Human immunodeficiency virus grown in CD4-expressing cells is associated with CD4

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Using a CD4-capture immunoassay for gp120, several strains of human immunodeficiency virus type 1 (HIV-1) grown in CD4-expressing T lymphoblastoid cells were found to contain little CD4-reactive gp120 (0.3–1.0 ng/ml) relative to virus titre (10^3–10^5 TCID_50/ml) and p24 antigen (80–1000 ng/ml). The measured CD4-reactive gp120 concentrations of HIV-1 suspensions grown in CD4-negative human neuroblastoma cells were 100- to 10,000-fold greater than those of HIV-1 grown in CD4-positive lymphoblastoid cells, even though both virus suspensions contained abundant viral gp120 as shown by immunoblot assay. It was postulated that CD4 derived from host cells might be associated with virions, concealing the binding domains of gp120. CD4 association with HIV-1 virions grown in CD4-positive cells was demonstrated directly by immunoblot assay of sucrose gradient-purified virus suspensions and by specific co-sedimentation of 125I-labelled OKT4 with virions propagated in CD4-expressing cells. CD4 coating of primary HIV-1 isolates grown in peripheral blood mononuclear cells was also observed. The biological significance of CD4 coating of HIV particles remains to be determined.

Introduction

The envelope glycoprotein of human immunodeficiency virus type 1 (HIV-1) mediates the early events of HIV-1 infection of cells. The HIV-1 envelope precursor glycoprotein (gp160) is cleaved by host cell proteases to form two non-covalently associated subunits, gp120 and the membrane-anchored gp41. HIV-1 infection of most cell types requires the high-affinity interaction (K_a = 4 x 10^-9 M; Lasky et al., 1987) between gp120 and CD4 (Bour et al., 1995), which may produce other pathological effects such as promoting fusion and syncytium formation between infected and uninfected cells (Sodroski et al., 1986; Crowe et al., 1992) and immunosuppression (Miedema et al., 1988; Chirmule et al., 1988).

In order to quantify HIV-1 gp120, we developed an enzyme immunoassay (EIA) which utilizes solid-phase recombinant soluble CD4 (rsCD4) as a capture system (Gilbert et al., 1991). This assay was found to be sensitive, detecting a reference sample of gp120 at concentrations of > 600 pg/ml, and could be used to detect variations in the affinities of different gp120 molecules. This assay also detected native gp120 in serum samples obtained from some patients with HIV-1 infection (Gilbert et al., 1991). Known inhibitors of CD4–gp120 binding such as rsCD4, Leu3a and aurintricarboxylic acid blocked the detection of gp120 in this assay system (Gilbert et al., 1993). However, using the CD4-capture EIA, suspensions of several strains of HIV-1 which had been propagated in immortalized, CD4-expressing lymphoblastoid cell lines were found to have little CD4-reactive gp120 relative to virus titre and p24 levels. This report presents data showing that the poor reactivity of these viruses in the CD4-capture EIA results from association of virions with host cell-derived CD4, limiting the reactivity of virion gp120 with other CD4 molecules.
Methods

**Viruses and cells.** HIV-1{sub}DV was provided by G. Reyes from GeneLabs, Redwood, CA (Crowe et al., 1990); HIV-1{sub}HIV-III (HIV-1{sub}HIV-III), HIV-1{sub}AY and HIV-1{sub}LB were provided by the AIDS Reference and Research Reagent Program, AIDS Program, NIAID, NIH. HIV-1{sub}PB2 was a highly cytopathic isolate from a patient studied in San Francisco (Tateno et al., 1989). The primary HIV-1 strain MA-1 was isolated by cocultivation of the peripheral blood mononuclear cells (PBMCs) from a patient with AIDS with PBMCs prepared from an HIV-1-seronegative buffy pack, followed by one passage through donor PBMCs (Crowe et al., 1994). The primary isolates SL-5 and SL-6 were obtained courtesy of Ms S. Land (Victorian Infectious Diseases Reference Laboratories, Fairfield Hospital) and were similarly prepared but had undergone one further passage in PBMCs.

Purified virus suspensions were prepared from infected culture supernatants following clarification at 10000 g for 30 min at 4 °C to remove cell debris. Virus was then pelleted from this supernatant at 75 000 g for 60 min at 4 °C, resuspended in 0·05 mM-Tris–HCl pH 7·4, 0·15 m-NaCl, 0·01% NaN₃ (TBSN₃) and overlaid onto a discontinuous gradient of 25–60% sucrose in TBSN₂. After centrifugation at 97 000 g for 2 h at 4 °C, the virus band directly over the 60% sucrose cushion was removed and pelleted before being passed over a continuous gradient of 20–60% sucrose in TBSN₁, (97 000 g, 2 h, 4 °C). Fractions were collected and the peak virus-containing fractions were pooled, pelleted and resuspended in TBSN₂. The resultant virus suspensions contained little, if any, detectable cellular debris when examined by electron microscopy (data not shown).

Virus suspensions were tested for p24 antigen concentration using an HIV-1 p24 antigen EIA (Abbott Laboratories). The infectious virus titre was determined by the method of Reed & Muench (1938) after terminal dilution in VB cells, or by plaque assay on MT4 cells (Tateno & Levy, 1988).

The CD₄⁺positive T lymphoblastoid cell line VB was provided by Dr J. Lifson, GeneLabs, Redwood City, CA. The SK-N-MC neuroblastoma cells were obtained from the American Type Culture Collection and MT4 cells from Dr N. Yamamoto, Japan. These cell lines were cultured in RPMI (Trace Biosciences) supplemented with 10% fetal calf serum (PA Biologicals), 2 mm-glutamine, 100 μg/ml penicillin and 100 IU/ml streptomycin. PBMCs were isolated from HIV-1-seronegative buffy packs (obtained from Red Cross Blood Bank, Melbourne) by Ficoll–Hypaque density centrifugation (Crowe et al., 1994).

**CD₄⁺-capture EIA.** This assay was performed as previously described (Gilbert et al., 1991). Briefly, immobilized rsCD4 is reacted with the gp120-containing sample and bound gp120 is detected by successive binding of sheep anti-gp120, biotinylated rabbit anti-sheep IgG, and avidin–alkaline phosphatase. The sheep anti-gp120 from a wide variety of HIV-1 strains (Moore et al., 1989).

**Solid phase rsCD4 binding assays.** rsCD4 cloned from human lymphocytes (a generous gift from Dr R. Sweed, SmithKline Beecham, King of Prussia, PA) or bovine serum albumin (BSA) (Sigma) were conjugated to cyanogen bromide-activated Sepharose orMB (Pharmacia) at a ratio of 1 mg protein to 0·5 ml gel following the manufacturer's instructions. A 2 ml sample of the virus suspension was mixed with 250 μl of either CD₄⁺ or BSA-conjugated Sepharose for 2 h at room temperature with continuous agitation. Output samples were tested for p24 antigen concentration and virus titre. To confirm that the conjugated rsCD4 could still bind gp120, a 2 ml sample of recombinant gp120 (produced in Drosophila ovarian cell culture system, a generous gift from Dr J. Culp, SmithKline Beecham, PA) at 4 μg/ml was similarly incubated with Sepharose beads, and assayed with the CD₄⁺-capture EIA (Gilbert et al., 1991).

**Antibodies.** The murine hybridomas OKT4 (anti-CD₄⁺, IgG₂a), OKM1 (IgG₂a), BMM1 (anti-β₂-microglobulin, IgG₁) were obtained from the ATCC. The MAb CG10 (anti-CD₄⁺-gp120 complex, IgG₁) was a generous gift from Prof. J. M. Gershoni and Dr G. Denisova. George S. Wise Faculty of Life Sciences, Tel Aviv University. MAb 244 (IgG₂a, anti-influenza haemagglutinin) was a gift of Dr E. M. Anders and Dr G. Kapakis-Delyannis, Department of Microbiology, University of Melbourne. IgG was purified from hybridoma tissue culture supernatants, pooled sera from HIV-1-infected patients (HIV + IgG) or sera from healthy laboratory volunteers (normal IgG) by affinity chromatography on Protein A-Sepharose (Pharmacia). Human anti-gp120 was affinity purified from sera of HIV-1-infected patients, by passage of the sera over a column of Sepharose–gp120SF₃ (a gift from Dr P. Poubournious, St Vincent's Institute for Medical Research, Victoria, Australia). IgG was radio labelled using a modification (Jackson, 1980) of the chloramine T method described by Greenwood et al. (1963).

**SDS-PAGE and immunoblotting.** SDS-PAGE was carried out as previously described (Laemmli, 1970) on 12·5% slab gels using a Mini-Protein II and Mini-Transblot system (Bio-Rad). After electrophoresis, proteins were transferred to nitrocellulose (Schleicher & Schuell), and the membrane was blocked with 5% skim milk in TBS for 2 h at 20 °C. Blots were probed overnight with either sera from HIV-1-infected subjects or hyperimmune rabbit serum to rsCD4 (a gift from Dr J. Culp, SmithKline Beecham) in 2·5% skimmed milk in TBS with 0·1% Tween 20 (TBS-T). The membranes were then washed in TBS-T and bound antibody was detected with 1·3 x 10⁶ c.p.m. of ¹²⁵I-labelled Protein A. After 1 h at room temperature, unbound ¹²⁵I-Protein A was removed by washing in TBS-T, the nitrocellulose membrane dried in air and autoradiographed overnight at −70 °C. Coomassie blue-pretained low range molecular mass markers (Bio-Rad) were used to estimate molecular mass.

**Virus capture assay.** Binding of the anti-CD₄⁺ MAb OKT4 to different virus strains was measured in a two-step EIA. Just prior to use in the capture assay, the virus-containing culture supernatant (0·6 ml) was clarified by centrifugation at 10000 g for 15 min at 4 °C to remove cellular debris, and the virus was pelleted from the supernatant at 23 500 g for 60 min at 4 °C in a Heraeus Sepatech Biofuge 22R. After removal of the supernatant, virus was resuspended in 0·6 ml of 5 mg/ml BSA in TBS (BSA-TBS). The capture assay was prepared by coating Nunc Maxisorp wells overnight with 100 μl of capture antibody (50 μg/ml) in 0·1 M-bicarbonate buffer pH 9·6, blocking for 1 h with 150 μl of 10 mg/ml BSA in TBS, and washing with TBS-0·05% Tween 20, followed by TBS without Tween 20. Virus (100 μl) in BSA-TBS was incubated in wells for 4 h at 37 °C, and the wells washed extensively with TBS. Bound virus was lysed on ice for 1 h with 0·5% Triton X-100 in TBS, and the p24 antigen levels in the supernatant were assayed in an Abbott EIA. Capture antibodies included IgG prepared from the plasma of healthy volunteers (HIV – IgG) or HIV-1-infected patients (HIV + IgG), OKT4 and an isotype-matched control MAb (244), as well as a no antibody (BSA block only) control.

**Co-sedimentation analysis of ¹²⁵I-labelled MAb and virus complexes.** Sucrose gradient-purified virus suspensions were incubated with 5 x 10⁵ c.p.m. of ¹²⁵I-labelled MAb for 16 h at 4 °C and overlaid onto a continuous gradient of 20–60% sucrose in TBSN₄. After centrifugation at 97 000 g for 2 h at 4 °C, gradients were collected into 300 μl fractions and each was assayed for radioactivity in a Packard AutoGamma Scintillation Spectrometer, and for p24 antigen levels. The
specificity of the antibody interaction was demonstrated by preincubation of the virus suspension with 20 μg of unlabelled competitor MAb IgG for 4 h at 4 °C, prior to the addition of the 125I-labelled MAb.

Results

HIV-1 strains propagated in CD4-expressing cell lines bind poorly to immobilized rsCD4

The EIA developed to measure gp120 following capture by solid-phase rsCD4 was able to detect reference gp120 at concentrations of > 600 pg/ml and could detect native gp120 in serum samples obtained from patients with AIDS (Gilbert et al., 1991). However, laboratory suspensions of a variety of HIV-1 strains, including HIV-1IIIB (Popovic et al., 1984) and HIV-1DV (Crowe et al., 1992) which were propagated in immortalized CD4-expressing, lymphoblastoid cell lines (H9 or VB) had little detectable gp120 (0.3–1.0 ng/ml), despite having high p24 HIV-1 antigen concentrations (80–1000 ng/ml), high titres of infectious virus (10^3.2–10^6.0 TCID_50/ml), and dense virus populations observed by electron microscopy (not shown). Assuming 2500–3000 Gag molecules (Bess et al., 1992) and 216 gp120 molecules (Gelderblom, 1991) per virion (about 14 p24 molecules for every gp120 molecule) virus suspensions would be expected to contain approximately 3-fold less gp120 than p24 by weight. However, suspensions of all HIV-1 strains examined with the CD4-capture EIA appeared to contain 250- to 2500-fold less gp120 than p24 antigen. These data suggested that either p24 was produced in relative excess to gp120, or that gp120 in virions was depleted, inaccessible to CD4, or possessed an altered structure which affected CD4 binding. Given that immunoblotting showed significant amounts of gp120 in these virus suspensions (data not shown), these virus strains have been shown to be neutralized by soluble CD4 in vitro (Weiss et al., 1988; Byrn et al., 1989; Crowe et al., 1990), that virion-associated gp120 has the same affinity for CD4 as soluble gp120 (Moore et al., 1991, 1992), and that the gp120 of one of the HIV-1 strains used (HIV-1IIIb) has been well-characterized with regard to its CD4 binding (Lasky et al., 1987), marked depletion of virion gp120 or alteration in its structure seemed highly unlikely.

The reactivity of the gp120 of these viruses with CD4 was examined further by testing their ability to bind to immobilized CD4. Passage of HIV-1DV over rsCD4-conjugated Sepharose reduced virus titres only minimally (from 10^{4.5} to 10^{2.2} TCID_{50}), and p24 titres from 1260 to 1220 ng/ml (equivalent to the 10^{4.5} reduction in virus titre and 40 ng/ml reduction in p24 concentration which occurred after the same virus sample was passed over BSA-conjugated Sepharose). Comparable results were obtained for HIV-1IIIB. This same Sepharose-immobilized CD4 bound more than 99% of 4 μg of recombinant gp120. Thus, these virus suspensions were apparently unable to bind efficiently to solid-phase CD4 which was able to bind gp120.

HIV-1 particles grown in CD4-expressing cells are associated with CD4

The failure of cultured virus to bind to immobilized rsCD4 conflicted with the accepted paradigm of HIV-1 infection. We hypothesized that the CD4-binding domain on gp120 was inaccessible, due to non-specific binding by either proteins

![Image](https://via.placeholder.com/150)

**Fig. 1.** Immunoblot assay of HIV-1SF33 propagated in CD4-positive VB cells. A virus suspension purified by two cycles of gradient centrifugation, and rsCD4, were subjected to SDS–PAGE under reducing conditions on 12.5% gels, transferred to nitrocellulose and immunoblotted with HIV-1-seropositive human plasma or hyperimmune rabbit serum to rsCD4.
present in the culture medium or by products derived from the cell suspension. As spent culture medium (MEM with 10% fetal calf serum) from H9 cells did not impair binding of recombinant reference gp120 to CD4 (data not shown), it was postulated that cellular CD4 itself might be associated with the virus particles in suspension, concealing the CD4-binding domains of HIV-1 gp120. To evaluate whether binding of host cell-derived CD4 to virus particles occurs, sucrose gradient-purified virus preparations were immunoblotted and probed with either HIV-1 immune plasma or hyperimmune rabbit serum raised to rsCD4 (Fig. 1). Purified VB cell-derived HIV-1SF33 contained immunoreactive CD4 at the expected molecular mass of 55 kDa, whereas rsCD4 had a lower molecular mass consistent with the absence of transmembrane and cytoplasmic domains on this variant of CD4 (Sweet et al., 1991) (Fig. 1). Slot-blot analysis suggested that HIV-1 gp120 was present in approximately 30-fold excess to CD4 in the purified virus suspension and also demonstrated the presence of $\beta_2$-microglobulin (data not shown), in agreement with the findings.

Table 1. Capture of primary and laboratory-adapted HIV-1 strains by selected antibodies.

<table>
<thead>
<tr>
<th>HIV-1 strain (host cell)</th>
<th>HIV+ IgG*</th>
<th>Normal IgG†</th>
<th>OKT4</th>
<th>244+</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF33 (VB cells)</td>
<td>1.92</td>
<td>0.24</td>
<td>0.68</td>
<td>0.32</td>
<td>0.22</td>
</tr>
<tr>
<td>MA-1 (PBMC§)</td>
<td>0.54</td>
<td>0.19</td>
<td>0.57</td>
<td>0.17</td>
<td>0.20</td>
</tr>
<tr>
<td>SL-5 (PBMC§)</td>
<td>1.12</td>
<td>&lt; 0.1</td>
<td>0.98</td>
<td>0.17</td>
<td>0.20</td>
</tr>
<tr>
<td>SL-6 (PBMC§)</td>
<td>0.48</td>
<td>&lt; 0.1</td>
<td>0.39</td>
<td>0.17</td>
<td>0.13</td>
</tr>
</tbody>
</table>

* IgG purified from the pooled plasma of six HIV-1-infected patients.
† IgG purified from the plasma of healthy volunteers, not HIV-infected.
‡ Isotype matched control for MAb OKT4 (IgG2a).
§ Primary isolates, see Methods.

Table 2. Comparison of virus titre, p24 antigen concentration and CD4-reactive gp120 concentrations of HIV-1SF33 grown in CD4-positive and CD4-negative cells.

<table>
<thead>
<tr>
<th>HIV-1SF33 produced in</th>
<th>Log$_{10}$ concentration or titre of:</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>p24 antigen (pg/ml)*</td>
</tr>
<tr>
<td>CD4-positive VB cells</td>
<td>4.9</td>
</tr>
<tr>
<td>CD4-negative neuroblastoma cells (SK-N-MC)</td>
<td>6.0</td>
</tr>
</tbody>
</table>

* Determined in an Abbott HIV-1 p24 Antigen EIA, according to manufacturer’s instructions.
† Determined by plaque assay in MT4 cells (Tateno & Levy, 1988).
‡ Determined by CD4-capture EIA (Gilbert et al., 1991).
Table 3. Relative binding to solid-phase CD4 of HIV-1sF33 suspensions grown in CD4-positive and CD4-negative cells.

<table>
<thead>
<tr>
<th>CD4-negative cells</th>
<th>CD4-positive cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>gp120 concentrations (ng/ml)†</td>
<td>Before*</td>
</tr>
<tr>
<td>Virus titre (log₁₀ p.f.u./ml)‡</td>
<td>1995</td>
</tr>
<tr>
<td></td>
<td>4-1</td>
</tr>
</tbody>
</table>

* Indicated values for gp120 concentration or virus titre of the HIV-1sF33 suspension produced in indicated cell type, before and after exposure to CD4-Sepharose.
† Determined in CD4-capture EIA (Gilbert et al., 1991).
‡ Determined by plaque assay in MT4 cells (Tateno & Levy, 1988).

Fig. 3. Co-sedimentation of 125I-OKT4 with purified suspensions of HIV-1sF33, propagated in either CD4-positive VB cells or CD4-negative neuroblastoma cells. Sucrose gradient purified virus was reacted with 5 x 10⁵ c.p.m. 125I-labelled OKT4 for 16 h at 4 °C prior to centrifugation at 97 000 g for 2 h at 4 °C over a continuous 20–60% sucrose gradient. Gradients were collected into 300 µL fractions and each fraction assayed for radioactivity (upper panels) and p24 antigen concentration (lower panels). (a) VB cell-(O) or neuroblastoma-(O) derived virus incubated with 125I-OKT4 (anti-CD4). (b) VB cell-derived virus preincubated with buffer (O) or 20 µg of unlabelled competitor MAb OKT4 (O) or its isotype-matched control antibody OKM1 (O O O O) for 4 h at 4 °C prior to the addition of 125I-OKT4.

of Arthur et al. (1992). Several MAbs have been described recently which bind CD4–gp120 complexes (Gershoni et al., 1993) and one of these, CG10, binds exclusively to gp120–CD4 complexes and is unable to bind gp120 or CD4 alone (Gershoni et al., 1993 and data not shown). CG10 reacted strongly with undisrupted sucrose gradient-purified HIV-1sF33 grown in CD4-positive cells, but as previously reported was unable to bind rsCD4 alone in the same assay (Fig. 2).
In addition to laboratory-adapted virus suspensions propagated in established CD4-expressing cell lines, several other virus strains grown in PBMCs were investigated. Like HIV-1sF33 cultured in CD4-positive cells, these PBMC-grown primary HIV-1 isolates were specifically captured by the anti-CD4 MAb, OKT4 (Table 1).

**Comparison of HIV-1 particles grown in CD4-positive and CD4-negative cell lines**

If HIV-1 cultured in CD4-positive cells is associated with cell-derived CD4, then virus propagated in CD4-negative cells should not be associated with CD4, should contain abundant CD4-reactive gp120, and should bind efficiently to solid-phase CD4. A strain of HIV-1, HIV-1sF33, was therefore examined which replicates to high titre in CD4-negative neuroblastoma (SK-N-MC) cells as well as in CD4-bearing lymphoblastoid cells (VB cells) (J. A. Levy, unpublished observation). Consistent with our hypothesis, the HIV-1sF33 virus suspension grown in the CD4-negative cells contained equivalent amounts of p24 antigen and CD4-reactive gp120, while the same virus strain grown in a CD4-positive cell line did not (Table 2). Moreover, virus propagated in the CD4-negative cell line adhered efficiently to immunoabsorbed CD4, resulting in a > 1000-fold reduction in virus titre (Table 3). The poor CD4-reactivity of CD4-positive cell grown HIV-1sF33 compared to CD4-negative cell-grown HIV-1sF33 could not be explained by an alteration in gp120 structure affecting CD4 neutralization, since both viruses were equally sensitive to rsCD4 (50% neutralization titre = 1 ~g/ml for both viruses). Furthermore, the poor reactivity of CD4-positive cell-grown HIV-1sF33 was not due to low levels of virion-associated gp120, since gp120 was readily detected in an immunoblot assay of this purified virus suspension (Fig. 1).

The specific association of cellular CD4 with virions was further demonstrated by gradient sedimentation of purified virions after reaction with an 125I-labelled anti-CD4 MAb, OKT4. 125I-labelled OKT4 co-sedimented with VB cell-derived HIV-1sF33, but not with that same HIV-1 strain propagated in the CD4-negative neuroblastoma cell line (Fig. 3). The co-sedimentation was specific for CD4, as 20 ~g of unlabelled OKT4 blocked co-sedimentation of 125I-labelled OKT4 with virus, whereas no such inhibition resulted from pre-incubation of virus with 20 ~g of an unlabelled isotype-matched control competitor MAb, OKM1 (Fig. 3).

**Discussion**

The data presented in this study show that common laboratory strains and some primary isolates of HIV-1 grown in CD4-bearing cells have virion-associated CD4 derived from host cells. The CD4 associated with the virus appears to be bound to virion gp120, as it inhibits binding of virus to solid-phase CD4; however, CD4 was probably not present at levels which would saturate all gp120 spikes on the virions. Virion-associated CD4 probably exists in equilibrium with CD4 receptors on the cell surface, and exchange of virus between CD4 receptors could occur at this site since rsCD4 has been shown to remove HIV-1 particles already bound to cell-surface CD4 (Byrn et al., 1989). Experiments showing a time-dependent increase in binding of CD4-coated virus to solid-phase CD4 (unpublished observations) suggest that HIV-1 coated with CD4 could infect CD4-bearing cells by such an exchange mechanism, depending on the relative concentration and gp120 affinity of cell-associated and free CD4. CD4-coated virions remain sensitive to neutralization by rsCD4, since the addition of excess quantities of rsCD4 would inhibit the exchange between virus and cellular CD4 and favour exchange with rsCD4. As Layne and co-workers have shown, above the rsCD4 concentration at which half of virion gp120 molecules are bound, neutralization by rsCD4 becomes synergistic (Layne et al., 1990). Moreover, low levels of CD4 have been shown to enhance the infectivity of HIV-2 and SIV. CD4 bound to gp120 changes the conformation of the gp120, facilitating fusion and enhancing infection by HIV-2 and simian immunodeficiency virus (Clapham et al., 1992; Werner et al., 1990).

The higher molecular mass of the virus-associated CD4 compared to rsCD4 (Fig. 1), indicates that virion CD4 possesses a transmembrane and cytoplasmic domain; as such, the C-terminal portion of the molecule is probably inserted into the viral envelope. CD4 on HIV-1 virions may result from several processes which are yet to be fully evaluated. Virion-associated CD4 may arise as a consequence of virus particles budding through a portion of the cell membrane which contains CD4, a mechanism which has been proposed for several other cell membrane proteins that associate with HIV-1 (Meerlool et al., 1992; Arthur et al., 1992, Hoxie et al., 1987). Alternatively, gp160–CD4 complexes may form in the endoplasmic reticulum (Criso et al., 1990; Jabbar & Nayak, 1990; Willey et al., 1992a). However, formation of CD4–gp160 complexes can retard the normal proteolytic processing of gp160, generating mature gp120 and gp41 envelope components only after Vpu-induced degradation of CD4 (Willey et al., 1992b). If virion CD4 resulted from this pathway, virions should contain unprocessed gp160; however, gp160 is rarely found on mature virus particles (Willey et al., 1988) and was not detected in the purified HIV-1sF33 preparation (Fig. 1).

It has been suggested that normal human proteins camouflage 'native' HIV-1 present in the plasma and other body fluids of infected patients (Spear, 1993; Meerlool et al., 1992; Arthur et al., 1992, Hoxie et al., 1987). Other infectious agents have been shown to escape host defences by binding serum or other membrane proteins of the host to their surface. Schistosoma mansoni absorbs host HLA class I heavy chains (Simpon et al., 1983), and Staphylococcus aureus absorbs host immunoglobulins through binding of the Fc portion to bacterial A and G proteins (Wilkinson, 1980). Laboratory suspensions of HIV-1 have been
shown to be associated with a number of different host cell proteins, including the \( \alpha \) and \( \beta \) chains of HLA-DR, \( \beta_2\)-microglobulin, HLA class I molecules, ICAM-1 and LFA-1 (Capobianchi et al., 1994; Arthur et al., 1992; Schols et al., 1992; Henderson et al., 1987). More recently, cyclophilin A was shown to be specifically incorporated into HIV-1 particles by its interaction with p55\(^{gp66} \), and was found to be necessary for the production of infectious HIV-1 particles (Franke et al., 1994; Thali et al., 1994). The addition of CD4 to this list of HIV-1-associated host proteins contrasts with other reports suggesting that little (Meerloo et al., 1992) or no CD4 (Capobianchi et al., 1994) associates with HIV-1 particles. However, detection of CD4 in these studies employed monoclonal antibodies known to block gp120–CD4 binding such as Leu3a. As our results suggest that the CD4 coating the virus particles is bound to viral gp120, such antibodies would not detect CD4 associated with virus particles, since the Leu3a epitope on CD4 would be blocked by bound viral gp120. In addition, the polyclonal antisera we employed in the western blot (Fig. 1) is more sensitive than a single MAb for detection of CD4 (Dr T. Hart, SmithKline Beecham Research Laboratories, King of Prussia, Penn. USA, personal communication).

There are a number of observations by other investigators which support the possibility that viral gp120 may be obscured as it exists in vivo. Many HIV-1-infected persons have moderate titres of free infectious virus circulating in their plasma (Ho et al., 1989; Coombs et al., 1989), despite the fact that these same individuals also have tissue and circulating cells expressing immunoreactive CD4. This is true both during primary HIV-1 infection, prior to seroconversion, as well as during all the later stages of infection (Connor & Ho, 1994). Furthermore, only a relatively small proportion of CD4-bearing cells are infected with HIV-1, even in patients with AIDS (Schnittman et al., 1990; Ou et al., 1988). In plasma, HIV-1 co-exists with a number of host cell proteins capable of binding to virus. HIV-1-infected patients have high titres of antibodies to gp120 which are capable of blocking the binding of gp120 to CD4 (Gilbert et al., 1993) and have elevated levels of serum CD4 (Reddy et al., 1990; Peakman et al., 1992). In addition to circulating gp120, gp120–immunoglobulin, and gp120–CD4 complexes have been detected in plasma fractions of patients with AIDS. Indeed, these complexes were found to inhibit the detection of plasma gp120 by a CD4-capture immunoblot assay of plasma fractions (Oh et al., 1992). Another possible candidate for an ‘HIV-binding protein’ is human serum mannos-binding protein, which binds to oligosaccharide side chains on gp120 and is capable of neutralizing HIV infection in H9 cells in vitro (Ezekowitz et al., 1989) and blocking gp120 binding to CD4 (Gilbert et al., 1993). Therefore HIV-1 in vivo could be associated with CD4 as it is in vitro, or may be coated by human serum proteins such as antibodies to viral envelope components or mannos-binding protein. Further in vivo studies of this phenomenon should shed additional light on the pathogenesis of HIV infection.

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References


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