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The Cytoplasmic Domain of the Human T-Cell Leukemia Virus Type I Envelope Can Modulate Envelope Functions in a Cell Type-Dependent Manner

CLAUDINE PIQUE, DANIÈLE PHAM, THOMAS TURSZ, AND MARIE-CHRISTINE DOKHÉLAR*

Centre National de la Recherche Scientifique UA 1156, Institut Gustave Roussy, rue C. Desmoulins, 94805 Villejuif, France

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C-terminal truncations of the human T-cell leukemia virus type I envelope affected the intracellular maturation and syncytium formation in a cell type-dependent manner. The intracytoplasmic domain appears dispensable for syncytium formation, but its truncation can modulate the envelope functionality in some cell types.

The human T-cell leukemia virus type I (HTLV-I) envelope proteins are synthesized as a precursor protein of 61 kDa (15), which is cleaved into a gp45 surface protein (SU) and a gp20 transmembrane protein (TM). Little is known about the functional domains of the SUgp45 and TMgp20 proteins of the HTLV-I envelope. In other retroviruses, the SU protein is implicated in receptor binding (6, 18, 19, 28), as well as in postbinding events, as was shown for the V3 loop in human immunodeficiency virus type 1 (HIV-1) (1, 22). With HTLV-I, we have shown that the SUgp45 envelope protein bears a region located between amino acids 195 and 205 which is important in envelope-dependent syncytium formation (25). This region is also recognized by a monoclonal antibody inhibiting HTLV-I infectivity (30).

The contribution of the TM proteins of retroviruses to the envelope functionality has been studied mostly with lentiviruses. The TM bears a C-terminal fusion peptide (9, 18), as well as regions implicated in the SU and TM association in HIV-1 (18), and a transmembrane anchorage domain that also plays a role in the fusion capacity of the HIV-1 envelope (13). The role of the intracytoplasmic (IC) domain remains unclear. This IC domain is dispensable for normal intracellular maturation of the envelope glycoprotein in HIV-1 (7, 12, 18), although its presence can stabilize the envelope proteins in certain cell types (8, 11). The exact role of this region in envelope-mediated functions is not clearly established yet. In the absence of the IC domain, the CD4 receptor-binding capacity of the HIV-1 envelope is not affected (7, 12, 18), whereas in HIV-2 deletion of the IC domain increases the CD4-binding capacity of the envelope, which leads to an augmentation of the envelope-mediated cytopathic effect (14). Envelope-mediated fusion is not affected by deletion of the IC domain in HIV-1, since the mutants with deletions are capable of normal syncytium formation (2, 12, 18). Despite a normal syncytium-forming capacity, however, viral transmission is highly affected when the IC domain is absent in HIV-1 (12). Altogether, the results suggest a complex role of the IC domain of the envelope TM protein of lentiviruses.

The IC domain of the TM envelope glycoprotein is very long in lentiviruses (166 amino acids in HIV-1) and shorter in type C and type D retroviruses, including HTLV-I (24 amino acids) and Rous sarcoma virus (RSV) (22 amino acids). As opposed to the situation described above for primate lentiviruses, in RSV no difference in infectivity between virions bearing wild-type envelopes and those bearing IC domain-deleted envelopes could be observed (23). Thus, the importance of the IC domain seems to vary among retroviruses. To obtain further insights into the role of the TM IC domain of retroviruses and define the functional domains of the HTLV-I envelope, we have performed truncations in the HTLV-I envelope IC domain.

Several deletion mutants were constructed by introduction of early termination codons into the HTLV-I envelope expressor plasmid HTE-1, previously described (5). HTE-438 (Fig. 1A) contains a stop codon located before a putative transmembrane region, composed of a hydrophobic stretch between amino acids 446 and 465 (amino acid 1 being the methionine initiation codon). HTE-438 was constructed by introducing an *Nhe*I 12-bp linker at position 6495 (numbered as in reference 27) by using an *Xho*I restriction site. The HTE-438 mutant was transfected into COS-1 cells (simian virus 40-transformed African green monkey kidney cells) (10) by the procedure described by Cullen (4), using DEAE-dextran and chloroquine (Sigma, La Verpillère, France). At 48 h posttransfection, the cells were labeled and lysed for immunoprecipitation with serum from a patient with tropical spastic paraparesis (TSP) as described previously (25). Immunoprecipitations were performed with both the transfected-cell lysate and the transfected-cell supernatant. As shown in Fig. 1, transfection of HTE-438 resulted in secretion of the SUgp45 and the truncated TM in the supernatant (Fig. 1B, lane 4), confirming an anchorage function for the domain located after amino acid 438. As a result of the deletion in the mutant protein, the apparent molecular masses of the precursor and the TM are reduced to 55 and 15 kDa, respectively (Fig. 1B, lane 3). TMgp15 is hardly detected in the HTE-438 mutant compared with the wild-type TMgp20, because the deletion removes a cysteine residue, the amino acid used for labeling the protein.

The HTE-466 and HTE-476 mutants (Fig. 2A) contain an early termination codon in the IC domain. HTE-466 was constructed by introducing an *Nhe*I 12-bp linker at position 6576 by using an *Alu*I restriction site. HTE-476 was constructed by changing the tyrosine codon at position 6607 for a nonsense codon by using site-directed mutagenesis as described previously (24).

* Corresponding author.

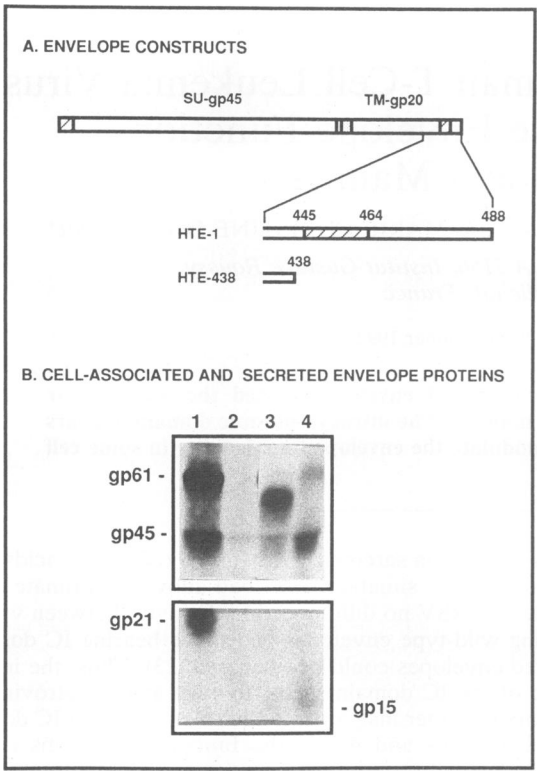

FIG. 1. Schematic representation of the HTE-438 mutant (A) and results of immunoprecipitation of the envelope proteins produced by the wild-type expressor and the HTE-438 mutant (B). The putative anchorage domain, located between amino acids 445 and 464, is indicated (hatched region). In HTE-438, a nonsense codon was introduced at position 438. Lanes 1 and 3, transfected-cell lysates; lanes 2 and 4, transfected-cell supernatants; lanes 1 and 2, wild-type expressor; lanes 3 and 4, HTE-438.

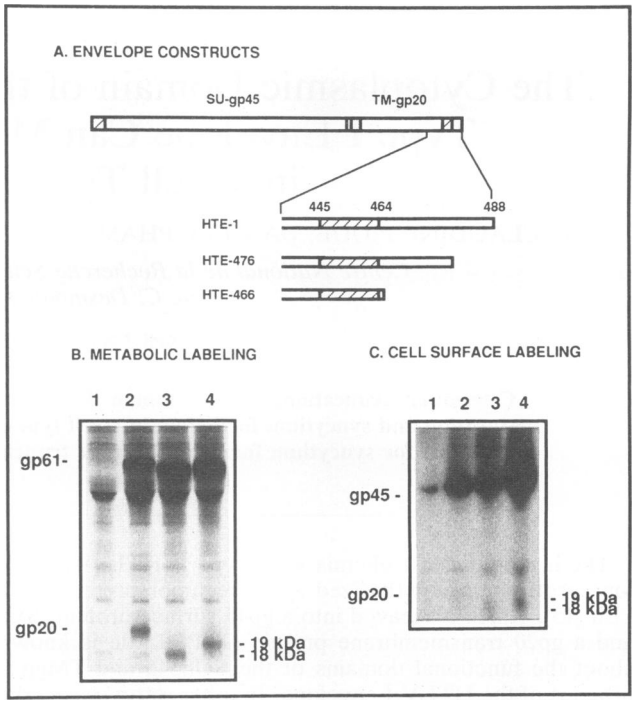

FIG. 2. Schematic representation of the HTE-466 and HTE-476 mutants (A) and intracellular maturation (B) and transport to the cell surface (C) of the envelope proteins produced by the mutants. Proteins were obtained from COS-1 cells transfected with a negative control (lanes 1), HTE-1 wild-type envelope expressor (lanes 2), HTE-466 (lanes 3), and HTE-476 (lanes 4). The putative anchorage domain is shown (hatched region).

The intracellular maturation of the HTLV-I envelope mutants (Fig. 2) having premature termination codons in the IC domain was analyzed after transfection of the corresponding vectors into COS-1 cells (10), K562 cells (20) (derived from a patient with chronic myeloid leukemia with a Philadelphia chromosome), HSB2 cells (16), or 1301 cells (31) (derived from a patient with T-cell leukemia). K562, 1301, and HSB2 cells were transfected by electroporation with 10 μg of plasmids added to 10^7 cells in 250 μl of culture medium. The pulse was performed at 250 V and 960 μF. At 48 h posttransfection, cells were used for immunoprecipitation with a TSP patient's serum after radiolabeling or for a syncytium assay as described previously (25).

Figure 2B shows that in COS-1 cells, the cleavage of the precursor protein was as efficient for the mutants as for the wild-type envelope expressor. As a result of the introduction of premature stop codons, the molecular sizes of both the precursor protein and the TM protein in the mutants were lower than those for the wild type (Fig. 2B, lanes 2 to 4). Pulse-chase experiments showed that the kinetics of envelope synthesis and of precursor cleavage are comparable among envelopes with a mutated IC domain and the wild-type protein (data not shown). SU and TM protein transport to the plasma membrane was also identical among mutated and wild-type envelopes, as shown by cell surface labeling experiments using iodine and lactoperoxidase as previously described (24) (Fig. 2C, lanes 2 to 4). This result also confirms that the region between amino acids 438 and 466 is sufficient to allow normal membrane integration of the envelope proteins.

Since in lentiviruses the IC domain modulates the envelope maturation in a cell type-dependent manner, the function of the HTLV-I IC domain was analyzed in a panel of different cell lines. In these cells, the transfectability is very low, and HTLV-I envelope expression could be detected by immunoprecipitation only in K562 cells (data not shown). Western blot (immunoblot) analysis (using a TSP patient's serum) had to be performed for correct detection of the gp61 envelope precursor in K562, HSB2, and 1301 cells (Fig. 3). Equal amounts of proteins (as measured by a Bio-Rad protein assay) were loaded in the lanes of the sodium dodecyl sulfate-polyacrylamide gel. Two independent experiments were performed. These experiments show that the amounts of precursor detected in two of these cell lines (K562 and 1301) are lower for the mutant proteins than for the wild type (compare, in each case, lanes 3 and 4 with lane 2 [Fig. 3]), as opposed to what was observed with COS-1 cells (Fig. 2B).

The syncytium formation capacities of the mutant and wild-type envelopes were compared after transfection into the different cell lines described above. COS-1-transfected cells were cocultivated with RSV-transformed XC cells (29), which form large numbers of syncytia and are used as indicator cells as previously described (25). For cells growing in suspension, another indicator cell line was used, since syncytia are more easily monitored when both the tested and

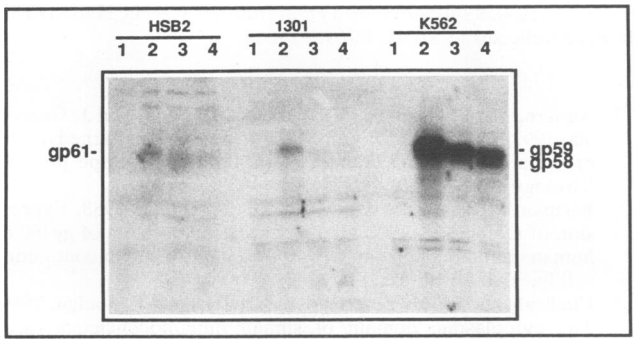

FIG. 3. Expression of envelope proteins after transfection of human cell lines with the HTE-466 and HTE-476 mutants. Western blot analysis was performed with a TSP patient's serum. Cells were transfected with a negative control (lanes 1), HTE-1 wild-type expressor (lanes 2), HTE-476 (lanes 3), or HTE-466 (lanes 4).

the indicator cells grow in suspension. The assay consisted of a coculture of 2×10^5 transfected cells (1301, HSB2, or K562) together with 10^6 indicator cells (1301). Syncytia were scored after 24 h of coculture, and four independent experiments were performed in each case. As expected, HTE-438, the anchorage-negative mutant, was unable to elicit syncytium formation when transfected into COS-1 cells (Table 1). A marked increase in syncytium formation capacity was observed in the two mutants with a deleted IC region, HTE-466 and HTE-476, compared with the wild type when COS-1 or K562 cells were transfected and, to a lesser extent, when HSB2 cells were transfected (Table 1). Transfection of 1301 cells with the mutants, however, resulted in syncytium formation comparable to that observed with the wild-type envelope (HTE-476) or slightly diminished syncytium formation (HTE-466). The addition of a TSP patient's serum to the coculture resulted in the inhibition of syncytium formation with both the wild-type and mutant envelopes, whereas the addition of normal serum to the coculture had no effect (data not shown). Taken together, these results show that the IC domain truncation results in unchanged or diminished (1301 transfected cells) or augmented (COS-1, K562, and HSB2 cells) syncytium formation capacity, with the phenotype conferred by the mutation being cell type dependent. The level of syncytium formation did not correlate with the amount of detectable envelope in the different cells, since in K562 cells, for example, the mutants expressed smaller amounts of envelope than the wild type but formed syncytia more efficiently (Fig. 3 and Table 1). The level of syncytium formation might be inversely correlated to the level of receptor expression reflected in the abilities of the nontransfected cell lines to form syncytia with infected cells (Table 1).

We have analyzed the role of the HTLV-I envelope IC domain in the envelope functions and localized the anchorage domain of the envelope at the plasma membrane. Our results show that when a nonsense codon is introduced at position 438 of the envelope protein, both SUgp45 and truncated TM are released in the supernatant, whereas a nonsense codon introduced at position 466 allows anchorage at the cell surface. This confirms the sequence analysis which predicts that the anchorage region corresponds to a cluster of hydrophobic amino acids located between amino acids 446 and 465. As expected, no syncytium formation was observed with the secreted envelope encoded by the HTE-438 mutant.

In COS-1 cells, in the absence of the IC domain, no modification in the kinetics of the envelope precursor cleavage or the transport of the HTLV-I envelope products at the cell surface was observed. In K562 and 1301 cells, however, a partial or complete truncation of the IC domain (12 or 21 of 22 amino acids in the case of the wild-type IC domain) results in markedly diminished envelope expression. These results show the cell type dependency of the IC domain in envelope intracellular maturation. Similar results have been obtained for HIV-1 (8, 11). For RSV, the IC domain is totally dispensable for normal maturation of the envelope proteins, at least in the cells tested so far (23). Whether the cell type dependency of the IC domain's effect upon envelope maturation is a general feature of retroviruses remains to be established.

We have examined the role of the HTLV-I envelope IC domain in the envelope functions by testing the abilities of cells with mutant proteins to form syncytia. Depending on the transfected cell type, truncation of the IC resulted in unchanged (transfected 1301 cells) or augmented (COS-1, HSB2, and K562 cells) syncytium formation capacity of the envelope. The IC domain is thus dispensable for the envelope-mediated binding and fusion capacity. However, the IC domain can modulate the envelope functions in a cell type-dependent manner. The augmented syncytium formation observed with the truncated envelopes was not due to differences in the envelope transport to the cell surface, at least not in the COS-1 system, in which the intracellular maturation could be fully studied.

The observed augmented syncytium formation could be due to an increased affinity of the envelope for its receptor, as was previously shown with HIV-2 (14). In our HTLV-I envelope mutants, the binding capacity of the envelope cannot be examined directly, since there is no binding assay available yet. One indirect argument would suggest in-

TABLE 1. Envelope expression and syncytium formation in different cell lines

Transfected cells	Envelope expression[a]				Syncytium formation[b]				Receptor expression[c]
	HTE-1	HTE-438	HTE-466	HTE-476	HTE-1	HTE-438	HTE-466	HTE-476	
COS-1	+++	+++	+++	+++	++	−	++++	++++	+
K562	+++	NT[d]	++	++	+	NT	++	+++	++
HSB2	+	NT	+/−	+	+/−	NT	+	++	+
1301	+	NT	+/−	+/−	++	NT	+	++	+++++

[a] Results from immunoprecipitation and Western blot experiments (Fig. 1 to 3).
[b] Numbers of syncytia per well were scored as follows: +++++, >80; ++++, >60; +++, >40; ++, >20; +, >10; +/−, >5; −, 0.
[c] Evaluated by the syncytium formation capacity of the cell line with HTLV-1-infected cells. Numbers of syncytia per optical field of the inverted microscope are scored as follows: +++++, >80; ++, >20; +, >10.
[d] NT, not tested.

creased receptor affinity of the truncated HTLV-I envelope. As stated above, augmented syncytium formation is observed only when the constructs are transfected in cells expressing small amounts of receptor but not when they are transfected in a cell line (1301 cells) expressing very large amounts of receptor. The syncytia form between an indicator cell line (expressing the putative HTLV-I receptor) and an envelope-transfected test cell line. However, the envelope-transfected cells also endogenously express the putative receptor at various levels. Envelope-transfected cell lines which express high levels of receptor (like 1301 cells) will have the capability of sequestering large amounts of the transfected envelope. In contrast, cells expressing low levels of receptor (COS-1, K562, and HSB2) will be, as test cells, very sensitive to the affinity of the envelope. In these cells, an increase in affinity will result in increased syncytium formation, such as that observed with the truncation of the IC domain.

An alternative explanation for the observed augmented syncytium formation in distinct cells would be an increased fusion capacity of the IC mutants, as observed with HIV-2, in which cytoplasmic-domain truncation enhances the fusion activity in a cell type-dependent manner (21). A role of the IC domain in the viral cell-to-cell and cell-free transmissions has also been proposed (3, 17); these are affected when the IC domain is absent in HIV-1 (12). These results were suggested to reflect the implication of the IC domain in postfusion events, since syncytium formation was not modified. Again, a role for the IC domain in particle release or viral transmission cannot be directly tested with HTLV-I because of the absence of an available infectious proviral clone.

Altogether, the results suggest a complex role for the IC domain of the envelope in retroviruses; the IC domain could be implicated in the following processes: (i) intracellular maturation in a cell type-dependent manner; (ii) viral-particle release, perhaps because of required interactions with the core proteins, as shown recently with HIV-1 (32) and a type D retrovirus (26); (iii) envelope-receptor affinity and maybe, consequently, in the cytopathic effects; and (iv) postfusion events necessary for viral entry. In contrast to the situation described above, in RSV, however (23), no difference in infectivity between virions bearing wild-type envelopes and those bearing IC domain-deleted envelopes could be observed. As discussed herein, our results showing increased syncytium formation when the HTLV-I truncated envelope is expressed in COS-1 or K562 cells tend to favor the hypothesis of a functional role for this short domain in HTLV-I, as was previously shown with primate lentiviruses. This role remains to be specified for precise stages of the viral life cycle.

Finally, in previous studies we have shown that the HTLV-I envelope is very sensitive to mutagenesis, since artificial introduction of mutations results in a nonfunctional protein in most cases. The IC domain, however, is an exception, since insertions of amino acids in this region (25) have no effect on syncytium formation. As shown here, however, a partial truncation of the IC domain can modulate syncytium formation. This could explain why this domain, although very short in HTLV-1, is very conserved and never truncated in the natural strains.

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