HTLV-1-induced cell fusion is limited at two distinct steps in the fusion pathway after receptor binding

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Accepted 1 November; published on WWW 9 December 1999

SUMMARY

Human T-cell leukemia virus type 1 (HTLV-1) is notable among retroviruses for its poor ability to infect permissive cells, particularly as cell free virus. The virus is most efficiently transmitted between individuals by infected cells, where it is presumed that intracellular particles and viral RNA are transferred to target cells following fusion. Although the mandatory first step for HTLV-1 fusion is the binding of envelope SU (gp46) to the receptor, the events which follow this interaction and lead to fusion and infection have not been well characterized. To investigate these events, we studied two HTLV-1 chronically infected cell lines with different abilities to fuse with K562 target cells. Although not inherently fusion incompetent, the HTLV-1 envelope protein on MT2 cells was poorly able to undergo a change in membrane hydrophobicity required for fusion with the target cell membrane after binding to the receptor. High level expression of a fusion-competent HTLV-1 envelope protein on MT2 cells had little effect on improving this suggesting that the defect was encoded by the parent cell. Visible syncytia were seen after incubation of these cells with K562 target cells but complete fusion as measured by transfer of cellular contents into the recipient cell was not observed. In C91-PL cells, binding of SU to the receptor resulted in a sustained hydrophobic change of envelope accompanied by a cytopathic effect in mixed cell cultures and complete fusion. However, in C91-PL cells, overexpression of envelope protein blocked the transfer of cell contents after receptor engagement and initiation of cytopathic membrane changes, indicating that post binding fusion events were blocked. These data suggest that HTLV-1 fusion is a multistep process which is susceptible to inhibition at two separate stages of the fusion pathway post receptor binding. This, and the inefficient infection by cell-free virions, may explain the poor infectivity of HTLV-1 in vivo and suggests strategies for preventative therapy.

Key words: HTLV-1, Cell fusion, Envelope protein

INTRODUCTION

Human T-cell leukemia virus type 1 (HTLV-1), like other retroviruses, enters permissive cells by binding to a cellular receptor on the target cell membrane (Sommerfelt et al., 1988). Binding of envelope SU (gp46) to the receptor is a mandatory prerequisite for fusion with and infection of target cells, although the identity of the receptor remains obscure. Complete cell-cell fusion following the interaction between gp46 and the cellular receptor and resulting in the transfer of cellular contents is modified by cofactor molecules which may be cell type specific (Daenke et al., 1999; Hildreth et al., 1997). One cofactor, CD98, has been shown to modify fusion events of several different viruses including HTLV-1 (Ito et al., 1992; Ohgimoto et al., 1996; Okamoto et al., 1997) suggesting that this molecule affects part of the fusion pathway which is well conserved in viral fusion mechanisms.

Retroviral fusion results from a conformational change in the transmembrane subunit (TM) of the envelope protein, triggered by the SU/receptor interaction at neutral pH. This engagement exposes a fusion peptide located at the N terminus of the TM protein (Jones et al., 1998). X-ray crystal analyses of TM proteins of several viruses indicate common elements in the structure and mechanism of viral fusion proteins (Bullough et al., 1994; Kobe et al., 1999; Peuvot et al., 1999). In the inactive and post-fusion conformation, the hydrophobic fusion peptide is either buried or located distal from the target membrane. Activation of the TM protein immediately prior to fusion rearranges the structure to reveal the fusion peptide in a position adjacent to the target membrane. The conformation of the fusion peptide itself is sensitive to membrane lipid composition and can switch from an α-helical structure to predominantly a β-structure (Nieva et al., 1994). Each of these structures differ in their efficiency to insert into the target membrane, and different viruses may adopt preferentially different structures. Sequence analysis of N-terminal fusion proteins and analysis of synthetic peptides suggests that the fusion peptide inserts obliquely into the lipid phase of the membrane (Peuvot et al., 1999).

In influenza HA-mediated fusion, which shares structural and functional similarities with retroviral fusion, evidence suggests the formation of a number of intermediates in the fusion cascade (Blumenthal et al., 1996). One or more of these transitional states may be reversible, and commitment to the
full fusion competent form may be influenced by the presence of cofactors or membrane composition at the interaction site (Korte et al., 1999). Previously, we had shown that the HTLV-I cell lines MT2 and C91-PL differ in their ability to fuse with the target cell line K562 in the presence or absence of cofactor molecules (Daenke et al., 1999). In this study, we evaluated fusion in these cell lines to determine at which stage the deficiency in MT2 occurred. Expression in both cell types of a fusion-competent envelope protein did not complement the deficiency in MT2 and paradoxically inhibited fusion in C91-PL cells. Analysis of changes in MT2 or C91-PL membrane hydrophobicity in response to binding of envelope proteins to target cells revealed that the block in fusion occurred post-binding to targets. This study suggests that HTLV-I fusion is a multistep process which can be arrested at one or more intermediate stages. Furthermore, poor HTLV-I infection may result from a combination of weak or unstable interactions of envelope with target cell receptor(s) and deficient post-binding fusion events required for transfer of cell contents from infected cells to target cells.

MATERIALS AND METHODS

Cells, antibodies and viruses

MT2, C91-PL, C8166 and EBV-transformed B-cell line (KW) were maintained in RPMI 1640 medium, 2 mM L-glutamine with 10% foetal calf serum (FCS; Globe Farm), MOP8 (mouse fibroblasts), K562 (human myelogenous leukemia) and KCAT (K562 cells stably transfected with a plasmid encoding a CAT reporter gene driven by the HTLV-I LTR promoter) were maintained in Dulbecco’s modified MEM (DMEM) supplemented with 2 mM L-glutamine and 5-10% FCS. For K562-CAT, selection was maintained with 1 mM G418 (Daenke et al., 1999). All cell culture was done in the absence of antibiotics. The vaccinia recombinant virus expressing HTLV-I gp68 envelope (WRproenv1) has been described (Shida et al., 1987). All other recombinant vaccinia viruses used were constructed using established protocols (Mackett et al., 1984) by recombination into the thymidine kinase locus of vaccinia virus. Recombinant vaccinia viruses expressing HTLV-I gag, influenza A nucleoprotein and HIV-1 gp160 (HXB2, T-cell tropic) were used. The monoclonal antibody 1C11 recognizes a defined epitope (aa 190-209) in HTLV-I envelope SU and was a gift from T. Palker (Duke University); the rat monoclonal antibody 39b recognizes a conformationally dependent epitope in HTLV-I TM between aa 348-442 and was a gift from T. Schulz (University of Liverpool). Human serum reactive with an undefined epitope(s) on HTLV-I envelope from a patient with HAM/TSP was obtained from S. Nightingale (Birmingham). Anti-human CD4 was a gift from G. Harcourt. FITC anti-mouse Ig and FITC anti-rat Ig were obtained from Sigma and used at the recommended concentrations.

Infection of cells with vaccinia viruses

MT2, C91-PL or B-cells were infected with vaccinia viruses at 2-5 plaque-forming units/cell. Virus was adsorbed to cells in serum-free DMEM, 1 mM L-glutamine, 0.1% bovine serum albumin for 1 hour at 37°C; cells were then supplemented with complete medium and incubated 16 hours at 37°C to initiate the infection. Expression was verified by FACs analysis on a Becton-Dickinson FACS CALIBUR using the CellQuest software analysis program.

Syncytia formation and CAT assay for fusion

MT2 or C91-PL cells with or without vaccinia virus infection were mixed with equal numbers of KCAT or MOP8 cells and allowed to fuse overnight at 37°C. Cytopathic changes were recorded visually and where appropriate, cells were then harvested for CAT activity as a measure of transfer of cell components between infected and target cells. CAT activity was measured as described previously using a scintillation microdiffusion assay (Daenke et al., 1999). In some experiments, CAT activity was measured using an ELISA assay for CAT enzyme (Boehringer Mannheim).

Immunochemical staining for CAT enzyme

5x10^4 KCAT cells were added to 96-well flat bottom tissue culture plates which had been coated with 10 μg/ml CD29-specific mouse IgG in 50 mM Tris-HCl, pH 9. C91-PL cells, with or without vac-env infection were added in equal numbers and the mixed cell cultures were incubated at 37°C. Alternatively, KCAT cells were incubated with cell culture medium alone (control), supernatant from a confluent C91-PL cell culture as a source of cell-free virions, or purified virions prepared from C91-PL culture supernatant by sucrose-density ultracentrifugation. After overnight incubation, cells were fixed onto the plate with 1% paraformaldehyde in PBS, washed and stained for CAT enzyme. Briefly, fixed cells were permeabilized with 1% parafomaldehyde, 0.01% Tween-20 in PBS, washed with PBS, 1% glycine. Digoxygenin-conjugated anti-CAT antibody (Boehringer Mannheim) was added at 1/100 final dilution in PBS, 0.1% BSA. After 2 hours at 37°C, unbound antibody was removed and HRPO-conjugated anti-digoxygenin antibody (Boehringer Mannheim) was added at the recommended concentration. Specific binding was detected using DAB substrate (Sigma).

Measurement of membrane hydrophobicity changes

Bis-ANS was dissolved in methanol (5 mg/ml) and added to envelope-expressing cells at 2 μg/ml in culture medium without serum. Fifteen minutes after addition, saturation levels of fluorescence were obtained and target cells were added. Fluorescence was measured at 370 nm excitation and 530 nm emission wavelength in a spectrophotometer 0-60 minutes following target cell addition. Change in fluorescence intensity (%) was calculated relative to the saturated fluorescence value of each HTLV-1 cell line before addition of target cells. Background fluorescence attributed to direct binding of bis-ANS to target cells was subtracted.

RESULTS

We have previously shown that two HTLV-I chronically infected cell lines, MT2 and C91-PL differ in their ability to fuse with K562 cells based on visual syncytium formation and on the transfer of cell contents measured by Tax-dependent transcriptional activation of a CAT reporter gene in the K562 target cells (Daenke et al., 1999). MT2 cells do not fuse efficiently with K562 cells unless provided with accessory factors, whereas C91-PL are constitutively fusion competent. Both cell types express significant amounts of envelope protein on their membranes and each has been shown to fuse with other cell types. MT2 cells have been shown to support at least two proviral copies of HTLV-1, one of which has a truncated TM protein. Expression from this proviral sequence may reduce the net amount of fusion competent envelope protein available on the surface of the cells (Carrington et al., 1994). MT2 cells were infected with recombinant vaccinia virus Wrproenv1 (vac-env) to complement a deficiency of functional envelope on the MT2 surface. FACs analysis using HAM/TSP antiserum or antibody 1C11 confirmed that envelope expression was increased on vac-env-infected MT2 cells (Fig. 1A). In a separate experiment, infection with vac-gag with a high moi had no effect on envelope expression (mean peak fluorescence...
Two checkpoints in the HTLV-1 fusion pathway

of 719, compared with 609 for MT2 and 1442 for MT2 vac-env). Antibody 39b, specific for the TM protein also showed increased staining on the cells. C91-PL were infected similarly, resulting in slightly increased expression of envelope determined by HAM/TSP antiserum and antibody 1C11 (Fig. 1B). It is noteworthy that while antibody 1C11 showed low intensity staining of MT2 cells compared with HAM/TSP antiserum, staining of C91-PL with the same antibodies showed roughly equivalent intensity of envelope-specific staining. Antibody 39b staining of both cell types was similar.

Vac-env-infected cells were tested for their ability to fuse with KCAT cells. Cultures were scored visually for syncytium formation prior to being processed for CAT activity. The results are shown in Fig. 2. MT2 cells fused poorly with K562 as determined visually (Fig. 2A, top panel) and by CAT assay (Fig. 2C). Infection with vac-env resulted in the presence of ballooned cells in the cultures, similar to those seen in fusion competent cell cultures after mixing with target cells (Fig. 2A, centre panel). No increase in CAT activity was seen. Vaccinia virus infection characteristically produces a cytopathic effect 24-48 hours after infection, however infection of MT2 with a recombinant vaccinia virus expressing influenza A nucleoprotein did not show similar cytopathic changes during the 16 hour incubation (data not shown). CAT activity was similarly unchanged in these cells (Fig. 2C). The small amount of CAT activity detected in MT2 cells was abrogated by the addition to cultures of an antibody to envelope SU, but not by normal human serum (Fig. 2C) or an influenza-specific antibody (not shown).

When C91-PL cells were infected with vac-env, CAT activity in mixed cell lysates was reduced by over 90%, although pathological changes similar to those seen in MT2-env cultures were also noted (Fig. 2B, centre panel). Infection

Fig. 1. (A) MT2 cells were stained with HAM/TSP antiserum (a), monoclonal SU-specific antibody 1C11 (b) or monoclonal antibody 39b to TM (c) followed by a FITC-anti-IgG antibody. FACS profiles are shown for wild-type MT2 cells (filled trace) and MT2 cells after infection with recombinant vaccinia Wrproenv1 (bold trace). The dotted trace shows cells stained with an irrelevant IgG antibody followed by FITC-anti-IgG. Staining of MT2 cells infected with vac-gag was similar to uninfected cells (see text). (B) C91-PL cells were stained as for MT2. (a-c) Staining with HAM/TSP antiserum, 1C11 or 39b monoclonal antibodies.
of C91-PL with vaccinia-influenza nucleoprotein slightly inhibited, and anti-SU antibody completely inhibited CAT activity, but addition of serum from an uninfected individual had no effect (Fig. 2C). In both cases, the cytopathology in vac-env-infected MT2 and C91-PL cultures was abrogated by anti-SU antibodies (Fig. 2A,B, bottom panels). This suggested that the ballooning effect was mediated by an interaction of SU with the target cell membrane, but that in some cases, complete fusion did not follow. It is important to note that vac-env-infected MT2 or C91-PL cells did not fuse with each other (data not shown).

One explanation for the inhibition of CAT activity in KCAT cells mixed with vac-env-infected C91-PL is the release of soluble SU protein from the cells. WRproenv1 has been shown to shed large amounts of SU into the cytoplasm (Shida et al., 1987) resulting in receptor blockade at the target cell membrane, and this could also contribute to the cytopathic effect visualised in mixed cell cultures. To test this, we pretreated K562 target cells with supernatants taken from control or vac-env-infected cells before adding C91-PL effector cells. Significant inhibition of CAT activity was not transferred in supernatants from infected cells. Similarly, supernatants did not produce a cytopathic effect when added to KCAT cells (data not shown). Vaccinia virus titres in the supernatants after 16 hours total incubation time were established by plaque assay to be less than 100/ml.

A second possibility is that the CAT activity and visual syncytium formation represent separate phenomena, with
syncytia resulting from cell-cell fusion and the majority of the CAT activity resulting from virion-cell fusion with no visible syncytia. To test this, we visualized CAT enzyme expression by immunochemical staining of mixed cell cultures. Fig. 3 shows that the majority of CAT staining in mixed cultures of C91-PL and KCAT cells was present within large syncytia (A). Little or no CAT was detected outside syncytia. When C91-PL cells were infected with vac-env, few cells were positively stained for CAT, despite the presence of syncytia in the cultures (B). KCAT cells incubated with a source of HTLV-1 virions (supernatant from C91-PL cultures; (C) or purified virions; not shown) showed neither syncytia or CAT staining. This suggests that CAT activity is not the result of Tax expression in KCAT following infection by virions. Furthermore, these data provide evidence that syncytium formation represents a mandatory step in the fusion cascade and that mixing of cell contents is a separate downstream event.

The fact that membrane changes were seen in vac-env-infected cells mixed with target cells and that this was abrogated by SU-specific antibodies suggested that this represents an intermediate stage in the fusion process. By analogy with other viruses, the initial interaction of envelope protein with the cellular receptor should result in a conformational change to the TM protein which exposes the fusion peptide for insertion into the membrane. Conformational changes of this type can be followed quantitatively and kinetically using the probe bis-ANS which fluoresces when bound to hydrophobic groups exposed as a result of the interaction (Korte and Herrmann, 1994). Bis-ANS fluorescence of C91-PL and MT2 cells was measured before and after the addition of target cells. MT2 cells showed a very slow increase in bis-ANS intensity following addition of target cells over 60 minutes (Fig. 4A). This was slightly enhanced when cells were infected with vac-env but not a vaccinia virus expressing HTLV-1 gag. In contrast, C91-PL cells showed faster binding and higher intensity fluorescence after addition of target cells, resulting in 100-fold increase after 60 minutes (Fig. 4B). Infection of C91-PL with vac-env or vaccinia HTLV-1 gag had no effect on bis-ANS binding. In all cases, addition to the cells of HTLV-1 neutralising antibody (HAM/TSP antiserum) inhibited target cell-induced fluorescent changes to the membrane. Addition of HTLV-1 non-permissive target cells (MOP8 cells) to C91-PL produced no increase in bis-ANS fluorescence (Fig. 4C). For comparison, bis-ANS binding to an EBV-transformed B-cell line infected with vaccinia-virus expressing HIVgp160 (HXB2 T-tropic strain) was rapid and intense after addition of C8166 target CD4+ cells (Fig. 4C). The HIV-1 gp160 interaction was inhibited by anti-CD4 antibody (data not shown).

DISCUSSION

HTLV-1 is notable among human retroviruses as being poorly infectious, particularly as cell-free virus (Clapham et al., 1983). This has been attributed to the low titre of viral particles released from infected cells (Sutton and Littman, 1996) and a restricted cellular tropism. Evidence now suggests that the HTLV-1 receptor is more widely distributed than previously thought, and that alternative receptors or co-receptors may be used under certain conditions (Sutton and Littman, 1996). It is clear that, secondary to the initial binding of envelope to the cellular receptor on target cell membranes, several cell surface molecules can regulate syncytium formation (Daenke et al., 1999; Hildreth et al., 1997; Imai et al., 1992) and others may influence the release or infectivity of virions (Sutton and Littman, 1996). Therefore, the restricted tropism of HTLV-1 largely but not exclusively to CD4+ T-lymphocytes may result from a combination of the receptor and secondary molecules present on target cells at the time of encounter.

We have studied two HTLV-1 chronically infected T-cell lines which differ in their ability to fuse with target cells. We hypothesized that the inability of MT2 cells to form syncytia with K562 may be due in part to the low density of fusion competent envelope protein on the cell membrane. Carrington et al. (1994) have described a truncated HTLV-1 envelope protein in C10/MJ2 cells which is fusion incompetent, in spite of which the cells express SU at their surface. The staining pattern of MT2 cells with envelope-specific antibodies (Fig. 1A) also suggests that the majority of the envelope proteins on the membrane are in a different form to those seen on fusion competent cells. For example, defective SU might be represented by 1C11-poorly reactive, HAM/TSP antisum-reactive envelope protein. This and the C10/MJ2 data also suggests that there is a mechanism for SU expression at the membrane in the absence of the TM membrane anchoring domain, possibly by association with another membrane protein.

Increased expression of an envelope protein from Wrproenlv vaccinia virus failed to increase fusion-dependent transfer of Tax protein from MT2 to target cells, but produced membrane changes in the mixed cell cultures similar to an early fusion phenotype. This effect was shown to be envelope SU-specific as infection with vaccinia viruses expressing non-envelope proteins did not replicate the effect, and neutralising antibodies specific for SU completely inhibited it. The phenotypic changes seen in the cultures may represent a partial step towards fusion with the target cells which is unable to
progress to completion as measured by transfer of Tax protein. Vac-env-infected MT2 cells showed increased staining with 1C11 antibody, but this did not approach the level seen in C91-PL cells. These data are consistent with an unconventional form of envelope protein present on MT2 cells and also suggests that the defect in these cells acts as a dominant negative effect on envelope expressed from vac-env. In support of this, the full length envelope sequence derived from MT2 expresses a fusion competent protein when transfected into Hela cells (Carrington et al., 1994) so the defect is encoded by the MT2 parent cell. In HOS cells, the incorporation of HTLV-1 envelope proteins into pseudotype virions was also thought to be dependent on other cellular proteins (Sutton and Littman, 1996) although the mechanisms involved were not clear.

C91-PL cells are constitutively fusion competent on K562 cells. However, overexpression of envelope protein from vac-env on the surface of the cells resulted in a decrease in fusion, although cytopathic membrane changes were visible in mixed cultures as for MT2/vac-env cells. Envelope SU expression on vac-env infected C91-PL was increased albeit less so than on MT2 cells and the constitutive level of envelope expression was higher. Unlike MT2 cells, no significant differences in staining with 1C11 and HAM/TSP antiserum were seen on C91-PL. Consequently the inhibition of CAT activity in C91-PL/vac-env cells is difficult to explain. One possibility is that overexpression of envelope disrupts the stoichiometric expression levels of a factor essential for complete fusion. Although a post-fusion role for envelope protein in the transfer of Tax from infected to recipient cells cannot be ruled out, this would seem unlikely. It should be noted that Tax is essentially a nuclear protein and therefore measurement of CAT activity in recipient cells may depend on disruption of infected cell nuclei during the fusion process. Supernatant transfer experiments showed that CAT inhibition was not a result of receptor blockade of target cells by soluble SU, and the envelope-specific cytopathic changes in target cells were still present after vac-env infection. CAT staining in mixed cultures indicated that this was present predominantly within syncytia and little CAT was detected in its absence. This suggests that CAT activity represents a fusion event downstream of the cytopathic changes. Similar to reports by others (Clapham et al., 1983), infection by cell-free virions was undetectable in our cultures and all of the envelope-specific events measured could be attributed to cell-cell interactions. Vaccinia infection of cells is known to alter a number of cellular functions and some downregulation of HLA class 1 expression was seen in vaccinia-infected cells (data not shown). However other vaccinia recombinants had neither the same inhibitory effect on CAT activity nor produced recognizable changes in membrane envelope expression in C91-PL cells.

The conformational change resulting from the interaction of viral envelope proteins with their specific receptors has been shown to correlate with the binding of bis-ANS on the virus-infected cell membrane (Jones et al., 1998). Binding is accompanied by a significant increase in bis-ANS fluorescence in support

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**Fig. 4.** (A) Time course bis-ANS fluorescence of MT2 cells following addition of K562 target cells. MT2-E = vac-env infected; MT2-G = vac-gag infected; +TSP= addition of HAM/TSP antiserum before addition of target cells. (B) bis-ANS fluorescence of C91-PL cells with conditions as for MT2 (A). (C) bis-ANS fluorescence of C91-PL cells mixed with non-HTLV-1 permissive target cells (MOP; closed squares). EBV-transformed human BCL infected with vac-HIVgp160 and mixed with C8166 target cells (open squares). % fluorescence increase was measured relative to the saturation equilibrium level before addition of target cells. Fluorescence due to direct binding of bis-ANS to target cells was subtracted. Values represent mean ± s.e.m. of duplicate wells.
associated with exposure of hydrophobic binding sites. The crystal structure of HTLV-1 TM shows hydrophobic residues positioned internally along the length of the coiled coil domain and clustered at the region of chain reversal proximal to the cell membrane (Kobe et al., 1999). It is this region which is thought to play a pivotal role in the transition from pre-fusogenic to activated fusogenic conformation.

The bis-ANS binding to MT2 cells showed a very slow increase in fluorescence after addition of target cells with no clear maximum being achieved after 60 minutes. The rate of change was slightly increased in MT2 cells infected with vac-env. By comparison, hydrophobicity measured by bis-ANS binding to C91-PL cells was more rapid, but no difference was seen in cells infected with vac-env. The kinetics of binding of vaccinia HIVgp160-infected B-cells to target cells was more rapid than in any of the HTLV-1-specific interactions. In HIV-1, binding of CD4 to gp120 is thought to occur within minutes of addition of target cells, and the resulting conformational change occurs more slowly over 20 minutes (Jones et al., 1998). Our data using strain HXB2-derived gp160, which represents an interaction at 25°C, is slower than the kinetics shown for the T-tropic HIV-1 strain VSC60 (Jones et al., 1998). Furthermore, a lag period of approximately 5 minutes was noted in our samples in contrast to the previous report. Conformational change is known to be temperature dependent, and we acknowledge that the kinetics of change in all our cell samples could be significantly more rapid at physiological temperature. However, the data support the suggestion that kinetics of interaction of HTLV-1 envelope with target cells resulting in a presumed conformational change associated with increased membrane hydrophobicity which precedes fusion is slower than in HIV-1 infected cells. The apparent deficiency in fusion of MT2 cells with K562 may therefore be partly due to poor interaction at the target membrane. However, the fact that vac-env-infected MT2 cells were slightly more efficient in bis-ANS binding but no significant increase in CAT activity was seen in target K562 cells also suggests that membrane changes can occur in the absence of fusion, and that post-conformational events contribute to this fusion deficiency. Furthermore, although the bis-ANS membrane changes in vac-env-infected MT2 cells were modest, a visible cytopathic effect was observed in the cultures. Therefore, preliminary syncytial changes may not require a long-lived stable conformational change in envelope protein. In C91-PL, no differences were seen in the bis-ANS binding of vac-env-infected and uninfected cells, but vac-env infected cells failed to fuse with K562 cells as determined by CAT activity. The inhibition of fusion in these cells is clearly mediated after binding of target cells and induction of a change in envelope-dependent membrane hydrophobicity.

Taken together, these data suggest that in comparison to HIV-1, the interaction of HTLV-1 envelope with the target cell membrane is either weak or unstable and that for fusion to proceed to completion, one or more cellular factors are required, perhaps to stabilize the fusion-activated form of the envelope protein or promote pore formation after partial membrane mixing has occurred. Comparison of the crystal structures of several viral fusion proteins fails to suggest particular features of HTLV-1 TM which might predispose to instability, although it is recognized that the crystal structures obtained are probably of post-fusion conformations.

Stabilization of the active envelope conformation on cell-free virions following receptor binding may be particularly poor if accessory factors are not present on the outer coat of the virion. This would contribute to inefficiency of infection by cell-free virus. A requirement of accessory factors could also play a significant role in the ability to mediate cell-cell infection by fusion and transfer of cell contents, and may explain the relatively restricted tropism of infected cells in vivo. In vitro, the tropism appears to be less restricted and could be due to the overrepresentation of accessory proteins in cultured cells. It is difficult to equate the fusion phenotypes of MT2 or C91-PL cells with infected T-cells in vivo. While expression levels of HTLV-1 envelope on T-cells in the peripheral blood are constitutively lower than either cell type examined here, the context of expression with respect to accessory molecules is not known and may change in response to many factors including cell cycle or activation state. We conclude that the poor infectivity of HTLV-1 is due in part to limitations in probable conformational and post-conformational events resulting from the engagement of envelope on virions and infected cells with the receptor on target cells and suggests that a strategy to destabilize the envelope-receptor interaction might be a simple and effective approach in preventing infection.

We thank T. Schulz, T. Palker, A. McMichael and G. Harcourt for gifts of reagents. J. Hemelaar helped with the spectrofluorimetry. This work was supported by a Wellcome Fellowship (Career Development Ref. 046194) to S.D.

REFERENCES


Daenke, S., McCracken, S. A. and Booth, S. (1999). Human T-cell leukaemia/virus type 1 syncytium formation is regulated in a cell-specific manner by ICAM-1, ICAM-3 and VCAM-1 and can be inhibited by antibodies to integrin β2 or β7. J. Gen. Virol. 80, 1429-1436.


Jones, P. L. S. J., Korte, T. and Blumenthal, R. (1998). Conformational changes in cell surface HIV-1 envelope glycoproteins are triggered by
cooperation between cell surface CD4 and co-receptors. J. Biol. Chem. 273, 404-409.


