *vif*-minus proviral clone but not of wild type. These results indicated that our new A3G-C1 cell line is eminently useful for various studies on the interaction of human APOBEC3G and HIV-1 Vif. J. Med. Invest. 54 : 154-158, February, 2007

Keywords **:** *HIV-1, Vif, APOBEC3G, 293T*

INTRODUCTION

The apolipoprotein B mRNA-editing enzyme-catalytic polypeptide-like 3G (APOBEC3G) is a potent anti-HIV-1 cellular factor that modifies minus strand viral DNA during reverse transcription, resulting in either its degradation or its integration into host chromosomal DNA as a hypermutated provirus (1-3). The deleterious effect of APOBEC3G on HIV-1 comes from its packaging into progeny virions at the stage of assembly in the viral replication cycle (4, 5). HIV-1 Vif impedes the encapsidation of APOBEC3G into nascent virions (6-9). In the APOBEC3G-expressing cells such as a lymphocyte cell line named H9 and peripheral blood mononuclear cells (PBMCs), HIV-1 without functional *vif* gene is unable to grow (10, 11), and therefore, these cells are called non-permissive. Natural target cells of HIV-1 in infected individuals are thought to be all non-permissive.

The non-permissive target cells, which have been routinely used for biological and/or biochemical studies on the interaction of Vif and APOBEC3G, are of lymphocyte cell lineage. It has been well established that transfection of lymphocytic cell lines and primary lymphocytes with test DNAs are very inefficient, and that reproducible results are difficult to obtain. The APOBEC3G-positive cell lines with high transfection efficiency were obviously required

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for molecular virological analyses of Vif and its target APOBEC3G. To the best of our knowledge, however, there has been only one reported monolayer cell line which steadily expresses APOBEC3G and is very susceptible to transfection (8). In this study, we have established stable APOBEC3G-positive 293T cell lines, which are very sensitive to and suitable for transfection analysis. We show here clearly that the cell line designated 293T/A3G-C1 produced a much higher level of APOBEC3G functional against HIV-1 than that by natural target cells.

MATERIALS AND METHODS

Cells

A monolayer cell line 293T (12) was maintained in Eagles's minimal essential medium containing 10% heat-inactivated fetal bovine serum. A lymphocytic cell line H9 (13) was maintained in RPMI-1640 medium containing 10% heat-inactivated fetal bovine serum. Human PBMCs were prepared essentially as previously described (14).

Transfection

293T cells were transfected by the calcium-phosphate co-precipitation method as previously reported (15).

Western immunoblotting

Immunoblotting analysis using anti-Myc (Invitrogen), anti-APOBEC3G (NIH AIDS Research and Reference Reagent Program, Catalog no. 9906), anti-β-actin (Sigma), or anti-HIV-1 Gag p24 (NIH AIDS Research and Reference Reagent Program, Catalog no. 6521) was done essentially as previously described (16).

Reverse transcriptase (RT) assay

RT assay using ^{32}P-dTTP has been previously described (17).

MAGI assay

Infectivity of virions produced in various 293T cell lines transfected with proviral DNA clones was determined in MAGI cells as previously described (18).

Immunofluorescence (IF) assay

IF assay has been carried out essentially as previously described (19). In this study, cells for IF assay were fixed with ice-cold methanol for 10 min. First antibody used for this assay was anti-APOBEC3G (no. 9906).

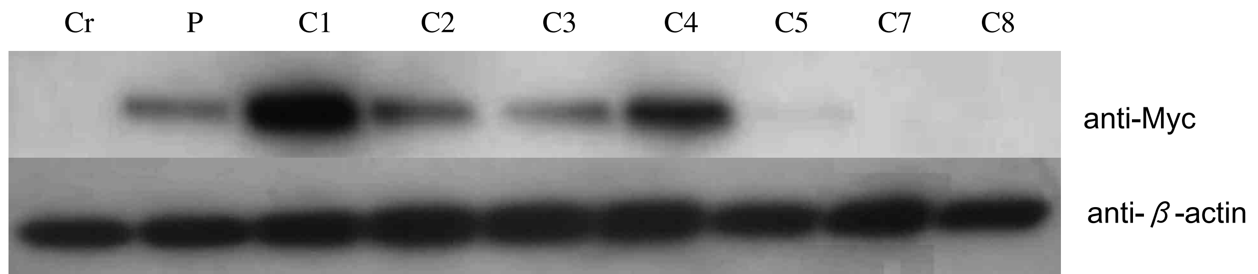
DNA constructs

An infectious DNA clone of HIV-1 designated pNL432 (15) and its *vif*-minus mutant designated pNL-Nd (11) has been previously described. An expression vector of human APOBEC3G designated pcDNA-APO3G (9) and a selection vector designated pTK-Hyg (Clontech) were used for the establishment of APOBEC3G-positive 293T cell lines.

RESULTS AND DISCUSSION

Most target cells routinely used for HIV-1 infection are of lymphoid cell lineage, and it is generally difficult to introduce molecular clones into them by transfection. We were interested in analyzing, by transfection experiments, the interaction of a cellular innate anti-retroviral factor APOBEC3G and HIV-1 Vif, which counteract the activity of APOBEC3G. Recent availability of the expression vector of APOBEC3G (pcDNA-APO3G) (9) prompted us to generate monolayer cell lines producing APOBEC3G. We selected 293T cell line (12) for this purpose, because its transfection efficiency is exceptionally high and it produces a huge amount of progeny virions upon transfection of HIV-1 proviral clones.

In order to establish stable cell lines expressing APOBEC3G, 293T cells were co-transfected by the calcium-phosphate co-precipitation method (15) with the pcDNA-APO3G (or pUC19 as control) and pTK-Hyg at the ratio of approximately 20 : 1, and cultured in the presence of hygromycin (200μg/ml) for selection. Of numerous cell clones thus obtained, seven were examined for the expression of APOBEC3G by Western immunoblotting analysis (Fig. 1A) as previously described (16). To see the average expression level, dozens of cell clones were pooled and cultured, and checked also for the APOBEC3G. Quite unexpectedly, only 4/7 were apparently APOBEC3G-positive. Among the four clones, two (A3G-C1 and A3G-C4) showed a higher expression level than that of the other clones and the pooled sample. The transfection efficiency of these cells were then compared. The cells were transfected by proviral clones as above, and RT production into the culture supernatants on day 2 post-transfection was determined as previously described (17). As shown in Fig.1B, no significant difference in the results was noted. The observed transfection efficiency for these cells was similar to that of parental 293T cells (data not shown).



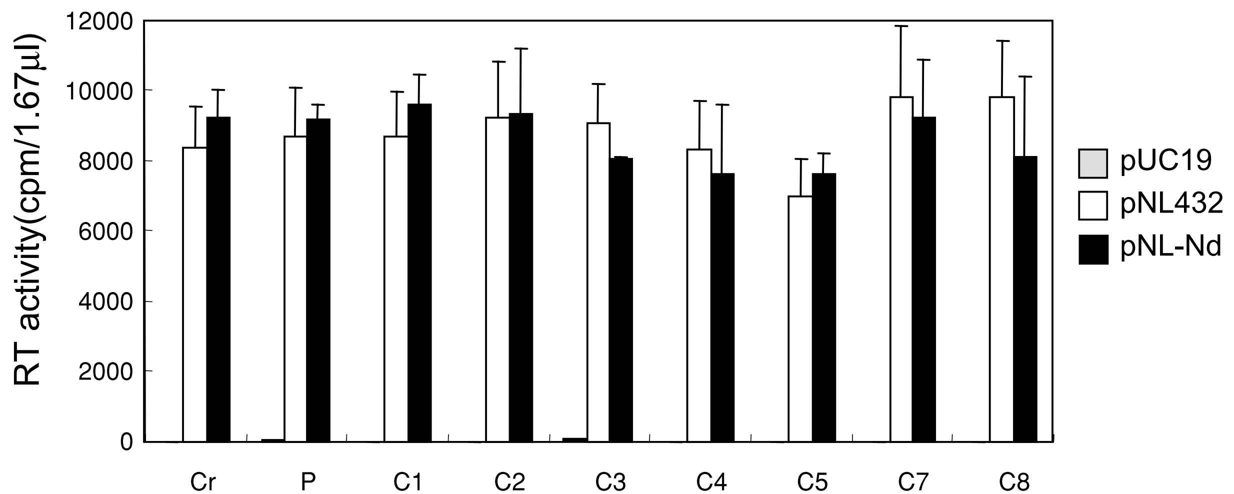


Fig. 1 : Characterization of various 293T clones established in this study. (A) Expressin levels of APOBEC3G in the cell lines established by co-transfection of pcDNA-APO3G containing Myc epitope sequence and pTK-Hyg (A3G-C1 to A3G-C8). Lysates were prepared from the cell lines, and analyzed by immunoblotting using antibodies indicated. Cr, the cell line was obtained by co-transfection of pUC19 and pTK-Hyg ; P, pooled A3G cell clones. (B) RT production in various cell lines upon transfection of proviral clones. Each cell clone indicated was transfected with pNL432 or pNL-Nd, and two days later, RT activity in the culture fluids was determined. Cr and P, the same as above.

To determine whether the A3G-C1 and A3G-C4 are expressing functionally active APOBEC3G, i.e., have the ability to suppress the replication of HIV-1 without the *vif*, the infectivity of progeny virions produced from transfected A3G-C1 and A3G-C4 was assessed in MAGI cells which are routinely used as indicator cells for HIV-1 infection (18). As is clear in Fig. 2, both cell lines consistently expressed biologically active APOBEC3G like naturally occurring non-permissive cells. In total, based on the results in Figs. 1 and 2, we concluded that A3G-C1 is the best clone here.

Next, we comparatively analyzed the expression of APOBEC3G in lymphocytic H9 cells, natural target PBMCs and newly established A3G-C1 cells. These

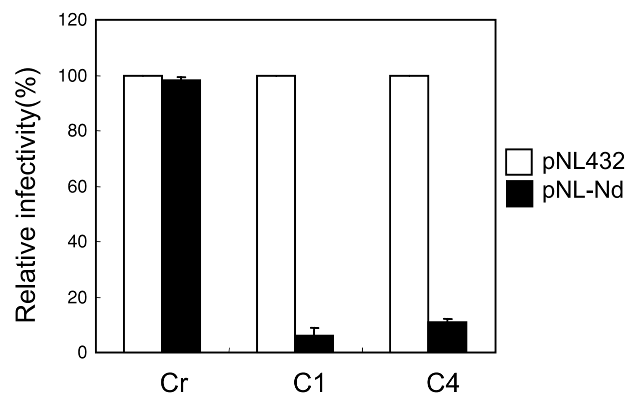


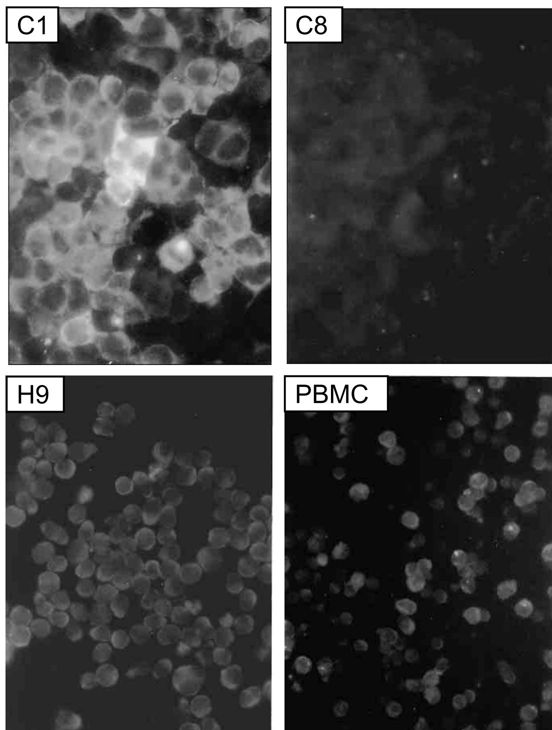
Fig. 2 : Viral infectivity of virions produced in various 293T cell lines. Virus samples were prepared from cells (A3G-C1, A3G-C4 and Cr) transfected with pNL432 or pNL-Nd, and their infectivity was determined in the MAGI indicator cells (18). Cr, the same as in Fig.1.

cells were examined by indirect IF (19) and Western immunoblotting (16) analyses. Fig. 3A shows the results of the IF assay. Although the intensity of IF in A3G-C1 cells was much stronger than that of H9 and PBMC, APOBEC3G predominantly localized in the cytoplasm of the three cell types as judged by IF. It was also noted that some cell populations in A3G-C1 was producing a particularly high level of APOBEC3G as judged by IF. The biological meaning of this observation is not presently known. To quantitatively compare the expression level of APOBEC3G in various cell types, lysates were prepared from them and subjected to immunoblotting analysis as shown in Fig. 3B. In good agreement with the results in Fig. 3A, A3G-C1 expresses far much higher level of APOBEC3G than that of the others.

We finally monitored the incorporation of APOBEC3G from A3G-C1 cells into viral particles in the absence of Vif. A3G-C1 and control 293T cells were transfected with pNL432 or pNL-Nd, and progeny virions produced on day 2 post-transfection were collected and concentrated by ultracentrifugation as previously described (20). As shown in Fig. 4, quite expectedly, only the virions released from A3G-C1 cells transfected with the *vif*-minus mutant clone contained the APOBEC3G. In addition, we noticed no abnormal Gag profile in the mutant virions produced from A3G-C1 cells, suggesting that the Vif may not affect the virion morphology of HIV-1.

In conclusion in this report, the A3G-C1 cell line, a newly established 293T subline, was demonstrated to be very suitable for biological and molecular biological studies on HIV-1 Vif. It expressed a high level of APOBEC3G (Figs. 1 and 3). The APOBEC3G expressed in it was biologically functional (Figs. 2 and 4). Furthermore, it was very sensitive to transfection and produced a high level of HIV-1 virions (Fig.1). By using A3G-C1 cells, we would be able to analyze the molecular basis for interaction of HIV-1 Vif and APOBEC3G more easily and definitely.

A



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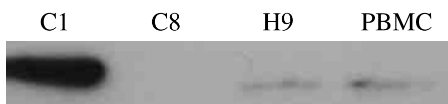

Fig. 3 : Expression of APOBEC3G in 293T/A3G-C1, 293T/A3G-C8, H9 and PBMC cultures. Expression of APOBEC3G in various cell types was monitored by indirect IF (A) and immunoblotting (B) assays. A3G-C8 was used as a negative control (see Fig.1). (A) For detection of APOBEC3G, anti-APOBEC3G (no. 9906) and FITC-conjugated anti-rabbit IgG antibodies were used as 1st and 2nd antibodies, respectively. (B) For detection of APOBEC3G, anti-APOBEC3G antibody (no. 9906) was used.

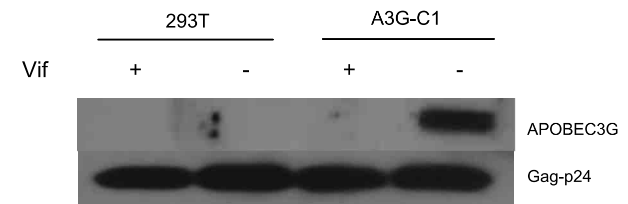

Fig. 4 : Incorporation of APOBEC3G into HIV-1 virions produced in the absence of Vif. Virus particles were prepared from 293T cells and A3G-C1 transfected with pNL432 (+) or pNL-Nd (-) as previously described (20), and their lysates were made for immunoblotting analysis. For detection of APOBEC3G and viral p24, anti-APOBEC3G (no. 9906) and anti-Gag p24 (no. 6521) antibodies, respectively, were used.

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