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HUMAN CELL LINES STABLY EXPRESSING HIV env and tat GENE PRODUCTS

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A DNA fragment containing the tat, rev and env genes of the human immunodeficiency virus type 1 was inserted into the retroviral vector pZIPneoAU3. The resulting plasmid penvAU3 was transfected into HeLa and ψCRIP cells. Resulting recombinant retroviruses were used to infect HeLa and Jurkat cells. Immunoprecipitation analysis of stable transformants showed the expression of HIV env glycoproteins gp160, gp120 and gp41. Transactivation assays with a plasmid containing the gene for chloramphenicol acetyltransferase linked to HIV promoter-enhancer sequences demonstrated the expression of functional tat. These cells constitute virus-free tools for functional and structural studies of native env and tat.

The envelope glycoprotein of HIV, the etiological agent of the acquired immunodeficiency syndrome (AIDS), is involved in the recognition and binding of CD4-bearing cells and in the formation of syncytia which lead to cell death (1-4). This envelope glycoprotein is synthesized as a 160 kD precursor which is cleaved by cellular proteases to generate gp120 and gp41, the transmembrane anchor protein for gp120 (5,6). A unique feature of gp120 is its heavy glycosylation, which accounts for about 50% of its molecular weight (2). There is evidence that proper glycosylation of gp120 is essential for CD4 receptor binding and syncytium formation (7-12). Both functions can be suppressed by enzymatic removal of the carbohydrate moiety (7), by treatment with the mannose-specific lectin concanavalin A (8,9), and by inhibitors of glycoprotein processing (10-13). Moreover, purified gp120 but not non-glycosylated recombinant HIV envelope fragments induced the production of interleukin-1 and arachidonic acid metabolites in monocytes (14). These findings stress the importance of HIV glycoprotein envelope molecules in the pathobiology of AIDS, and the need to further study their function.

High levels of envelope glycoprotein expression have been obtained by transfecting CHO cells with a recombinant envelope gene engineered to yield a soluble secreted form of gp120 (15). Since glycoprotein processing is not only a function of the host cell species or tissue of origin, but also

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depends on the primary aminoacid sequence of the protein backbone, these CHO cells may not be an adequate source for studying certain env properties such as glycan structure. To obtain human cell lines capable of synthesizing native envelope glycoproteins, a DNA fragment containing the tat, rev, and env genes was cloned into the retroviral vector pDOL, and the resulting recombinant retroviruses were used to infect CEM cells (18). In another approach, a plasmid harboring the envelope gene under the transcriptional control of the HIV long terminal repeat (LTR) was transfected into tat expressing HeLa cells (19). Recombinant vaccinia virus vectors have also been used to prepare cell lines expressing the HIV envelope products (16-17).

In this paper, we describe alternative approaches to prepare human cell lines (HeLa and Jurkat T cells) stably expressing native HIV envelope glycoproteins in addition to the tat gene product. Such cell lines, constructed independently via transfection or retroviral transduction, represent convenient sources for isolating HIV envelope glycoproteins for structural and functional analysis.

MATERIALS AND METHODS

1. Vector construction. Plasmid pHXBc2 (20) harboring a complete infectious HIV-1 provirus was double digested with SalI-XbaI and subcloned into pBluescript (Stratagene, CA). After digestion with XhoI, the resulting 3.1 kB DNA fragment harboring the tat, rev and env genes was purified by 0.7% agarose gel electrophoresis, electroelution, Elutip column chromatography and ethanol precipitation. After end-repair with T4 DNA polymerase, BclI linkers were added. The DNA was then digested with BclI and cloned into the BamHI site of pZipneoAU3, a retroviral vector derivative of pZipneoSV(X)1 (21), which harbors in its 3'LTR the U3 region of the non-leukemogenic Murine Leukemia Virus Akv (22). The resulting plasmid construct penvAU3 (Figure 1), was propagated in E. coli DH5α cells and isolated by CsCl-ethidium bromide isopycnic centrifugation.

2. Transfection of HeLa cells. Ten μg of penvAU3 were transfected into $1x10^5$ HeLa cells by calcium phosphate coprecipitation (23). HeLa clones expressing the gene for neomycin resistance (neo^R) were selected in Dulbecco's Modified Eagle (DME) medium containing 10% fetal calf serum, L-glutamine, penicillin/streptomycin and 400 μg/ml of the neomycin analogue G418. Expression of HIV gp160, gp120, and gp41 by cloned HeLa neo^R cells was analyzed by immunoprecipitation with patient anti-HIV sera. About $5x10^5$ cells growing in log phase were labelled for 12h in cysteine-free RPMI 1640 medium supplemented with 10% fetal calf serum, penicillin-streptomycin, L-glutamine and 200 μci/ml ^{35}S-L-cysteine (988.9 Ci/mmol). The cells were then washed with phosphate-buffered saline (PBS), lysed with RIPA buffer (0.05 M Tris-HCl, pH 7.2, 0.15 M NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 1% SDS) and cleared by centrifugation for 1h at 100,000 x g as described (25). The cleared lysates were reacted with anti-HIV serum bound to protein A-Sepharose CL-4B, and the immunoprecipitated proteins analyzed by SDS-polyacrylamide gel electrophoresis. After fixation in 10% acetic acid, 50% methanol, the gel was treated with Enhance (New England Nuclear), dried and autoradiographed at -70°C. In another set of experiments, cleared lysates from about $1x10^7$ HeLa cells producing HIV envelope (HeLa env clones) labelled with ^{35}S-cysteine or with ^{3}H-mannose (9) were chromatographed through lentil-lectin Sepharose CL-4B (Pharmacia) (2). After washing the columns with 10 volumes of RIPA buffer without sodium deoxycholate, the bound glycoproteins

were eluted with 3 ml of 0.05 M Tris-HCl, pH 7.2, 0.15 M NaCl, 1% Triton X-100, 0.2 M methyl-α-D-mannoside. The eluted fraction was analyzed by immunoprecipitation (2,25).

3. Transduction of HeLa and Jurkat cells. Plasmid penvAU3 was transfected into the amphotropic murine packaging cell line ψCRIP (26), and stable transformants were selected in G418 as described. Recombinant retroviruses released from neo^R ψCRIP cells were titrated on NIH 3T3 cells for the number of neo^R colonies, yielding about 10^2 - 10^3 colony-forming units/ml. Clones with the highest titers were used to infect HeLa and Jurkat cells. Stable neo^R clones were selected as described above. In the resulting cell lines (Hesenv and Jurkat env cell lines), the expression of env, tat, rev as well as that of the neo^R gene is driven by the Akv U3 region since the U3 region derived from the 3'LTR of the provirus is duplicated in the 5' LTR as a result of retroviral replication. Envelope expression in Jurkat env clones was analyzed by immunoprecipitation after lentil lectin chromatography as before (2).

4. Assay of tat expression. The presence of tat activity in HeLa env, Hesenv and Jurkat env clones was studied as published (27). In brief, 2.4 μg of pU3RCAT [a plasmid harboring the chloramphenicol acetyl transferase (CAT) gene under transcriptional control of HIV LTR promoter/enhancer region] was transfected into $5x10^5$ cells via calcium phosphate coprecipitation. Transfection of 5 ug of pU3RCAT into $5x10^6$ Jurkat env cells was performed by the DEAE-dextran method (24). After 48h, the CAT activity was assayed as described previously (27).

RESULTS

An expression retroviral vector, penvAU3 (Figure 1), harboring the HIV-1 tat, rev and env genes under the transcriptional control of a Moloney Murine Leukemia Virus LTR (21), was transfected into HeLa cells. Neo^R cells were

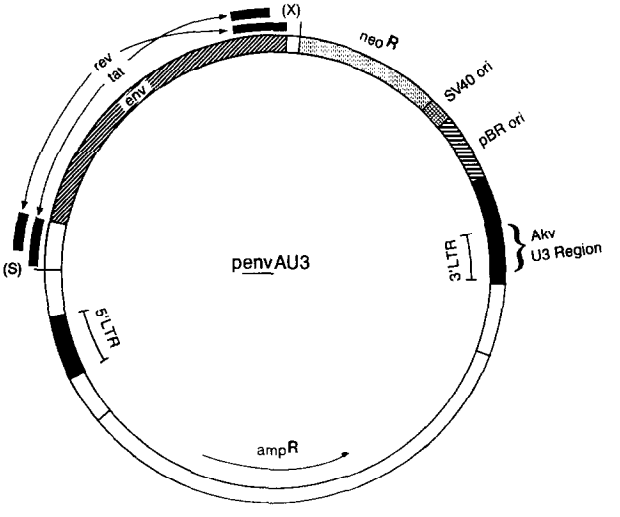

Figure 1. Schematic representation of the plasmid penvAU3. (S) and (X) represent the SalI and XhoI sites of a DNA fragment derived from pHXBc2 harboring the HIV env, tat, and rev genes. Amp^R, ampicillin resistance gene; pBRori, origin of replication of plasmid pBR322; SV40 ori, origin of replication of SV40; neo^R, gene encoding resistance to neomycin or its analogue G418.

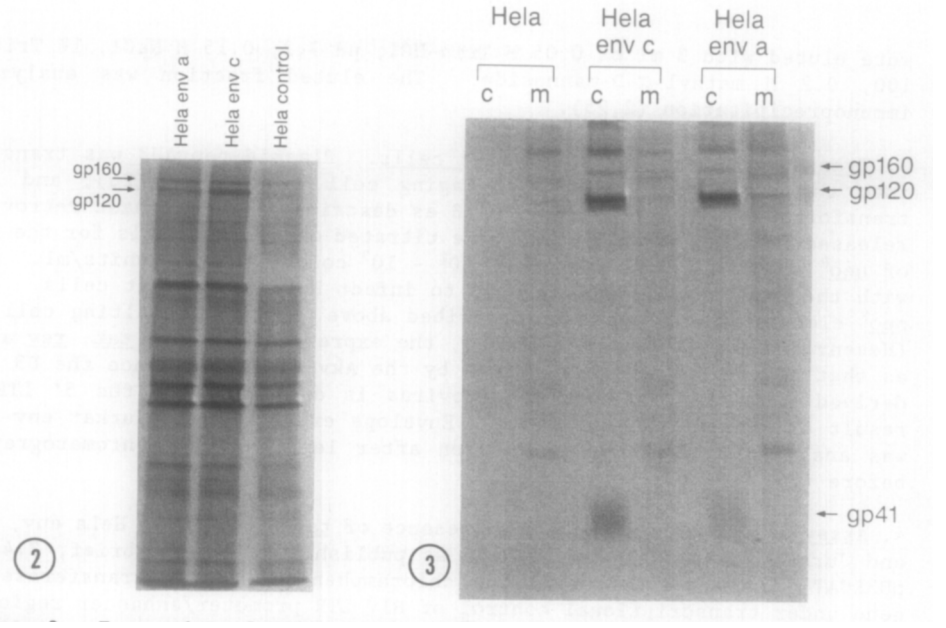

Figure 2. Expression of HIV envelope products by HeLa env cells. Control HeLa, HeLa env a and HeLa env c cells were labelled with ^{35}S-cysteine and env expression was analyzed by immunoprecipitation as described.

Figure 3. Immunoprecipitation analysis of lentil lectin purified HIV env products from HeLa env cells. c, ^{35}S-cysteine labelled cells; m, ^{3}H-mannose labelled cells.

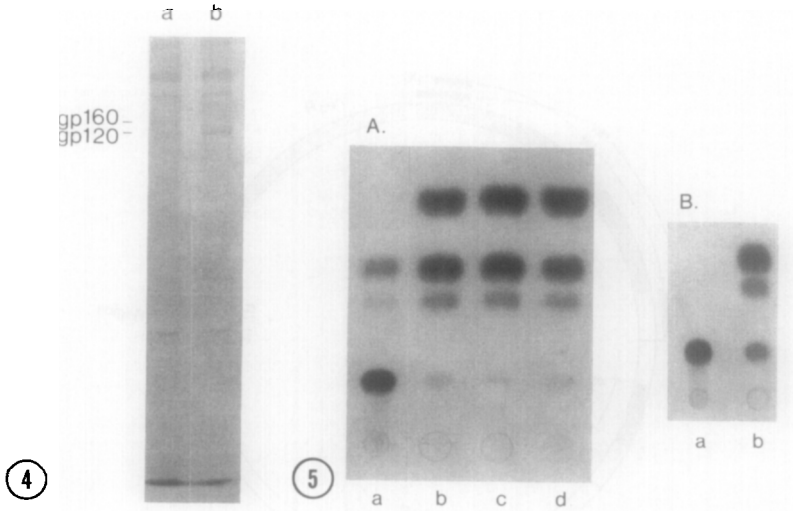

Figure 4. Immunoprecipitation analysis of lentil lectin purified HIV env products from ^{35}S-cysteine labelled Jurkat env cells. a, Jurkat env cells; b, Jurkat control cells.

Figure 5. Transactivation of the HIV-LTR by human cell lines expressing the HIV tat product. Cells were transfected with the pU3RCAT plasmid, harvested 48h after transfection and assayed for CAT activity (15 min). A. HeLa env and Hesenv cells. a, HeLa wild type; b, HeLa env c; c, HeLa env clone 1; d, Hesenv clone 7. B. Jurkat env cells. a, Jurkat control; b, Jurkat env.

selected in medium containing the neomycin analogue G418 and analyzed for HIV-1 env expression by immunoprecipitation of ^{35}S-cysteine labelled proteins with patient anti-HIV serum. This antiserum immunoprecipitated high molecular weight protein bands corresponding to HIV gp160 and gp120. No specific protein bands were seen in wild type HeLa cells (Figures 2 and 3). ^{35}S-cysteine or ^{3}H-mannose labelled glycoproteins isolated from HeLa env clones by lentil-lectin chromatography were also analyzed by immunoprecipitation, which revealed specific bands of 160 kD, 120 kD and 41 kD molecular weight (Figure 3) (2,5,6). Immunoprecipitation of ^{35}S-cysteine labelled Jurkat env cells after lentil-lectin column chromatography also showed expression of HIV envelope glycoproteins (Figure 4), albeit at lower levels as compared to HeLa env cells.

Transfection of HeLa env, Hesenv, and Jurkat env cells with pU3RCAT resulted in high levels of CAT activity. In contrast, transfection of wild type HeLa or Jurkat cells with the same plasmid resulted in the expression of significantly lower CAT activity (Figure 5). In the pU3RCAT construct, the expression of the CAT gene is driven by the HIV promoter/enhancer region which in turn requires tat activity for efficient expression of downstream genes (27-30). The high CAT enzyme levels seen in HeLa env, Hesenv and Jurkat env clones led us to conclude that in addition to env, tat is expressed in these cells.

DISCUSSION

We have constructed HeLa and Jurkat cell lines stably expressing the HIV env and tat gene products (HeLa env, Hesenv, and Jurkat env). Immunoprecipitation analysis of total proteins and of glycoproteins isolated by lentil lectin column chromatography shows the expected molecular weights for the envelope gp160 precursor and for the processed gp120 and gp41 products (2,5,6). These results confirm the correct processing of gp160 by HeLa and Jurkat cells. The higher expression of HIV envelope in HeLa env cells as compared to Jurkat env cells may indicate that cells coexpressing env and CD4 at high levels are selected against. Such cells may die as a consequence of syncytium formation or due to membrane disruption after gp120 fuses to CD4 located on the same cell surface. The latter mechanism for single-cell death of CD4^{+} cells has been postulated previously (31). Our subsequent experiments support the hypothesis that coexpression of CD4 and HIV env by T-cells is disadvantageous: After several months in culture, Jurkat env cells ceased to produce envelope, and Southern blot analysis revealed a deletion of env sequences. The cells, however, retained G418 resistance (data not shown).

Tat activity in HeLa env, Hesenv and Jurkat env clones was demonstrated in transient CAT expression assays. Since env expression has been shown to

require rev activity (32-35), we expect HeLa env, Hesenv and Jurkat env clones to also express the rev gene, an issue we are presently investigating.

Our human cell constructs, expressing HIV env and tat, constitute a biohazard-free model system to study candidate antiviral agents targeted against those functions. Furthermore, since HIV-infected cells may contain an heterogeneous population of envelope glycoproteins as a consequence of reverse transcriptase errors (36,37), these env-expressing cell lines are a unique source of homogeneous envelope glycoproteins for structural analysis and for the study of the cellular pathology induced by these molecules. In addition, penvAU3 and its amphotropic retrovirus derivative may be used to prepare stable cell lines of different tissue- and species origin to study the influence of the host cell on HIV env properties such as e.g. cell-type specific glycosylation.

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