Demonstration of Two Distinct Cytopathic Effects with Syncytium Formation-Defective Human Immunodeficiency Virus Type 1 Mutants

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The mechanism of human immunodeficiency virus type 1 (HIV-1) cytopathicity is poorly understood and might involve formation of multinucleated giant cells (syncytia), single-cell lysis, or both. In order to determine the contributions of the fusion domain to syncytium formation, single-cell lysis, and viral infectivity and to clarify the molecular details of these events, insertion mutations were made in the portion of env encoding this sequence in the functional HIV-1 proviral clone HXB2. Viruses produced from these mutant clones were found to have a partial (F3) or complete (F6) loss of syncytium-forming ability in acutely infected CEM, Sup T1, and MT4 T-cell lines. During the early stage of acute infection by F6 virus, there was a loss of the syncytial cytopathic effect, which resulted in increased cell viability, and a 1.9- to 2.6-fold increase in virus yield in the cell lines tested. In the late stage of acute infection, the single-cell cytopathic effect of F6 virus was similar to that of the parental HXB2 virus. The F3 and F6 viruses were also found to have a 1.7- to 43-fold reduction in infectivity compared with the HXB2 virus. The mutant F3 and F6 and parental HXB2 envelope proteins were expressed in vaccinia virus, and the mutant envelope proteins were observed to be defective in their ability to form syncytia. BSC-40 cells infected with vaccinia virus recombinants revealed no differences in kinetics of cleavage, cell surface expression, or CD4 binding capacity of the mutant and parental envelope proteins. These results demonstrate that a loss of syncytium formation results in an attenuation of infectivity and a loss of the syncytial cytopathic effect without a loss of single-cell lysis. These mutants may reflect in tissue culture the changes observed in the HIV isolates in vivo during disease progression, which exhibit marked differences in syncytium production.

Human immunodeficiency virus type 1 (HIV-1) is the etiologic agent of AIDS (3). The disease is characterized by an initial infection followed by a latency period of up to several years and then by severe depletion of the CD4+ T cells. As a consequence of T-cell depletion and resulting immunodeficiency, patients become susceptible to life-threatening opportunistic infections. Virus isolates from HIV-positive patients, obtained at the asymptomatic stage of infection to the disease stage of AIDS or AIDS-related complex, have been found to differ in infectivity, tissue tropism, and replication kinetics (7, 31, 36). Sequential isolates from patients that progressed from asymptomatic infection to AIDS or AIDS-related complex revealed a transition from virus strains that replicated slowly, with only low levels of reverse transcriptase (RT) production (slow/low viruses), to viruses that replicated rapidly, yielding high levels of RT (rapid/high viruses: 7, 31). Another characteristic feature of virus isolates from the late symptomatic stages but not early asymptomatic infection period of disease was the capacity to induce syncytium formation (7, 31, 35, 36). Although multinucleated giant cells have been observed in histologic sections from the brains of infected individuals (24, 32), the precise role of syncytium induction in disease onset in vivo is not known.

The HIV envelope glycoproteins are initially synthesized as a 160-kDa precursor (gp160), which is subsequently cleaved into a 120-kDa surface protein (gp120, designated SU), and a 41-kDa transmembrane protein (designated TM [23]). Eighty-five percent of the synthesized gp160 is degraded in lysosomes, with only cleaved forms of the envelope proteins reaching the cell surface (37). The SU protein is bound noncovalently to the TM protein on the cell surface and is responsible for virus binding with its CD4 receptor (17). The envelope protein expressed in the absence of other HIV proteins, by using a recombinant vaccinia virus (VV), also induced syncytium formation in CD4+ lymphoid cell cultures (19).

The N-terminal domain of the TM protein exhibits substantial homology with the fusion domain of the paramyxovirus envelope, or F protein (14). The use of site-directed mutagenesis to disrupt the putative SIV-mac and HIV type 1 (HIV-1) fusion domains supports the role of this domain in syncytium formation (4, 6, 13). In light of the association of syncytium-forming capacity with disease progression, it was of interest to examine the role of syncytium formation in the HIV-1 life cycle. The current study examined mutants with partial or complete loss of syncytium induction activity in tissue culture. Defects in cell-cell fusion resulted in diminished virus-cell fusion, demonstrating similarities in the mechanism of virus infectivity and multinucleated giant cell formation. The decreased infectivity of the mutant viruses also resulted in an altered tropism for different CD4+ cell lines. However, loss of cell-cell fusion had no effect on nonsyncytial single-cell lysis, thus demonstrating two distinct cytopathic mechanisms. The mutations produced no abnormalities in TM structure or expression. Thus, the effects on syncytium formation are ascribed to altered TM function.

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AMINO ACID POSITION

HXB2 507 G R K A V G I A L F L G F L G
F3 507 G R K A V G A V G I A L F
F6 507 G R K A G A A G A A V G I A L F
F6B 507 G R K A G A A A A A A V G I A L F
F6D 507 G R K A T A G T M A V G I A L F

FIG. 1. Mutations in the TM envelope protein. The envelope precursor (gp160) and cleavage products SU and TM are shown. The bottom portion of the figure shows the amino acid number (according to reference 27) and sequences of the wild-type HXB2 and the mutant clones F3, F6, F6B, and F6D, with the inserted amino acids underlined. The inserted nucleotides for the clones are as follows: F3, GCCGGCGTG; F6, GCCGGCGTGCCGGCGCT; F6B, GCCGGCGGCCGGCGCC; and F6D, GCCGGCGGCC

MATERIALS AND METHODS

Cells. CEM and Sup T1 T-lymphoid cell lines were provided by J. Hoxie (University of Pennsylvania). Molt 3 cells were provided by J. Lempp (Electronucleonics Inc.), and MT4 cells were provided by R. Desrosiers (New England Primate Research Center). Lymphoid cells were cultivated in RPMI 1640 medium supplemented with 10% fetal calf serum, 4 mM glutamine, 50 μM 2-mercaptoethanol, 50 U of penicillin per ml, and 50 μg of streptomycin per ml. BSC-40 cells were provided by T. Braciale (Washington University) and were cultivated in Dulbecco modified Eagle medium supplemented with 10% fetal calf serum, 1 mM pyruvate, 50 U of penicillin per ml, and 50 μg of streptomycin per ml.

Plasmids. The parental HIV-1 proviral clone used for mutagenesis was HXB2 (11, 27). The tat-expressing CV-1 plasmid was provided by S. Arya (National Cancer Institute [1]).

Mutations were made in HXB2 by cloning the BamHI-to-SalI fragment into the TZ18 phagemid vector (Bio-Rad) and using oligonucleotide site-directed mutagenesis (18). The mutations inserted unique restriction sites into the env gene: Nael for F3, F6, F6B, and F6D, as well as a PstI site for F6 (Fig. 1). A mutation which inserted a unique XbaI site prior to the envelope gene was made at positions 5791 to 5793. After this mutation was cloned into HXB2, the env gene was isolated by an XbaI and XhoI restriction digest and cloned into plasmid SC11.4 used for recombination with VV (20). SC11.4 was constructed by modification of SC11, with a poly linker placed in the unique Smal site (provided by T. Braciale, Washington University). The env gene was cut out of HXB2 with an XbaI and XhoI digest, isolated, and inserted into SC11.4 plasmid at the HindIII and Asp-718 sites, with the use of adaptive linkers to produce SC11.4 env.

The mutations in env of F3 and F6 were cloned in SC11.4 env between the HindIII and Stul sites.

Viral stocks. Parental HIV-1 and HIV-1 mutants were generated by transfecting Cos-7 cells with 25 μg each of cesium chloride gradient-purified proviral clone and 5 μg each of CV-1 DNA by the DEAE-dextran transfection procedure (29). Four hours after transfection, the cells were incubated in media containing 80 μM chloroquine for 1.5 h, shocked with 10% dimethyl sulfoxide in Dulbecco modified Eagle medium for 2 min, and washed. After 2 days of culture, either virus was taken directly from the Cos-7 supernatant after sterile filtration (for infection of MT4 cells), or the Cos-7 cells were first cocultivated with Sup T1 cells for 48 h. Culture supernatants were taken from the infected Sup T1 cells and titered by an RT assay (26). Recombinant VV were rescued on CV-1 cells and detected by color selection by using the β-galactosidase marker gene (21) and plaque purified, and stocks were grown and titers were determined on BSC-40 cells (20).

HIV-1 infectivity and replication assays. RT titers of three viral stocks for each mutant and parental virus were determined, and equivalent titers were used for infectivity assays with CEM, Sup T1, and Molt 3 cells. Infections were done in triplicate with fivefold serial dilutions of the virus. Incubations were continued for 21 days, with sampling of culture supernatants for RT activity every 3 days.

For replication assays, Sup T1 and CEM cells were infected with an equal number of infectious units, or MT4 cells were infected with equal levels of RT activity. Samples of culture media were removed every three days for RT assays. Thus, RT activity measured at a particular time represents the total amount of activity accumulated since the last sampling. Viable cell counts were determined by trypsin blue dye exclusion. All syncytia with a diameter of more than four uninfected-cell diameters were also counted. Virus yield was measured by determining the area under the curve of RT production over time as determined by the mass (in milligrams) in each graph. Data are provided as mean values ± the standard errors (Fig. 2).

Syncytium formation assays. Syncytium formation assays were performed by infecting each T-cell line (105 cells) with the VV recombinants at a multiplicity of infection of 30 PFU per cell. After adsorption for 1 h, cells were pelleted, washed, and resuspended in 1 ml of medium and cultured at 37°C. After 24 h, 10% of the culture was removed and all multinucleated cells with a diameter of more than four uninfected-cell diameters were counted. BSC-40 cells were infected at a multiplicity of infection of 2 PFU per cell for 1 h, washed, resuspended in media, and cultured for 5 h. T cells were added at a ratio of two T cells for each BSC-40 cell and were incubated overnight. The following day, 10% of the plate was scored for all multinucleated cells.

Radioimmunoprecipitation. Cells (3 × 106) were labeled with Tran35S-label (ICN; specific activity, 1,112 Ci/mmol) in cysteine- and methionine-free medium. Immunoprecipitation was carried out as previously described (2). Antibodies used for radioimmunoprecipitation were from a pool of sera collected from HIV-positive patients and designated MA. All samples were boiled for 2 min prior to loading on the sodium dodecyl sulfate (SDS) polyacrylamide gel for electrophoresis (SDS-PAGE).

Surface iodination. BSC-40 cells were infected with 2 PFU per cell and cultivated overnight. The cells were then washed twice with phosphate-buffered saline prior to iodination by the lactoperoxidase method (15) with 125I-Na (New England Nuclear; specific activity, 5 to 12 Ci/mg).
to 15 mg of beads for 1 h, and the beads were washed. Forty micrograms of scD4 was added and allowed to bind to the OKT4 antibody beads for 4 h, washed twice, and then used to immunoprecipitate the SU proteins. Lysates of VV-infected BSC-40 cells metabolically labeled with Tran35S-label were precipitated with the scD4 beads with equal number of counts of each cell lysate.

Sucrose sedimentation gradients. BSC-40 cells were infected with 2 PFU of VVwt, VV3, VV6 and VVenv per cell for 1 h. (VVwt is wild-type VV; VV3 and VV6 are VV recombinants expressing the envelope proteins from clones of F3 and F6, respectively, and VVenv is a recombinant VV expressing the HXB2 envelope protein.) The infected cells were placed in Dulbecco modified Eagle culture media for 2 h. Prior to being labeled, cells were cultivated in Dulbecco modified Eagle media lacking cysteine and methionine. The cells were then labeled for 4 h in cysteine- and methionine-free media with 150 μCi of Tran35S-label per ml. The cells were then harvested in lysis buffer (100 mM Tris HCl [pH 8.0], 100 mM NaCl, 1 mM CaCl2, 250 mM octyl glucoside). Debris were removed by centrifugation for 5 min in a microfuge. Supernatants were loaded onto 10 to 35% (wt/vol) sucrose gradients containing 40 mM octyl glucoside and were centrifuged for 12 h at 45,000 rpm in an SW50.1 rotor at 4°C. Thirteen fractions per tube were collected and immunoprecipitated with patient antisera. The gradient standards were thyroglobulin (19S), catalase (11S), and bovine serum albumin (4S).

FIG. 2. Replication and cytopathicity of HXB2, F3, and F6 HIV-1 mutants in CEM cells infected with equal amounts of infectious units as determined in the experiment whose results are shown in Table 1. (A) Syncytia during infection were measured as all cell masses four uninfected-cell diameters or larger. (B) Cell viability as determined by trypan blue dye exclusion (arrows indicate viable cell number drops to zero). (C) RT production. The inset in graph B represents viable cell numbers for F6- and HXB2-infected cells on a different scale for days 25 to 40 after infection. Each value is the average of triplicate datum points for three distinct virus preparations arising from three different-transfections in each case. Similar results were obtained in two additional replicate experiments with CEM cells using different DNA preparations and virus stocks. Total virus yields (see Materials and Methods) were as follows for F6 and HXB2, respectively: 76 ± 12.6 and 40 ± 1.7 for CEM cells; 199 ± 22.3 and 86 ± 3.1 for MT4 cells; and 210 ± 37.5 and 79 ± 19.8 for Sup T1 cells.

SU-CD4 binding assay. Soluble CD4 (scD4) was provided by Raymond Sweet (Smith Kline & French). The scD4 was attached to protein A-Sepharose beads by using the OKT4 antibody. Ten micrograms of antibody was allowed to bind

RESULTS

Mutations in the TM protein. Previous studies of the fusion domain of TM have utilized nonconservative amino acid substitutions (4, 13). In order to examine more subtle conformational effects, the current study examined conservative mutations with insertions of three to six hydrophobic amino acids at the amino terminus of TM (Fig. 1). Mutations were confirmed by restriction enzyme analysis and DNA sequence determination (22, 30). Southern blotting was used to confirm the presence of the mutations in proviral DNA in infected cells in order to demonstrate that reversion did not occur during the course of the experiments (data not shown; 29).

Infectivity of HIV-1 mutants. Infectivity of the HIV-1 mutants was initially screened by cocultivation of T-cell lines with transfected Cos-7 cells, followed by 4 weeks of continuous cell culture. During this period, supernatant samples were assessed for RT activity. Cocultivation of the F6B- and F6D-transfected Cos-7 cells with MT4, Molt 3, CEM, and Sup T1 cells resulted in no RT production by the T cells. However, the transfected Cos-7 cells produced virus particles in each case (data not shown). These results suggest that the F6B and F6D virus particles were not infectious. These mutants were not examined further.

Cells cocultivated with F3- or F6-transfected Cos-7 cells produced RT, with the exception of Molt 3 cells exposed to the F6 virus. Syncytium formation was not observed with any cell line infected with F6 virus (data not shown).

Virus stocks were then produced for HXB2, F3, and F6 viruses by cocultivating Sup T1 cells with transfected Cos-7 cells. Titers of the virus samples were determined by RT activity and used to infect CEM, Molt 3, and Sup T1 cells. The number of infectious units in each virus sample is shown in Table 1. The F3 virus showed a reduction in infectivity of 1.7- to 5-fold relative to that of HXB2 virus. The F6 virus was not infectious for Molt 3 cells and showed 43- and
8.5-fold reductions in infectivity relative to that of HXB2 in CEM and Sup T1 cells, respectively.

Virus replication, syncytium formation, and cytopathic effects. CEM, Molt 3, and Sup T1 cells were infected with an equal number of infectious units as determined by limiting dilution of each virus preparation, and MT4 cells were infected with equivalent numbers of virus particles as determined by RT activity. Similar results were obtained for all four cell lines in three replicate experiments in each cell line. Representative data for one experiment performed with CEM cells are shown in Fig. 2. During acute infection, a reduction in syncytium formation was observed with F3 virus-infected cells relative to HXB2 virus-infected cells, whereas with F6 virus-infected cells, no syncytium formation was observed (Fig. 2A). The F3 virus also had a reduced syncytium-forming capacity on Molt 3 cells, though numbers of syncytia on Sup T1 cells with F3 were equivalent to those on Sup T1 cells with HXB2 virus infection (data not shown). The F6 virus also showed no syncytium formation on Sup T1 and MT4 cells (Fig. 3 and data not shown).

Cytopathic effects of the mutant virus preparations were also determined from the number of viable cells after infection of CEM cells (Fig. 2B). The F3-infected cells demonstrated a slight delay in cell death compared with HXB2-infected cells; this delay correlated with the reduction in syncytium formation activity (Fig. 2A). Cell death in F6-infected CEM cells was somewhat delayed. However, cell lysis in F6 infection still resulted in the death of all cells in the culture. Similar results were also observed for the acutely infected Sup T1 and MT4 cells.

Virus production from cells infected with each mutant virus was also analyzed (Fig. 2C). No significant differences in the kinetics or amount of virus released by F3- and HXB2-infected CEM cells were noted. However, virus production from F6-infected cells was delayed, and the total amount of virus released was 1.9-, 2.3-, and 2.6-fold more than HXB2 in CEM, MT4, and Sup T1 cells, respectively (see legend to Fig. 2). In contrast, virus particles were produced from F3-, F6-, and HXB2-transfected Cos-7 cells in equivalent amounts (data not shown).

Syncytium formation assays. Syncytium formation assays were performed with recombinant VV which result in a highly efficient infection at equal levels in the absence of other HIV-1 proteins (Table 2). Assays were performed by direct infection of CD4+ cell lines or by infection of a nonlymphoid cell line, BSC-40, followed by cocultivation with CD4+ lymphoid cells. CEM cells formed few syncytia, even when infected with VVenv, a recombinant VV expressing the HXB2 envelope. In contrast, Molt 3 and Sup T1 cells readily formed syncytia after VVenv infection. However, few or no syncytia were seen in lymphoid cultures infected with VV3 and VV6, recombinant VV expressing the envelope proteins from clones F3 and F6, respectively.

Syncytium formation from infected BSC-40 cells cocultivated with lymphoid cells more closely mirrored the results observed for the acute infection assays with the HIV-1 mutants (Fig. 2 and Table 2). Compared with VVenv-infected cells, a reduction in syncytium-forming capacity was observed with VV3-infected BSC-40 cells cocultivated with CEM and Molt 3 cells. Similar numbers of syncytia were seen with VVenv- and VV3-infected BSC-40 cells cocultivated with Sup T1 cells. Compared with VVenv-infected BSC-40 cells, VV6-infected BSC-40 cells showed a

<table>
<thead>
<tr>
<th>HIV-1 mutant</th>
<th>Infectivity for cell type</th>
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<tbody>
<tr>
<td></td>
<td>CEM</td>
</tr>
<tr>
<td>HXB2</td>
<td>625 ± 0</td>
</tr>
<tr>
<td>F3</td>
<td>125 ± 0</td>
</tr>
<tr>
<td>F6</td>
<td>14.6 ± 6.6</td>
</tr>
</tbody>
</table>

* * * * *

* Infectivity of the HIV-1 mutants expressed as the means of triplicate experiments with standard errors. All values, expressed in infectious units per milliliter, are the inverse of the greatest dilution of virus inoculum capable of generating RT activity more than or equal to fivefold above background over the 21-day assay.

![FIG. 3. Loss of syncytium formation in Sup T1 cells resulting from a mutation in TM. While syncitia are evident in (b) HXB2-infected cells, cell aggregates but no syncitia were apparent in (a) uninfected and (c) F6-infected cells. For the infected cells shown in panels b and c, photographs were taken at the time of peak virus production at days 3 and 7 after infection, respectively. Magnification, ×100.]
dramatic reduction in syncytium formation with all cell lines used for cocultivation.

Characterization of parental and mutant envelope proteins. Having established that the HIV-1 envelope protein was expressed by VV in BSC-40 cells and that cocultivation with CD4+ cells resulted in syncytium formation, this system was chosen for molecular characterization of envelope protein expression. The kinetics of synthesis and processing of the mutant and parental envelope proteins were examined by pulse-chase analysis (Fig. 4A). Infected cells were labeled with [35S]methionine and [35S]cysteine for 2 h and then incubated in the absence of radioisotope for 0.5, 2.0, or 4.0 h. No significant differences were observed in cells expressing mutant and parental envelope proteins, in the rate of synthesis of the 160-kDa precursor envelope proteins, and processing into the SU and TM products. Furthermore, the rate of degradation or release of TM and SU proteins did not differ significantly between the mutant and parental recombinant VV-infected cells over the 4-h chase period.

Expression of envelope at the cell surface was examined by iodinating intact VV-infected BSC-40 cells (Fig. 4B). Equivalent levels of SU and TM proteins were expressed by VV3, VV6, and VVenv. Iodination efficiency was examined with gels of nonimmunoprecipitated protein lysates and was.

### TABLE 2. Syncytium formation assays*

<table>
<thead>
<tr>
<th>Cells infected with VV</th>
<th>Cells cocultivated</th>
<th>No. of syncytia per assay with:</th>
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<tr>
<td></td>
<td></td>
<td>VVwt</td>
</tr>
<tr>
<td>CEM</td>
<td>None</td>
<td>2 ± 0.6</td>
</tr>
<tr>
<td>Molt 3</td>
<td>None</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>Sup T1</td>
<td>None</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>BSC-40</td>
<td>Molt 3</td>
<td>0 ± 0</td>
</tr>
<tr>
<td>BSC-40</td>
<td>Sup T1</td>
<td>0 ± 0</td>
</tr>
</tbody>
</table>

* Syncytium formation assays with cells infected with recombinant VV. The number of syncytia per assay are reported as the means of triplicate assays with standard errors; assays were performed blinded with coded samples. VVwt is the nonrecombinant VV; VV3, VV6, and VVenv are recombinant VV expressing the envelope proteins derived from clones F3, F6, and HXB2, respectively.
found to be equivalent in each case (data not shown). A small amount of iodinated 160-kDa precursor was detected and possibly resulted from a low level of cell disruption in VV-infected cells prior to labeling. However, separation of viable and nonviable cells by centrifugation into a Ficoll gradient, followed by iodination, still resulted in the labeling of some gp160 precursor protein. These results suggest that some gp160 is being expressed on the cell surface. Results of iodination of F3-, F6-, and HXB2-infected Sup T1 cells revealed no precursor envelope on the cell surface, and no reduction of expression of SU on the cell surface was observed with F3 and F6 compared with HXB2 (data not shown).

The ability of the SU protein from the mutant and wild-type envelope proteins to bind to CD4 was also examined using sCD4 bound to OKT4-protein A-Sepharose beads (Fig. 4C). No differences were observed in the ability of the F3, F6, and parental SU protein to bind to the CD4 receptor. Fifty percent VVenv and 20% VVenv aliquots of labeled infected cell lysates gave rise to bands of comparably reduced intensity, demonstrating that the sCD4 protein was used in vast excess of the levels of SU protein in these assays.

Finally, it has been reported that the HIV-1 envelope protein is assembled into dimers, trimers, and tetramers (10, 25). Therefore, to determine whether changes in oligomerization may have occurred as a result of these mutations, the mutant and wild-type envelope proteins were sedimented onto a sucrose gradient to separate monomeric envelope from higher-molecular-weight complexes. Immunoprecipitation of envelope proteins found in the different fractions from the gradient revealed no differences in the structure between the mutant (VV6 and VV3) and wild-type (VVenv) envelope proteins (Fig. 5). The SU protein is found primarily in fractions 8 to 9, consistent with a monomeric form as reported previously (10). The gp160 precursor is found in fractions 4 to 6, with a sedimentation behavior consistent with that predicted for a dimeric form (10), and fractions 6 to 8, at which the monomeric form would likely migrate. Lesser amounts of higher-molecular-weight complexes are present in fractions 2 to 4. These complexes have been reported to be trimers and/or tetramers (10). Similar results were obtained with extracts prepared in nonionic detergents and analyzed by PAGE with only 0.1% SDS (data not shown), conditions previously established for analysis of oligomers of HIV-2 and SIV-mac envelope proteins (28).

**DISCUSSION**

The results presented here demonstrate that the fusogenic function of the TM protein may be altered by insertion of hydrophobic amino acids at the amino terminus (Table 2 and Fig. 2 and 3). These data suggest that differences in processing, stability, surface expression, receptor binding, and higher-molecular-weight-complex formation are not likely explanations of the loss of fusogenicity observed with the HIV-1 mutants (Fig. 4 and 5). These results demonstrate an attenuation of a distinct fusogenic function of the TM protein.

The mechanism by which the fusogenic function is attenuated is not clear but may involve alterations in the angle of insertion of the fusion domain into the lipid bilayer (5). These results are consistent with those of previously reported studies on the fusion domain (4, 13). Reduction in infectivity (Table 1), coincident with loss of syncytium formation capacity (Table 2 and Fig. 2 and 3), allows one to conclude that the fusion domain is important for virus-cell fusion as well. The results presented here for the F6 virus also show that syncytium formation is a more sensitive indicator of proper fusogenic function since fusion can be eliminated while the virus still maintains infectivity. This conclusion is further supported by our work with the glucosidase inhibitor N-butyl deoxynojirimycin. Treatment of infected cells with this agent abolished cell-cell fusion at considerably lower doses than those which affected virus-cell fusion (9).

The syncytium formation-defective phenotype of the F6 clone has allowed us to define two distinct cytopathic effects of HIV-1 in tissue culture (Fig. 2). Initially in acute HIV infection, the cytopathic effect associated with syncytium formation is observed. This was demonstrated by the formation of multinucleated cells which were incapable of excluding trypan blue dye, while single cells retained their viability. This effect manifested itself as a marked decline in viable cell number with HXB2-infected cells over a 9-day period. Loss of the syncytial cytopathic effect resulted in a more gradual decline in viable cell number with F6-infected cells over a period of 15 days. Furthermore, the increased viable cell number observed with F6 virus infection resulted in a significant increase in total virus yield. In the absence of syncytia, single-cell lysis was still observed with the F6 virus. This demonstrates that mechanisms other than syncytium formation result in HIV-1-induced cytopathicity.
These findings are in agreement with other studies suggesting two distinct cytopathic mechanisms. Syncytium formation in the absence of the single-cell cytopathic effects may not result in the death of the culture. Fisher and colleagues demonstrated that mutations in the C terminus of TM attenuated single-cell killing but not syncytium formation (12; unpublished observations). Furthermore, cytopathic effects have also been observed in the absence of syncytia in N-butyl deoxynojirimycin-treated, HIV-1-infected cell lines (9). Other studies have found a poor correlation between the susceptibility of different lymphoid cell lines to HIV-1-mediated cytopathicity and HIV-1-induced syncytium formation (33). The mechanism by which single-cell lysis occurs is not known but has been suggested to be a function of extremely high levels of viral RNA and protein synthesis (33) or superinfection (34).

It has recently been reported that syncytium formation is the sole cytopathic mechanism in HIV-1-infected cells (16). By using an HIV-1 clone that is only partially defective for syncytium formation, reduced cytopathic effects were demonstrated with Jurkat cells and primary lymphocyte cultures. The usefulness of Jurkat cells in this study in determining cytopathicity is not clear, since not all wild-type viruses used in this study demonstrated a substantial cytopathic effect (e.g., the HIV-CAT virus). The data of Kowalski and colleagues with peripheral blood mononuclear cells appears to show significant cytopathic effect, albeit delayed relative to that of the wild-type virus, with the syncytium formation-defective clone (517A). In fact, 517A virus replication kinetics on Jurkat cells show a delay in the peak syncytium formation. This suggests that the 517A virus exhibited a reduction in infectivity, though this parameter was not specifically examined in this study. This may explain the delay in cytopathic effects observed with the 517A virus on peripheral blood mononuclear cells. It is unclear whether the residual cytopathic effects of 517A are due to its residual syncytium induction activity, as suggested by Kowalski and colleagues (16), or to an alternate pathway of cytopathogenicity, as suggested by the current study.

The results presented in the current study may mimic observations of increasing fusogenicity of sequential viral isolates taken from patients infected with HIV. Thus, on the basis of results presented here, it is possible that naturally occurring virus isolates which induce syncytium formation may have increased infectivity and enhanced cytopathicity. The observed changes in infectivity also resulted in changes in tropism among the T-cell lines tested (Table 1). The loss of infectivity by the F6 virus was not equivalent in all cell lines, varying from complete to partial loss of infectivity. Thus, differences observed in cell tropism in vivo could be the result of an enhancement of infectivity and not of alternative receptors or cell-specific replication properties.

These results have several implications for studies of HIV replication in infected individuals. First, development of therapeutic agents which eliminate virus-induced syncytia may not necessarily eliminate the cytopathic effect of the virus. Second, antibodies designed to neutralize HIV, while eliminating syncytia, will not necessarily eliminate virus entry. Thus assays relying on syncytia as an indication of neutralization of HIV should use additional markers of infection such as p24 antigen production. Third, agents or antibodies that eliminate syncytia could enhance virus production in vivo. Furthermore, it is possible that antibodies that impair syncytium formation could result in increased virus yield (Fig. 2). This could result in a novel form of antibody-dependent enhancement of virus production, which has not previously been described. Such antibodies which block syncytium formation but do not neutralize virus infectivity have been studied with visna virus infections (8).

Syncytium formation is probably not the sole determinant of disease progression. Immune selection, enhanced replication properties, and changes in cell tropism are likely to be important as well. The depletion of CD4+ cells may be due to the enhanced cytopathic effects caused by syncytia. An alternate possibility is that enhanced infectivity may accelerate virus spread, followed by single-cell lysis, which may play a more important role than syncytium formation.

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11. Fisher, A. G., B. Ensoli, L. Ivanoff, M. Chamberlain, S. Petic, L. Ratner, R. C. Gallo, and F. Wong-Staal. 1987. The susceptibility of different lymphoid cell lines to HIV-1 infection such as p24 antigen production. Third, agents or antibodies that eliminate syncytia could enhance virus production in vivo. Furthermore, it is possible that antibodies that impair syncytium formation could result in increased virus yields (Fig. 2). This could result in a novel form of antibody-dependent enhancement of virus production, which has not been described previously. Such antibodies which block syncytium formation but do not neutralize virus infectivity have been studied with visna virus infections (8).

Syncytium formation is probably not the sole determinant of disease progression. Immune selection, enhanced replication properties, and changes in cell tropism are likely to be important as well. The depletion of CD4+ cells may be due to the enhanced cytopathic effects caused by syncytia. An alternate possibility is that enhanced infectivity may accelerate virus spread, followed by single-cell lysis, which may play a more important role than syncytium formation.


