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Part of the C-terminal tail of the envelope gp41 transmembrane glycoprotein of human immunodeficiency virus type 1 is exposed on the surface of infected cells and is involved in virus-mediated cell fusion

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The C-terminal tail of the gp41 transmembrane glycoprotein of the human immunodeficiency virus type 1 (HIV-1) virion is usually thought to be inside the virion, but it has been shown recently that part of the tail is exposed on the virion exterior. Here, using a panel of antibodies, it was demonstrated that the same part of the tail is exposed on the surface of HIV-1-infected C8166 lymphoblastoid cells and HeLa cells infected with a gp41-expressing vaccinia virus recombinant. Both types of infected cell failed to react with p17 matrix protein-specific IgGs until permeabilized with saponin, confirming the integrity of the plasma membrane. Cell-surface exposure of the gp41 tail was independently demonstrated by inhibition of HIV-1-mediated cell–cell fusion by one of the gp41 tail-specific antibodies. These data also implicate the exposed region of the gp41 C-terminal tail either directly or indirectly in the viral fusion process. Its surface exposure suggests that the gp41 C-terminal tail may be a candidate for immune intervention or chemotherapy of infection.

INTRODUCTION

The envelope glycoprotein of human immunodeficiency virus type 1 (HIV-1) is synthesized as the gp160 polypeptide and is subsequently cleaved to form a non-covalently linked heterodimer. The mature envelope glycoprotein is a non-covalently linked trimer of heterodimers, comprising three copies each of the external gp120 polypeptide and the transmembrane, anchoring gp41 polypeptide, and is transported to and inserted into the plasma membrane. gp120 contains binding sites for attachment to the CD4 primary receptor and the CCR5 or CXCR4 coreceptors. The binding to receptors activates the fusogenic regions of gp41, through conformational changes that lead to the insertion of its N-terminal hydrophobic sequence into the lipid bilayer of the target cell, fusion of the viral and cell membranes and entry of the viral genome into the cell. In addition, both gp120 and gp41 are active antigenically, with epitopes that allow virus infectivity to be abrogated by interaction with neutralizing antibody (Levy, 1998).

Conventionally, virion and cellular gp41 of HIV-1 and the related simian immunodeficiency virus (SIV) are viewed as having three domains: an ectodomain that contains the N-terminal fusion sequence and whose structure has been partially solved (Caffrey *et al.*, 1998; Chan *et al.*, 1997; Malashkevitch *et al.*, 1998; Tan *et al.*, 1997; Weissenhorn *et al.*, 1997), a transmembrane domain of 22 aa and a long C-terminal tail of approximately 144 aa (Gallaher *et al.*, 1989). However, our group has argued that the structure of the C-terminal tail of the virion is more complex than generally appreciated and that the part of the tail including the Kennedy sequence, ^{731}PRGPDRPEGIEEEGGERDRDRS^{752} (Chanh *et al.*, 1986; Kennedy *et al.*, 1986), is exposed on its outer surface. Part of the evidence for this is adduced from the neutralization of virus infectivity by antibodies to the Kennedy sequence (Buratti *et al.*, 1998; Chanh *et al.*, 1986; Cheung, 2002; Cleveland *et al.*, 2000a, b, 2003; Dalgleish *et al.*, 1988; Durrani *et al.*, 1998; Evans *et al.*, 1989; Ho *et al.*, 1987; Kennedy *et al.*, 1986; McLain *et al.*, 1995, 1996a, b, 2001; Newton *et al.*, 1995). Since particles of infectious virus are by definition intact and IgG does not cross lipid bilayers, it follows that the neutralizing epitope is expressed on the outside of the virion. More specifically, we have shown that antibody directed to a specific conformation of the tail sequence, ^{746}ERDRD^{750}, binds to virions and neutralizes infectivity (Cheung, 2002; Cleveland *et al.*, 2000b, 2003; McLain *et al.*, 2001). The ERDRD epitope is exposed constitutively and does not require contact with cell receptors or an elevated

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temperature (Cleveland *et al.*, 2003). Antibody-binding studies have revealed two other gp41 tail epitopes on the surface of the virion. One, formed by the sequence ^{740}IEEE^{743}, is recognized by mAb 1575 (Vella *et al.*, 1993). The other, formed by ^{734}PDRPEG^{739}, is recognized in Western blots by mAb C8 (Abacioglu *et al.*, 1994). However, mAb C8 also reacts with virions of a neutralization escape mutant selected by antibody to an adjacent epitope (McLain *et al.*, 2001).

Using the above data and standard protein structure prediction programs, we have proposed a model for the gp41 C-terminal tail of the HIV-1 virion (Cleveland *et al.*, 2003). In brief, this suggests that the conventional transmembrane region has a central β turn that permits the current transmembrane region to form two short transmembrane sequences, the second of which takes the tail outside the virion. This external tail region is a predicted hydrophilic loop of approximately 40 residues that carries the three epitopes described above. A predicted third transmembrane region takes the most C-terminal part of the tail (approx. 100 residues) back inside the virion.

To date, work on the disposition of the C-terminal tail of gp41 in the plasma membrane of infected cells has concentrated on the interaction of gp41 with the matrix MA protein and the role of various sequences involved in trafficking of the envelope glycoprotein (see Discussion), and the gp41 tail has generally been assumed to be entirely cytoplasmic. However, using a panel of antibodies, we found here that the same region of the C-terminal tail that is exposed on the surface of virions is also exposed on the surface of HIV-1-infected T cells and HeLa cells infected with a gp41-expressing vaccinia virus recombinant, although the exposed part of the tail on the infected-cell surface was antigenically different from that of the virion. Furthermore, we have shown here that a virus-neutralizing, gp41 tail loop-specific antibody inhibited HIV-1-mediated cell–cell fusion, confirming with this independent assay that part of the gp41 tail is exposed on the cell surface and demonstrating for the first time that the tail loop is involved in the fusion process itself or is closely apposed to the regions of the main gp41 ectodomain that are involved in the fusion process. These new data can be reconciled with the other reported activities and properties of the gp41 tail. The finding of part of the C-terminal tail on the surface of HIV-1-infected cells in a form that is both immunologically active and associated with cell fusion suggest that it merits investigation as a possible antiviral target.

METHODS

Viruses and cells. HIV-1 IIIB was obtained from the AIDS Reagent Project (NIBSC, Potters Bar, UK). The human T-cell lines, H9 and C8166 (AIDS Reagent Project), were grown in RPMI 1640 medium (Gibco-BRL), with 2 mM glutamine and 10 % (v/v) heat-inactivated fetal calf serum (LabTech International), but no antibiotics. HIV-1 stocks were produced by cocultivating persistently infected and non-infected H9 cells for 48 h. Medium was replaced 24 h before harvesting to ensure a fresh virus yield and clarified culture fluid was stored in liquid nitrogen. Vaccinia virus recombinants expressing gp41 (vac-gp41; VVTG6127, Transgene) or p17 matrix protein (vac-p17; Nixon *et al.*, 1988) and wt vaccinia virus (vac-wt) were also used. All were grown in HeLa cells in Dulbecco's modified Eagle's medium, 4 mM glutamine and 5 % (v/v) heat-inactivated fetal calf serum.

Antibodies. The following antibodies specific for the gp41 C-terminal tail were used: mAb C8 to ^{734}PDRPEG^{739} (Abacioglu *et al.*, 1994; numbering system of Ratner *et al.*, 1985), mAb 1575 to ^{740}IEEE^{743} and mAb 1577 and mAb 1583 to ^{746}ERDRD^{750} (Vella *et al.*, 1993); for the gp41 ectodomain: mAb 2F5 (Muster *et al.*, 1993); for the gp120 CD4-binding site: mAb b12 (Burton *et al.*, 1994); and for p17: mAb 4C9 (Ferns *et al.*, 1987) and mAb MH-1 (J. Cottingham, unpublished data). Epitope-purified ERDRD-specific (EPES) IgG to the gp41 C-terminal tail was prepared as previously described using a cowpea mosaic virus (CPMV)–HIV chimera (Cleveland *et al.*, 2003), except that mice were immunized with the chimera (CPMV–HIV/29) expressing the HIV-1 gp41 tail peptide ^{745}GERDRDR^{751}. Serum antibody was purified on a flock house virus (FHV) coat fusion protein (FHV-L2-A) expressing the gp41 tail sequence ^{740}IEEEGGERDRDR^{751} in its neutralizing conformation (Buratti *et al.*, 1998). EPES antibody consisted predominantly of IgG1, IgG2a and IgG2b. Antibodies were quantified by solid-phase ELISA or by measurement of A_{280}.

ELISA for gp41 expressed on infected cells. C8166 cells were infected with HIV-1 IIIB [0·005 syncytium-forming units (s.f.u.) per cell] and incubated at 37 °C for 3 days. At this time, approximately 40 syncytia, with 14 % of cells (but not syncytia) stained with trypan blue. Of the mock-infected cells, 3 % stained with trypan blue. HeLa cells were infected with recombinant or wt vaccinia viruses at an m.o.i. of 10 for 18 h at 37 °C. There was no visible cytopathology at this time. Antibodies were reacted with cells prior to fixation or after fixation with 2 % paraformaldehyde (BDH) at 4 °C. Cells were permeabilized with 0·2 % saponin (CalBiochem) for 5 min at 4 °C as required. U-bottomed microtitre plates (Greiner Labortechnik Ltd) were blocked with 3 % Marvel skimmed milk (Premier Brands Ltd) in PBS containing 1 % Tween 20. Cells were aliquotted at 5×10^4 cells per well, pelletted and antibody added for 1 h at room temperature. After washing, cell-bound IgG was detected with HRP-conjugated anti-mouse IgG and *o*-phenylenediamine according to the manufacturer's instructions (Sigma).

Assay for the fusion of HIV-1-infected cells with non-infected cells. This was based on an earlier method (Armstrong *et al.*, 1996). Briefly, C8166 cells were infected with HIV-1 IIIB (0·005 s.f.u. per cell) and incubated at 37 °C for 3 days. Cells (6×10^4 in 50 µl) were then incubated with antibody in medium or with medium alone (50 µl) for 1 h at 37 °C. Non-infected cells (6×10^5 cells in 50 µl) were added and incubation continued for 3·5 h to allow fusion to take place. A replicate culture was kept at 4 °C as a negative control. To help visualize syncytia and nuclei, cells were incubated with 0·2 % Wright's stain in methanol, rehydrated and then stained with 0·05 % aqueous Giemsa. At least 1000 cells were counted per sample using low-power microscopy. A syncytium was defined as a cell containing three or more nuclei. Syncytia contained 3–8 nuclei, with a mean of 4 ± 1 nuclei per syncytium.

Neutralization of HIV-1 infectivity. Infectivity was assayed by the production of syncytia in C8166 cell monolayers. There is a linear relationship between the number of syncytia and the amount of inoculum, meaning that each syncytium is the product of a single infectious unit (McLain & Dimmock, 1994). The assay thus reflects a single cycle of replication. Virus (300 s.f.u. in 50 µl) was incubated with an equal volume of antibody or medium for 1 h at 37 °C. This was then incubated with C8166 cells (6×10^5 in 50 µl) for 1 h at

37 °C. Cells were washed, resuspended in 1·2 ml medium, aliquotted at 200 µl per well into 96-well plates (Gibco-BRL) and incubated at 37 °C. It took 3 days for primary syncytia to develop fully; secondary syncytia did not appear until after this time. Virus controls had approximately 50 s.f.u. per well. Syncytia contained three or more nuclei and their identity was confirmed by cytostaining. However, they were readily recognized unstained under low-power microscopy. The same virus titre was obtained when culture fluid p24 was assayed by ELISA (data not shown). Neutralization was expressed as the percentage reduction in syncytium count (five replicates) in wells containing the virus–antibody mix compared with that of the virus control without antibody. Values were corrected for the few syncytia (mean <1 per well) that occurred in cell controls.

RESULTS

Binding of gp41 C-terminal tail-specific antibodies to HeLa cells infected with vaccinia virus expressing HIV-1 gp41

HeLa cells infected with vaccinia recombinants expressing HIV-1 p17 or gp41, or infected with wt vaccinia virus, were reacted with specific mAbs to determine whether or not HIV-1 proteins were exposed on the exterior of the cell. The p17-specific mAbs 4C9 and MH-1 (not shown) did not react significantly with non-fixed, non-permeabilized vaccinia p17-expressing cells until after they were permeabilized, showing that infected cells are normally impermeable to IgG (Fig. 1a). However, mAb C8, specific for the Kennedy sequence of the gp41 C-terminal tail, reacted strongly with non-fixed, non-permeabilized vaccinia gp41-infected cells (Fig. 1a). The small reaction of the Kennedy sequence gp41-specific mAb 1577 to the same cells may not be significant. Permeabilization of cells increased reactivity with mAb C8 and revealed a positive reaction with mAb 1577, presumably because of the larger amount of antigen available intracellularly (Fig. 1a). However, fixation decreased reaction with 1577, but not C8, suggesting that the 1577 epitope may be unstable in the presence of paraformaldehyde (Fig. 1b). There were positive reactions with other Kennedy sequence-specific mAbs with fixed vaccinia gp41-infected cells (1575 to epitope IEEE and EPES IgG to epitope ERDRD) (Fig. 1c), showing that these epitopes were also unaffected by fixation. As before, there was no reaction with mAb 1577, nor was there any reaction with the other ERDRD-specific mAb, 1583. [It should be noted that antibodies 1577, 1583 and EPES IgG, which all have epitopes based on the ERDRD sequence, have different reactivity patterns (see Discussion). In addition these ERDRD-specific antibodies differ notably in other ways – for example, only EPES IgG neutralizes virus infectivity.] As expected, cells reacted with mAb 2F5 to the gp41 ectodomain (Fig. 1c). None of the antibodies reacted with wt vaccinia virus-infected HeLa cells. The main conclusion was that part of the tail of vaccinia-expressed gp41 is exposed on the outside of the cell and that expression of the gp41 tail loop on the cell surface is not dependent on any other HIV-1-specified molecule.

Binding of gp41 C-terminal tail-specific antibodies to C8166 cells infected with HIV-1

Non-fixed, non-permeabilized HIV-1-infected C8166 cells did not react significantly with the p17-specific mAbs 4C9 and MH-1 until cells were permeabilized with saponin

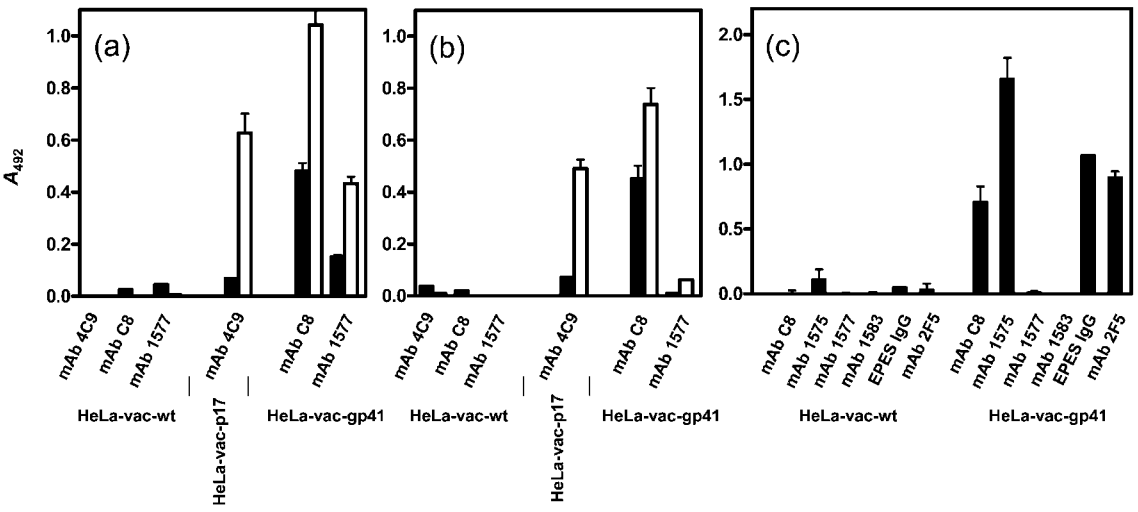

Fig. 1. Reaction of HeLa cells infected with wt vaccinia virus (HeLa-vac-wt) or vaccinia virus recombinants expressing p17 (HeLa-vac-p17) or gp41 (HeLa-vac-gp41) with p17-specific antibody (mAb 4C9) or gp41-specific antibodies (mAbs C8, 1575, 1577, 1583, 2F5 and EPES IgG). All cells were infected for 18 h. Cells in (a) were not fixed, while those in (b) and (c) were fixed with paraformaldehyde. Filled columns represent non-permeabilized cells and open columns represent saponin-permeabilized cells. All antibodies were used at a concentration of 1 µg per well. The background with normal mouse IgG of $<0{\cdot}2$ A_{492} has been subtracted. Experiments were repeated at least twice and data represent the mean ± SEM.

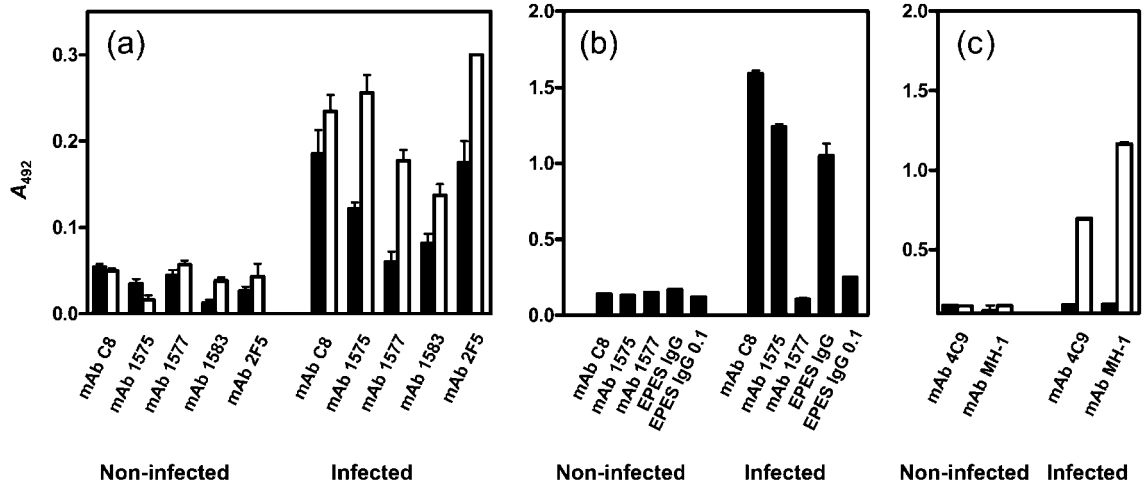

Fig. 2. Reaction of HIV-1 IIIB-infected C8166 cells or non-infected cells with gp41-specific antibodies (mAbs C8, 1575, 1577, 1583, 2F5 and EPES IgG) and p17-specific antibodies (mAbs 4C9 and MH-1). Cells were incubated for 3 days. Cells in (a) were not fixed, while cells in (b) and (c) were fixed with paraformaldehyde. Filled columns represent non-permeabilized cells and open columns represent saponin-permeabilized cells. For other information, see Fig. 1.

(Fig. 2c). Thus, these HIV-1-infected cells are normally impermeable to IgG. Non-fixed, non-permeabilized, HIV-1-infected C8166 cells reacted strongly with the gp41 Kennedy sequence-specific mAbs C8 and 1575, but reacted only weakly with mAbs 1577 and 1583 (Fig. 2a). mAb 2F5 to the gp41 ectodomain reacted with non-fixed, non-permeabilized infected cells as expected (Fig. 2a). Reactivity to all mAbs increased when cells were permeabilized (Fig. 2a). All the antibodies tested, except mAb 1577, reacted with fixed cells (Fig. 2b), as found with vaccinia-expressed gp41 (Fig. 1b and c). Non-infected cells gave only low-level reactivity. Overall, we conclude that the part of the gp41 C-terminal tail defined by mAbs C8 and 1575 and by EPES IgG is expressed on the outside of HIV-1-infected human T cells.

EPES IgG inhibits HIV-1-mediated cell–cell fusion

Fusion of HIV-1-infected cells with non-infected $CD4^+$ cells is mediated by viral envelope glycoproteins and results in syncytium formation. Here, infected C8166 cells were incubated with various concentrations of the virus-neutralizing EPES IgG and then mixed with an excess of non-infected C8166 cells at 37 °C for 3·5 h to allow fusion to take place. A standard virus neutralization assay with the same antibody concentrations was carried out in parallel. Fig. 3 shows that EPES IgG inhibited cell–cell fusion in a dose-dependent manner and at 10 μg ml^{-1} inhibited cell–cell fusion by 86·4 %. Neutralization of infectious virus proceeded in parallel, with 91 % neutralization at 10 μg ml^{-1}. A similar concordance of fusion-inhibiting and neutralizing activity has been reported with gp120-specific antibodies (Armstrong *et al.*, 1996; Jackson *et al.*, 1999; McInerney *et al.*, 1997; S. J. Armstrong, unpublished data). mAbs C8, 1575, 1577, 1583 and normal mouse IgG (all at 100 μg ml^{-1}) did not inhibit cell–cell fusion or neutralize HIV-1 (data not shown). mAb b12 IgG, included as a positive control, inhibited cell–cell fusion by 93·5 % at 1 μg ml^{-1} and resulted in 98·9 % neutralization, as found by others (McInerney *et al.*, 1997). Thus, antibody to the C-terminal tail inhibited virus-mediated cell–cell fusion, confirming independently that the region of cellular gp41 carrying the ERDRD sequence is located on the outside of the cell.

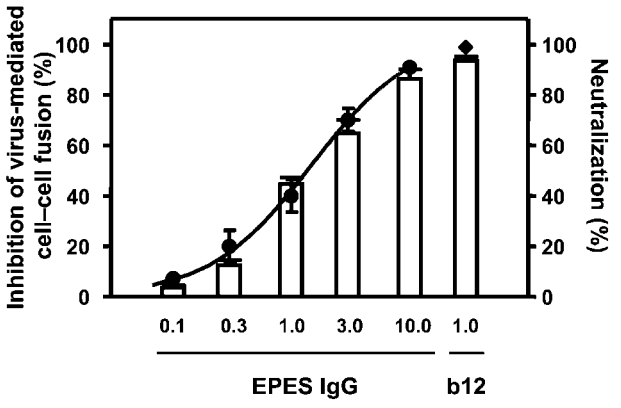

Fig. 3. EPES IgG inhibits fusion of HIV-1 IIIB-infected C8166 cells with non-infected C8166 cells. Columns show inhibition of fusion relative to a fusion control in the absence of antibody. Neutralization of HIV-1 infectivity was measured independently. mAb b12 was used as a positive control (●, EPES IgG; ◆, mAb b12). Numbers on the ordinate show μg IgG ml^{-1}. Data represent the mean ± SEM of two experiments.

DISCUSSION

The ELISA and fusion-inhibition data described here show clearly that part of the C-terminal tail of the gp41 transmembrane glycoprotein is exposed on the surface of HIV-1-infected T cells and vaccinia virus gp41-infected HeLa cells. The latter demonstrated that expression of the tail loop on the cell surface and its antigenicity are achieved independently of other HIV-1-encoded molecules, including gp120, and that these are also not restricted by cell type. The exposure of a region of the gp41 C-terminal tail agrees with earlier work showing that the same tail region is exposed on the surface of infectious HIV-1 virions (Cleveland *et al.*, 2000b, 2003; McLain *et al.*, 2001; Reading *et al.*, 2003). However, the possibility that the tail of some gp41 molecules is entirely inside the cell or virion cannot be excluded (see below).

It is unlikely that the gp41 tail exposure observed above in HIV-1-infected C8166 cells was due to attached progeny virions, as mAb C8 reacted positively with these cells but does not react with virions (see below and McLain *et al.*, 2001). Furthermore, HeLa cells infected with the vaccinia gp41 recombinant produced no virions, but gave the same reactivity with the panel of tail-specific antibodies as HIV-1-infected C8166 cells. gp41 is a transmembrane anchored glycoprotein and is not shed, and cells do not have receptors for gp41. It is also unlikely that cells had disrupted and were revealing cytoplasmic gp41, as neither vaccinia recombinant-infected HeLa cells nor HIV-1-infected C8166 cells reacted with p17-specific antibodies until after they had been permeabilized by saponin. Cytopathology was minimal in both cell systems.

There was a striking difference between cell-surface and virion gp41 tail loop antigenicity as shown by mAb C8, which recognized cell-expressed gp41 but not virion-associated gp41. However, C8 reacts with both cell and virion gp41 in Western blots (Abacioglu *et al.*, 1994; McLain *et al.*, 2001), suggesting that the C8 epitope on the cell surface is non-conformational, while the virion epitope has a different conformation or is not available to antibody. It is not known when in the course of virion budding or maturation the change in the C8 epitope takes place. It appears that either the cell conformer of gp41 is incorporated into nascent virions and is later converted into the virion conformation or virions selectively incorporate a minority species of gp41 from the cell with a different antigenicity.

The other evidence that placed part of the gp41 tail on the outside of the cell was the dose-dependent inhibition of HIV-mediated cell–cell fusion by the virion-neutralizing EPES IgG. This was important independent confirmation of the ELISA data above. Recently we found that another ERDRD-specific IgG, mAb SAR1, inhibits HIV-1-mediated cell–cell fusion (C. H. Heap, S. A. Reading and N. J. Dimmock, unpublished data). mAb SAR1 sees a different epitope conformation of ERDRD from EPES IgG as it gives post-attachment neutralization, but, unlike EPES IgG, it gives little or no neutralization of free virions (Reading *et al.*, 2003). Exactly how the external gp41 tail loop is involved in fusion and how its cognate antibody inhibits the fusion process is not clear. It appears that the tail loop either functions in the fusion process directly or is close enough to the fusogenic regions of the main gp41 ectodomain for bound antibody to interfere sterically with the fusion process. However, mAbs C8 and 1575, which bind to epitopes adjacent to ERDRD, do not inhibit fusion, although they might be expected to provide a similar steric barrier to ERDRD-specific IgG. This argues for specificity of the gp41 tail loop in the fusion process. Neither the possible direct involvement of the tail loop in the fusion process nor its proximity to the fusogenic regions of the gp41 ectodomain have been recognized before, although gp41 mutations are known to affect fusogenicity (Mulligan *et al.*, 1992; Sodroski *et al.*, 1986; Wilk *et al.*, 1992; Zingler & Littman, 1993). It may also be relevant that alterations to the C-terminal tail of HIV-1 and SIV can affect the conformation of both the gp41 ectodomain and gp120 (Edwards *et al.*, 2001, 2002; Spies *et al.*, 1994; Vzorov & Compans, 2000).

The ERDRD sequence in the exposed gp41 tail loop appears to give rise to a number of different epitopes. One is recognized by the virion-neutralizing EPES IgG (Cheung, 2002; Cleveland *et al.*, 2000b, 2003; McLain *et al.*, 2001), another by mAb SAR1 that gives little or no neutralization of free virions but gives post-attachment neutralization (Reading *et al.*, 2003), and a third by mAbs 1577 and 1583, which are non-neutralizing except in the presence of complement (Cleveland *et al.*, 2003). However, 1577 and 1583 may not see the same epitope (Vella *et al.*, 1993). Only the 1577 and 1583 epitopes were apparently destroyed by paraformaldehyde, as already mentioned. Others, using cell sorting, also found that 1583 did not react with fixed infected cells (Sattentau *et al.*, 1995). However, paraformaldehyde fixation does not destroy the 1583 epitope on virions (Cleveland, 1999). The reason for the difference in stability of this gp41 epitope expressed on cells and virions is not clear. It is interesting that a short sequence like ERDRD can express such a range of different epitopes, but, apart from differences in conformation, it may be that ERDRD is only the core epitope and that other residues contribute to antibody reactivity.

The proposal that part of the gp41 tail is on the outer surface of the cell membrane is not inconsistent with the reported interactions of the gp41 tail with the p17 protein (Bukrinskaya & Sharova, 1990; Cosson, 1996; Dorfman *et al.*, 1994; Freed & Martin, 1995a, b, 1996; Mammano *et al.*, 1995; Murakami & Freed, 2000a; Wyma *et al.*, 2000), providing that these are mediated by the more C-terminal portion of the tail (approx. 100 residues) and that this is back inside the virion. The C-terminal domain of gp41 of HIV-1, HIV-2 and SIV has been implicated in other viral properties, including the incorporation of the envelope

glycoprotein into virions (Celma *et al.*, 2001; Iwatani *et al.*, 2001; Manrique *et al.*, 2001; Murakami & Freed, 2000b; Piller *et al.*, 2000; Yu *et al.*, 1993; Zingler & Littman, 1993), fusogenicity (Mulligan *et al.*, 1992; Sodroski *et al.*, 1986; Wilk *et al.*, 1992; Zingler & Littman, 1993) and infectivity (Celma *et al.*, 2001; Iwatani *et al.*, 2001; Piller *et al.*, 2000). Our proposal that part of the gp41 C-terminal tail is looped out also has implications for the trafficking of gp41, in particular for the internalization of gp41 after it has been inserted in the plasma membrane. This activity is directed by sequences such as YxxΦ and LL (Di Fiore & Gill, 1999; Fultz *et al.*, 2001; Heilker *et al.*, 1999; Sauter *et al.*, 1996; Berlioz-Torrent *et al.*, 1999). The proposed looping out of part of the gp41 tail places the most N-terminal region of the tyrosine-sorting signal (^{719}YxxΦ^{722}) outside the membrane and hence renders it inoperative. There is, however, a second potential tyrosine sorting signal (^{775}YxxΦ^{778}), which would be cytoplasmic if residues 753–763 became the third transmembrane region and could function as an internalization signal as discussed previously (Cleveland *et al.*, 2003).

The data described here suggest that HIV-1-infected cells might be susceptible to antibodies that recognize the PRDPEG, IEEE or ERDRD epitopes exposed on the cell surface, either directly or through antibody-dependent cellular cytotoxicity and/or complement-mediated cellular cytotoxicity. ERDRD would be the epitope of choice as EPES IgG neutralizes 91 % of virus infectivity and inhibits nearly 90 % of cell–cell fusion that leads to syncytium formation and cell death (Fig. 3). However, IEEE is highly immunogenic and is known to exist in only one conformation (Cleveland *et al.*, 2000a). Both EPES- and IEEE-specific antibodies are readily stimulated by plant virus chimeras expressing short gp41 sequences (Cheung, 2002; Cleveland *et al.*, 2000a) and these chimeras can be cost-effectively produced (Porta & Lomonossoff, 1998). However, it will be necessary to establish if the gp41 of primary virus strains is expressed in the same way as that of the T-cell-line-adapted virus studied here.

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