Human T cell leukaemia virus type I env gene-transfected HeLa cells display a decrease in cell fusion ability

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The envelope (env) gene of human T cell leukaemia virus type I (HTLV-I) was inserted into an expression vector, referred to as pHMTenv, under the transcriptional control of the human metallothionein IIa gene promoter (hMT-IIa). When this vector was transiently transfected in HeLa cells treated with hMT-IIa inducers, formation of multinucleated cells was observed, indicating the expression of functional surface and transmembrane glycoproteins. Of several HeLa cell clones transfected with pHMTenv together with a plasmid carrying the neomycin resistance gene and isolated after selection in G418-containing medium, env mRNA was detected in only two, in the presence of hMT-IIa inducers. Viral glycoproteins were found to be weakly expressed, as detected in immunoprecipitation assays of 125I-surface-labelled cells. These env-transfected HeLa cell clones, although unable to form syncytia when cocultivated with untransfected control HeLa cells, retained the capacity to fuse with HTLV-I-producing C91PL T cells. However, a significant decrease in their fusogenic ability was observed, after treatment with hMT-IIa inducers. Under identical experimental conditions, control HeLa cell clones stably transformed with the same plasmid, but lacking the env gene, were still able to fuse with C91PL cells. These observations suggest that a post-transcriptional step in HTLV-I env expression is impaired, probably leading to the establishment of superinfection interference.

The retrovirus human T cell leukaemia virus type I (HTLV-I) is the aetiological agent of adult T cell leukaemia (Poiesz et al., 1980) and tropical spastic paraparesis/HTLV-I-associated myelopathy (Gessain et al., 1985; Osame et al., 1986). As with other retroviruses, HTLV-I entry into susceptible cells results from two consecutive events: the surface glycoprotein gp46 mediates the binding of viral particles to the cellular receptor and then the transmembrane gp21 mediates the fusion between the viral envelope and the cell membrane. Thus, formation of syncytia in cocultures of HTLV-I-producing cells with uninfected indicator cells provides evidence for the presence of receptors for HTLV-I particles on the membrane of the latter cells (Weiss, 1993). To circumvent cell death subsequent to the formation of multinucleated cells, stable expression of the envelope glycoproteins of human retroviruses has been achieved in cells lacking receptors for viral particles (Vile et al., 1991; Ahmad et al., 1993; Bird et al., 1992). Alternatively, transfection of receptor-expressing cells has been performed with plasmids constructed such that the envelope gene is placed under the control of an inducible promoter (Koga et al., 1990).

In our present work, we have used the latter strategy to study the expression of the HTLV-I env gene in clones of HeLa cells transfected with a vector, referred to as pHMTenv, in which the env gene was inserted under the control of the human metallothionein inducible promoter (hMT-IIa). This env vector does not contain the 5' or the 3' LTR and therefore expresses the envelope glycoprotein independently of both Tax and Rex viral regulatory proteins (for review see Cullen, 1992). The transcriptional activity of the human metallothionein promoter hMT-IIa is upregulated in the presence of heavy metal ions and glucocorticoids; the poly(A) signal sequences were those of the thymidine kinase gene of herpes simplex virus. The level of expression of envelope glycoproteins by HeLa cells transfected with this vector was evaluated by counting the number of syncytia in the cultures. As shown in Table 1, a significant number of syncytia was observed, but only when cells were treated with dexamethasone. For the sake of comparison, the transient expression of envelope glycoproteins was also evaluated in HeLa cells transfected by three other expression vectors, which are either Tax- and Rex-dependent (pHenvH), Tax-independent (pMTenvpX) or
Table 1. Syncytia formation in HeLa cells transiently transfected with HTLV-I env-expressing vectors*

<table>
<thead>
<tr>
<th>Vectors</th>
<th>Additional treatment</th>
<th>Number of syncytia†</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>~</td>
<td>0:2</td>
</tr>
<tr>
<td>pHenvH</td>
<td>~</td>
<td>0:2</td>
</tr>
<tr>
<td>pHenvH</td>
<td>Cotransfection with pMTpX‡</td>
<td>132:5±7:5</td>
</tr>
<tr>
<td>pHMTenvpX</td>
<td>~</td>
<td>81:5±5</td>
</tr>
<tr>
<td>pCMVenv</td>
<td>~</td>
<td>250±10</td>
</tr>
<tr>
<td>phMTenv</td>
<td>~</td>
<td>1:5±1:5</td>
</tr>
<tr>
<td>phMTenv</td>
<td>Addition of hMT-IIa inducers§</td>
<td>220±40</td>
</tr>
</tbody>
</table>

* HeLa cells were transfected with env-expressing vectors using the calcium phosphate technique. The pHenvH vector was made by inserting the env gene between the two HTLV-I LTRs. The pHMTenvpX vector, which was derived from the pMTpX construct provided by M. Yoshida, contains the entire 3′ HTLV-I genomic proviral sequence, encompassing both env and pX ORFs, under the transcriptional control of the mouse metallothionein promoter. The pCMVenv vector contains the env gene under the control of the human cytomegalovirus immediate early promoter. The 3′ LTR was replaced by the simian virus 40 transcription termination signals. The phMTenv vector construct carries the env gene under the control of the human metallothionein gene promoter (see text).

† Counting of syncytia obtained 42 h after transfection was performed after May-Grunwald–Giemsa staining. Only multinucleated cells containing more than four nuclei were scored. Values given are the average of three experiments (± SD).

‡ The pMTpX encodes for the Tax and Rex proteins. Syncytia formation was not observed in HeLa cells transfected with pMTpX alone (data not shown).

§ The transfected cells were treated 24 h after transfection and for the last 18 h were treated with 150 µM-ZnCl₂ and 1 µM-dexamethasone. Syncytia formation was not observed in untransfected HeLa cells treated with the hMT-IIa inducers alone (data not shown).

Tax- and Rex-independent (pCMVenv). The highest number of syncytia was observed in cultures of HeLa cells cotransfected with pHenvH and a Tax and Rex expression vector (pMTpX).

These observations led us to use the phMTenv vector to determine the effect of stable expression of the HTLV-I env gene in HeLa cells. Several G418-resistant clones were obtained from HeLa cells after cotransfection with the phMTenv and the pSV2neo vectors and subsequent selective growth in G418-containing medium. Four clones that displayed the same morphological characteristics and growth kinetics as the parental cells were selected for further studies. Total RNA extracted from cells of these clones, either untreated or treated with dexamethasone, was analysed either by Northern blotting or by RT–PCR to evaluate the level of transcription of the HTLV-I env sequences (Fig. 1). Both assays indicated that env mRNA was present only in clones 1 and 26, which were treated with the inducers, and could not be detected in clones 11 and 20. By RT–PCR, a faint band corresponding to the env mRNA was observed in uninduced cells of clones 1 and 26, indicating that the hMT-IIa promoter is not completely silent in these cells.

Expression of envelope proteins at the membrane of cells from clones 1 and 26 was then investigated after labelling of surface proteins with ¹²⁵I and immunoprecipitation with an HTLV-I envelope-specific rabbit antiserum. As shown in Fig. 2, proteins of 46 kDa and 21 kDa were revealed, corresponding to surface and transmembrane glycoproteins, respectively. The labelling of the transmembrane glycoprotein was very weak as its extracellular domain contains only one tyrosine residue whereas the surface glycoprotein contains 16. A longer exposure time was therefore necessary to reveal the 21 kDa protein confirming the presence of the recombinant envelope glycoprotein. Taken together, these observations indicate that a low level of envelope glycoproteins is produced by env gene-transfected HeLa cell clones.

To investigate further how the expression of envelope glycoproteins may affect the cell fusion ability of the HeLa cell env transfected clones 1 and 26, syncytium assays were performed. When the HTLV-I-producing C91PL cells were cocultivated with control HeLa cells (Fig. 3, column 1), a significant number of multinucleated cells was observed. Furthermore, cell fusion was observed in cocultures of env-negative HeLa cells (HeLa, clones of HeLa p36.7 cells and phMTCAT) and cells of clones 1 and 26 with C91PL cells, although in the cocultures of these env-transfected cells the number of syncytia was 37% lower than that observed in cocultures with the env-negative cells. However, an 80% decrease in the number of syncytia was observed in cocultures of C91PL cells with cells from clones 1 or 26, treated with the hMT-IIa inducers (Fig. 3). This decreasing ability of cells to fuse could not be correlated with a non-specific effect of the hMT-IIa inducers, since the number of syncytia was not impaired after addition of these inducers to cocultures of env-negative HeLa cells with C91PL cells (Fig. 3).
Fig. 1. Effects of hMT-IIa inducers on env mRNA expression by phMTenv HeLa cell transfectants. (a) Analysis by Northern blot hybridization. Total RNA of the four phMTenv HeLa cell transfectants (1, 11, 20 and 26), cultivated in the presence (+) or absence (−) of hMT-IIa inducers, was denatured, electrophoresed, transferred to a nylon membrane and hybridized with a 32P-labelled 720 bp XhoI fragment of the env ORF. Chronically HTLV-I-infected T cells (C91PL) and HeLa cells (H36.7) were included in this study as positive and negative controls, respectively. Positions of 18S and 28S rRNA are shown on the left. (b) PCR analysis of env expression in the cells in (a), and in a clone of HeLa cells containing the integrated phMTCAT vector. cDNA prepared by reverse transcription of total RNA incubated in the presence of Moloney murine leukaemia virus reverse transcriptase was amplified using a pair of env primers. The following HTLV-I envelope oligonucleotides (Genset) were used as primers: 5' ATGGGTAATTTCTCGCCACTTT 3' and 5' ACCCCAGGTATGGGCACTTT 3'. Lane M, bp markers.

Fig. 2. Expression of HTLV-I envelope glycoproteins on the cell surface. Cells were labelled with 125I using iodo-beads, lysed and used for immunoprecipitation with an HTLV-I-envelope-specific env rabbit antiserum. The proteins were resolved on a 12% acrylamide gel and detected by autoradiography. The autoradiograph was exposed overnight, but includes a section (the darker part) which was exposed for 1 week to help detection of faint bands. Lane 1, HeLa-hMTCAT (env-negative) cells treated with hMT-IIa inducers; lane 2, HeLa-hMTenv (clone 26) cells; lane 3, HeLa-hMTenv (clone 26) cells treated with hMT-IIa inducers; lane 4, HTLV-I-infected C91PL cells. The sizes of molecular mass markers are shown on the left.

These results therefore suggest that the expression of envelope glycoproteins in HeLa cells stably expressing the HTLV-I env gene negatively interferes with the fusion ability of the cells and that membrane antigens involved in virion entry are blocked by envelope glycoproteins expressed in these env-transfected clones. In addition, the stable expression of the HTLV-I env gene by these HeLa cell clones might lead to the establishment of a superinfection interference, which often occurs through the formation of complexes between viral receptors and envelope glycoproteins expressed at the cell surface (Delwart & Panganiban, 1989; Stevenson et al., 1988). Isolation and identification of such complexes could be of potential interest for the characterization of the HTLV-I receptor (Sommerfelt et al., 1988; Fukudome et al., 1992; Gavalchin et al., 1993). Another possibility is that a direct interaction of the expressed envelope products with a cell membrane component might occur at the cell surface, which could be responsible for the loss of the HTLV-I envelope-mediated fusion properties of HeLa cells. Recently, it was reported that the association of truncated HTLV-I envelope glycoproteins with the cellular membrane produced by the HTLV-I-producing C10/MJ2 T cells may explain the poor fusogenic
phenotype of these cells (Carrington et al., 1994). Alternatively, it cannot be excluded that the processing of the glycoproteins, such as their folding in the endoplasmic reticulum, may be altered and thus interfere with the membrane expression of receptors involved in binding and/or fusion events. This has been shown with the human immunodeficiency virus type 1 envelope glycoprotein precursor gp160 and CD4 antigens (Crisè et al., 1990; Koga et al., 1990). Observations reported in the present study suggest that the regulation of envelope glycoprotein synthesis should be strictly tuned in vivo to avoid death of target cells and to perpetuate the infectious process.

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References


