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Virions of Primary Human Immunodeficiency Virus Type 1 Isolates Resistant to Soluble CD4 (sCD4) Neutralization Differ in sCD4 Binding and Glycoprotein gp120 Retention from sCD4-Sensitive Isolates

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Primary isolates of human immunodeficiency virus type 1 (HIV-1) are much less sensitive to neutralization by soluble CD4 (sCD4) and sCD4-immunoglobulin (Ig) chimeras (CD4-IgG) than are HIV-1 strains adapted to growth in cell culture. We demonstrated that there are significant reductions (10- to 30-fold) in the binding of sCD4 and CD4-IgG to intact virions of five primary isolates compared with sCD4-sensitive, cell culture-adapted isolates RF and IIIB. However, soluble envelope glycoproteins (gp120) derived from the primary isolate virions, directly by detergent solubilization or indirectly by recombinant DNA technology, differed in affinity from RF and IIIB gp120 by only one- to threefold. The reduced binding of sCD4 to these primary isolate virions must therefore be a consequence of the tertiary or quaternary structure of the envelope glycoproteins in their native, oligomeric form on the viral surface. In addition, the rate and extent of sCD4-induced gp120 shedding from these primary isolates was lower than that from RF. We suggest that reduced sCD4 binding and increased gp120 retention together account for the relative resistance of these primary isolates to neutralization by sCD4 and CD4-IgG and that virions of different HIV-1 isolates vary both in the mechanism of sCD4 binding and in subsequent conformational changes in their envelope glycoproteins.

In the principal route of infection of susceptible cells by human immunodeficiency virus type 1 (HIV-1), pH-independent fusion of virus and cell membranes is initiated by interaction of virus external envelope glycoprotein gp120 with cell surface antigen CD4 (10, 14, 26, 28, 33, 35–37, 59). The broadly conserved nature of the gp120-CD4 reaction across HIV-1, HIV-2, and simian immunodeficiency virus (SIV) isolates (53) has provided a target for therapeutic intervention against HIV infection (3, 40). One experimental approach has been expression of soluble CD4 (sCD4) receptor molecules to act as inhibitors of the virus-cell binding reaction (15, 17, 23, 58, 60). These molecules, and immunoglobulin (Ig) chimeras (CD4-IgG and CD4-IgM) (6, 8, 61) potently neutralize cell culture-adapted HIV-1 isolates in vitro and neutralize HIV-2 and SIV with reduced efficacy (6–8, 11, 15, 17, 23, 58, 60, 61). Consequently, sCD4 (24, 55) and CD4-IgG are undergoing clinical trials for efficacy as antiviral agents in HIV-infected individuals. Furthermore, CD4-IgG protects chimpanzees from subsequent challenge with HIV-1 IIIB (62).

Whether sCD4 and related antagonists of virus-cell binding will be as effective in vivo as they are in vitro is unknown. Mathematical models have shown that competitive inhibition of virus-cell binding by agents such as sCD4 is relatively ineffective, as the cell density approaches those found in lymphoid organs (31), and this has been confirmed experimentally (29, 30). Furthermore, primary isolates of HIV-1 that replicate in activated peripheral blood lymphocytes (PBL) but not necessarily in transformed T-cell lines were found to be less sensitive to neutralization by sCD4 in vitro (13), as are some monocyte-tropic HIV-1 strains (19). Moreover, under some circumstances, sCD4 enhances infection by SIV and some HIV-2 isolates in vitro (2, 56, 63), perhaps by activating the fusion potential of the virus (1, 2, 47). However, enhancement of HIV-1 infection by sCD4 is rare (47, 56) and CD4-IgG does not enhance HIV-1 or HIV-2 infection (56).

HIV-1 isolates adapted for growth in transformed cell lines are neutralized by sCD4 by a complex mechanism that has at least two components: low sCD4 concentrations (below approximately 10 nM) inhibit the virus-cell interaction competitively and probably reversibly (7, 29, 30, 46), whereas at higher concentrations sCD4 strips gp120 from the virion surface and inhibits infection irreversibly (20, 25, 46, 48). The relative resistance of primary isolates to sCD4 might be attributable to alteration in the efficiency of one or both of these neutralization mechanisms (38). To test this, we investigated the binding of sCD4 to primary isolate virions and the ability of sCD4 to induce gp120 shedding from the virion envelope. We found a significant reduction (10- to 30-fold) in the binding of sCD4 to primary isolate virions compared with prototypic cell culture-adapted isolates IIIB and RF but a difference of only 1.5- to 3-fold between the affinities of the soluble gp120s from these isolates for sCD4 and CD4-IgG. The primary isolates also retained their gp120 to a much greater extent in the presence of sCD4. These properties may be sufficient to account for the reduction in sensitivity

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of these primary HIV-1 isolates to neutralization by sCD4 and CD4-IgG.

MATERIALS AND METHODS

Virus cultures. Primary HIV-1 isolates LS, WM, JSH2, MDP1, and ACP1 were cultured only once or twice for 5 to 7 days each time in PBL cultures. They had never been passaged through transformed T-cell lines. Extracellular virus stocks were prepared as previously described (13). Stocks of HIV-1 RF and IIIB, isolates adapted for growth in transformed cell lines (52), were prepared by cocultivation in H9 cells as described elsewhere (39). Viruses were stored in 1-ml aliquots under liquid nitrogen, and each aliquot was used for a single experiment only. Infectious virus titers (50% tissue culture infective doses per milliliter) for PBLs, assessed by p24 production after 7 days of culture, were as follows: LS, $10^{4.5}$; WM, 10^6; JSH2, 10^3; MDP1, 10^6; ACP1, 10^6; and RF, 10^6. The titer for RF was also estimated by syncytium formation on C8166 cells and was $10^{6.5}$ 50% tissue culture infective doses per milliliter.

Virus characterization by gel filtration and ELISA. The total gp120 and p24 contents of virus culture supernatants were estimated by enzyme-linked immunosorbent assay (ELISA) (39, 48) and were as follows (for gp120 and p24, respectively, in nanograms per milliliter): LS, 21 and 450; WM, 20 and 210; JSH2, 12 and 280; MDP1, 13 and 140; ACP1, 9 and 190; and RF, 90 and 1,700. Analysis of virion-bound and soluble gp120 by gel filtration on Sephacryl S-1000 (39, 46, 48) showed that the following percentages of the total gp120 were virion bound: LS, 75%; WM, 82%; JSH2, 78%; MDP1, 67%; ACP1, 82%; and RF, 67%. Data for virion-bound p24 were similar. As the free gp120 concentration in the culture supernatant was similar for each isolate and significantly lower than the concentration of virion-bound gp120, free gp120 was unlikely to interfere differentially with the experiments described below. It is also notable that the virion gp120/p24 ratio (average spike density) is similar (±twofold) for each of the isolates, although there are limitations to the precision of absolute gp120 measurements for different gp120 isolates (39).

Neutralization of HIV-1 isolates by sCD4. Neutralization by sCD4 was assayed as described previously (13). Virus (50 50% tissue culture infective doses per milliliter) was incubated with sCD4 in a volume of 200 μl for 30 min at 37°C prior to addition to 2×10^6 PBL. The inoculum was removed by washing after 24 h. Production of extracellular p24 was assayed after 4 to 7 days, depending on the replication kinetics of the viral isolate, by using a commercial immunoassay kit (Abbott Laboratories, North Chicago, Ill.).

sCD4 binding to virions and gp120 shedding. HIV-containing culture supernatants (100 μl) were incubated with or without sCD4 as described elsewhere in the text and then applied to columns of Sephacryl S-1000 (39, 46, 48). The RF stock was diluted to give approximately the same amount of virion gp120 in 100 μl as in the primary isolate stocks. Virion-bound (600 μl) and soluble antigen (1.2 ml) fractions were eluted with Tris-buffered saline. Unless otherwise specified, the columns and the eluant were precooled on ice. The fractions were inactivated by the addition of Nonidet P-40 (NP-40) and fetal calf serum to final concentrations of 1% and stored frozen unless otherwise described. In these assays, virus concentrations were several orders of magnitude greater than those in the neutralization experiments.

sCD4 and CD4-IgG binding to recombinant soluble gp120. The gp120-encoding sequences of primary isolates WM and ACP1 were amplified by polymerase chain reaction, cloned into expression vectors, and expressed in Chinese hamster ovary cells as described in detail elsewhere (5). The recombinant gp120 proteins were purified by immunoaffinity chromatography (5, 28). Binding to CD4-IgG was analyzed in competition assays in which gp120 of the IIIB isolate labelled with ^{125}I served as a tracer, as described previously (4, 5). Briefly, CD4-IgG was bound to microtiter wells coated with anti-IgG antibody and then a mixture of tracer (2.5 nM) and competitor recombinant gp120 (0 to 50 nM) was bound to the immobilized CD4-IgG. Nonspecific binding was determined by omitting CD4-IgG and was less than 10% of the total binding. Binding of the recombinant proteins to sCD4 was measured by a similar method (5).

Determination of gp120 shedding by ELISA. The gp120 contents of virion and soluble antigen fractions were estimated by ELISA by using sheep antibody D7324 as the capture antibody and pooled HIV-1-positive human serum and goat anti-human Ig-alkaline phosphatase conjugate as the detection system (39, 45, 48). For experiments with CD4-IgG, a directly conjugated human serum pool (QC256-alkaline phosphatase) was used to avoid cross-reaction of the anti-human Ig reagent with the Fc portion of CD4-IgG. The assays were calibrated with recombinant gp120 (IIIB) expressed in CHO cells (45). sCD4 did not significantly interfere with the gp120 quantification assay for any of the isolates.

To calculate shedding, the amounts of gp120 in the virion (V) and soluble antigen (S) fractions were summed to give the total gp120 recovery from the column. Virion-bound gp120 was then calculated as $[V/(V + S)] \times 100\%$. This value for virions in the absence of sCD4 was usually defined as 100%, and loss of gp120 was expressed relative to it. All samples were assayed by ELISA in triplicate, and the standard deviation was usually less than 15% of the mean value. A reproducible drop in $V/(V + S)$ of >15% was regarded as significant and an indication of shedding. All experiments were repeated at least three times with similar results.

Determination of gp120-sCD4 complexes by ELISA. Aliquots (100 μl) of the NP-40-treated virion fractions from S-1000 columns were mixed with 10 μl of 20% nonfat milk powder (Marvel) and added to solid-phase-adsorbed anti-gp120 antibody D7324. After capture, sCD4 bound to gp120 was detected with rabbit anti-sCD4 serum (CBL-34) and sheep anti-rabbit alkaline phosphatase conjugate (43, 46). Bound CD4-IgG was detected via its Fc portion with goat anti-human Ig-alkaline phosphatase conjugate. Mean results of ELISAs carried out in triplicate are shown, and the error bars represent standard deviations. Where this is not shown, the error bars lie within the symbol. In some experiments, aliquots (100 μl) of NP-40-treated column eluates or unfractionated virus were treated with sCD4 or CD4-IgG before ELISA to determine binding to solubilized gp120. These assays were carried out on single samples but were repeated several times with similar results.

The above-described assays depend on the capture of gp120 and gp120-sCD4 complexes on a solid phase by an antibody to the carboxyl terminus of gp120 (43, 46). On intact gp120-gp41 complexes, this epitope is occult, probably because of steric hindrance by gp41 (54). We have not been able to assess the efficiency with which detergent releases gp120 from gp41 on primary isolate virions. If the gp120-gp41 interaction on primary isolates is so stable as to resist nonionic detergent, then a significant proportion of the total virion-bound gp120 may not be detectable in our assays.

However, if this were true, then our estimates of relative gp120 loss would be overestimates and our conclusion would be unaltered. When we detected an increase in soluble gp120 after sCD4 binding (see, e.g., Fig. 3 and 4), there was a corresponding decrease in virion gp120. Nor is resistance to detergent disruption likely to account artifactually for our observation of reduced binding of sCD4 to primary isolate virions (Fig. 1); this could be a factor only if sCD4 binding decreased the efficiency of detergent disruption, and we found no evidence of this.

Reagents. CD4-IgG is described elsewhere (6). sCD4 expressed in CHO cells was provided by R. W. Sweet (Smith Kline Beecham) (15) or Genentech (58). These versions of sCD4 behaved equivalently in neutralization assays. In sCD4-binding experiments, Smith Kline Beecham sCD4 was used because the Genentech molecule reacted poorly with the rabbit anti-sCD4 (Smith Kline Beecham) polyclonal antiserum (CBL-34) used in our standard assay. This may have been due to differences in glycosylation between the two molecules. However, both sCD4 preparations reacted identically with monoclonal antibody L120 to domain 4 of sCD4 (21). Key experiments were repeated by using Genentech sCD4 and monoclonal antibody L120, with results similar to those shown.

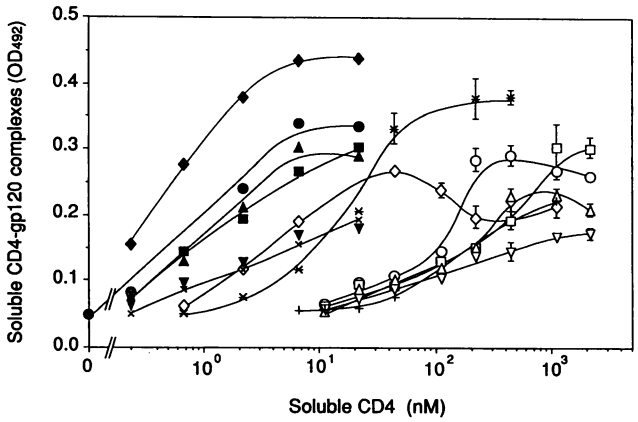

FIG. 1. Binding of sCD4 to intact and detergent-solubilized virions. Virus-containing supernatants (100 μl) were incubated for 1 h at 37°C with the sCD4 concentrations indicated before isolation of virion-sCD4 complexes by gel filtration (open symbols). Alternatively, virions were isolated by gel filtration, disrupted with NP-40, and then incubated with the sCD4 concentrations indicated for 1 h at 37°C (closed symbols). Symbols: ○ and ●, LS; □ and ■, WM; △ and ▲, JSH2; ▽ and ▼, MDP1; × and +, ACP1; ◇ and ◆, RF; *, intact RF virions incubated with sCD4 for 1 h at 4°C instead of 37°C. OD_{492}, optical density at 492 nm.

RESULTS

Neutralization of primary and laboratory isolates by sCD4. We confirmed that the primary HIV-1 isolates analyzed in this study, LS, WM, JSH2, MDP1, and ACP1, were resistant to neutralization by sCD4 relative to prototype cell culture-adapted isolate RF. The concentrations (nanomolar) of sCD4 for 50 and 90% inhibition of PBL infection by these viruses were as follows: RF, 1 and 7; MDP1, 220 and 1,500; JSH2, 600 and >2,000; WM, 600 and 1,500; ACP1, 700 and 1,500; and LS, 950 and >2,000. Thus, 200- to 1,000-fold more sCD4 was required to give comparable inhibition of infection of PBL by the primary isolates than by RF. The sensitivity of RF to neutralization by sCD4 was independent of both the cell type in which it was cultured (13) and the target cells used in the assay; RF infection of the C8166 T-cell line was neutralized by sCD4 at concentrations similar to those described above for PBL infection. CD4-IgG also neutralized these primary isolate stocks with significantly reduced efficiency compared with RF and IIIB (5, 13).

sCD4 binding to soluble gp120. One possible explanation for the resistance of primary isolates to sCD4 neutralization was that the individual gp120 molecules had a reduced affinity for sCD4. To test this, virus- and soluble gp120-containing culture supernatants (100 μl) were treated with NP-40 nonionic detergent to provide a source of soluble virion-derived gp120 and then titrated with sCD4. After capture of the gp120-sCD4 complexes on a solid phase by an anti-gp120 antibody, sCD4 was detected immunologically. Half-maximal sCD4 binding was found at the following sCD4 concentrations (nanomolar): RF, 0.5; IIIB, 0.6; LS, 1.0; WM, 1.3; JSH2, 0.7; and MDP1, 1.3 (see also Table 1). Although RF gp120 had an affinity for sCD4 that was consistently 1.5- to 3-fold greater than that of gp120 from the primary isolates, it seemed extremely unlikely that this could account for the 200- to 1,000-fold decrease in the neutralization sensitivity of the primary isolates by sCD4.

sCD4 binding to intact virions. We measured sCD4 binding to intact virions at 37°C by using gel filtration on Sephacryl S-1000 to separate virion-sCD4 complexes from sCD4 and soluble gp120 not associated with virions. The isolated virions were disrupted with 1% NP-40 before detection of gp120-sCD4 complexes by ELISA (Fig. 1, open symbols). To compare sCD4 binding to intact virions with binding to solubilized gp120, the same concentrations of virions isolated by gel filtration in the absence of sCD4 were disrupted with NP-40 and then titrated with sCD4 (Fig. 1, closed symbols). Intact virions of all five primary isolates bound sCD4 at 37°C half maximally at sCD4 concentrations of 120 to 260 nM. However, the equivalent concentrations of solubilized virion glycoproteins bound sCD4 half maximally at 0.9 to 1.6 nM (Fig. 1; Table 1), consistent with the data described above. In contrast to the primary isolates, intact RF virions bound sCD4 half maximally at approximately 10 nM at 37°C (Fig. 1; Table 1). The biphasic binding curve for RF at 37°C is due to sCD4-induced shedding of gp120-sCD4 complexes from the virions and their consequent loss from the assay system (cf. Fig. 1 and 2). At 4°C, a temperature at which RF does not shed gp120 (20, 46), the virion-sCD4 complexes are stable and sCD4 binds half maximally at 45 nM (44, 46) (Fig. 1; Table 1). Similar data were obtained with IIIB (Table 1), but lowering the temperature of the incubation to 4°C had only a small effect on sCD4 binding to the primary isolates (data not shown). These results indicate that the differences in sensitivity to sCD4 neutralization for RF and the primary isolates are due, at least in part, to variation in the binding of sCD4 to virions.

There are limitations on gel filtration methods for measuring ligand-receptor interactions. (i) The columns reduce the free sCD4 concentration in the virion fraction by only 100- to 200-fold. At input sCD4 concentrations of >100 nM, there is sufficient free sCD4 in the virion fraction to bind detectably to the primary isolate gp120 after detergent solubilization (data not shown). (ii) The gel filtration assays cannot be performed under equilibrium binding conditions: dissociation of sCD4 from the virions may occur to a significant extent during the 10 to 15 min required for passage of the virions through the columns. These two factors oppose one another and modulate the binding curves to an extent which

TABLE 1. Binding of sCD4 and CD4-IgG to intact and detergent-solubilized virions

Ligand and virus (temp, °C)	Concn (nM) for half-maximal binding[a]		
	Intact virus	Detergent-solubilized virus	Recombinant soluble gp120
sCD4			
LS	120	1.3	ND[b]
WM	260	1.6	3.1
JSH2	180	1.4	ND
MDP1	140	0.9	ND
ACP1	110	1.5	2.7
RF	10	0.5	ND
RF (4)	45	ND	ND
IIIB	5	0.7	3.3
IIIB (4)	25	ND	ND
CD4-IgG			
WM	25	0.3	4.1
ACP1	ND	ND	4.3
JSH2	15	0.25	ND
RF	0.7	0.1	ND
RF (4)	2.4	ND	ND
IIIB	0.8	0.1	3.6

[a] Except where indicated otherwise, data for binding to intact virions were obtained from 1-h incubations at 37°C. For RF and IIIB at 37°C, the half-maximal sCD4-binding concentrations are derived from estimates of the binding curve that would have ensued if gp120 shedding had not occurred (46). Data for recombinant, soluble gp120 are equilibrium dissociation constants, determined by Scatchard (IIIB) and competition (WM and ACP1) analyses.
[b] ND, not determined.

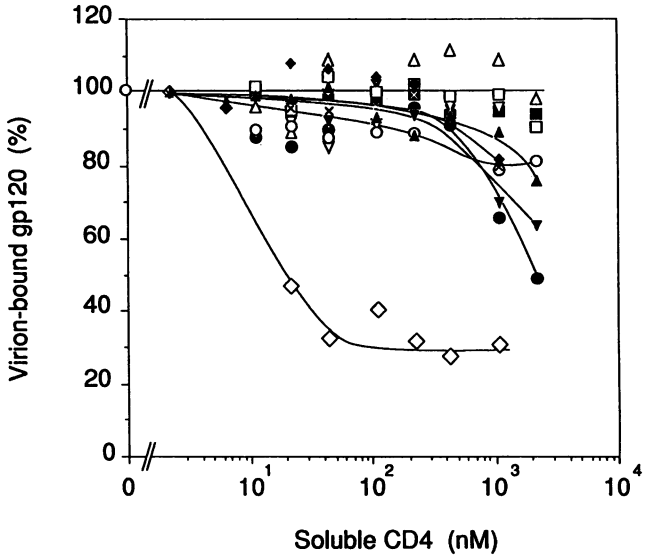

FIG. 2. Effect of sCD4 concentration on virion gp120 retention. Virions (100 μl) were incubated with the sCD4 concentrations indicated for 1 h at 4°C (closed symbols) or 37°C (open symbols) before gel filtration. The gp120 contents of the virion and soluble protein fractions were determined, and shedding was calculated as described in Materials and Methods. Symbols: ○ and ●, LS; □ and ■, WM; △ and ▲, JSH2; ▽ and ▼, MDP1; ×, ACP1; ◇ and ◆, RF.

is difficult to quantify. Thus, the half-maximum sCD4 binding concentrations for intact virions (Fig. 1) are not true dissociation constants.

CD4-IgG binding to intact and detergent-solubilized viruses. Primary isolates are also relatively resistant to neutralization by CD4-IgG compared with cell culture-adapted isolates (13), exemplified by the 300-fold higher CD4-IgG concentrations required to neutralize isolates ACP1 and WM compared with IIIB (5). We investigated the binding of CD4-IgG and sCD4 to intact and detergent-solubilized virions of the RF, WM, and JSH2 isolates. In addition, we measured the binding of CD4-IgG to recombinant gp120 from isolates WM, ACP1, and IIIB (Table 1). As with sCD4, CD4-IgG binding to detergent-solubilized gp120 was similar for the different viruses (Table 1). Consistent with this observation, recombinant, soluble gp120 proteins from the different isolates had similar CD4-IgG- and sCD4-binding affinities (Table 1) (5). In contrast, there was an approximately 20-fold reduction in CD4-IgG binding to intact WM and JSH2 virions compared with RF virions (Table 1). These results suggest that, as for sCD4, variation in CD4-IgG binding to intact virions contributes to the differences in sensitivity to neutralization by CD4-IgG of primary and cell culture-adapted HIV-1 isolates.

It is notable that the absolute concentrations of CD4-IgG required for half-maximal binding to intact virions and to detergent-solubilized virion gp120 were about 10-fold less than those for sCD4 under the same conditions. Consistent with this, the half-maximal concentrations required to neutralize HIV-1 are 5- to 50-fold lower for CD4-IgG than for sCD4 (5, 13). This is probably due to the greater avidity of bivalent CD4-IgG for gp120 oligomers on intact virions and for NP-40-solubilized gp120 (which may be oligomeric; 51) in the antigen capture assay system. In the competition assay, which is not influenced by bivalency, sCD4 and CD4-IgG have similar affinities for recombinant gp120, reflecting the common structure of the gp120-combining sites on the two molecules (Table 1).

Effect of sCD4 on virion gp120 retention. Spontaneous gp120 loss from HIV-1 is correlated with a reduction in virus infectivity of HIV-1 (30, 39). Cell culture-adapted HIV-1 isolates rapidly lose a major proportion of their gp120 contents on binding sCD4 at 37°C, and this neutralizes the virus irreversibly (20, 25, 46, 48). We reasoned that variation in the ability to shed gp120 as a result of sCD4 binding might also contribute to the variation in sensitivity to neutralization by sCD4. The effect of sCD4 on gp120 retention by primary isolate virions was therefore tested. At 37°C, none of the primary isolates lost significant amounts of gp120 within 1 h of treatment with sCD4 at concentrations of up to 2,200 nM, except for LS and ACP1, which lost about 20% of their gp120 at 1,100 to 2,200 nM sCD4 (Fig. 2). Similar data were obtained when a gp120 assay based on sCD4 binding was substituted for the standard serum-antibody gp120 assay, eliminating the possibility that an sCD4-sensitive subpopulation of gp120 molecules was selectively lost from the virions on binding of sCD4 (data not shown). In contrast, as previously reported (46, 48), RF shed gp120 at 37°C in response to sCD4 concentrations above approximately 5 to 10 nM (Fig. 2). At 4°C, however, there was some shedding of gp120 from LS, MDP1, and JSH2 at high sCD4 concentrations, but not from RF or WM (Fig. 2), which is evaluated in more detail below. ACP1 was not tested for shedding at 4°C. At 37°C, none of the primary isolates, LS, MDP1, JSH2, and WM, shed significant amounts of gp120 within 1 h of addition of CD4-IgG concentrations of up to 67 nM, whereas shedding from RF was detectable at CD4-IgG concentrations of greater than 1 nM (data not shown).

Shedding of gp120 from primary isolates at 37°C was detectable during prolonged exposure to very high (1,100

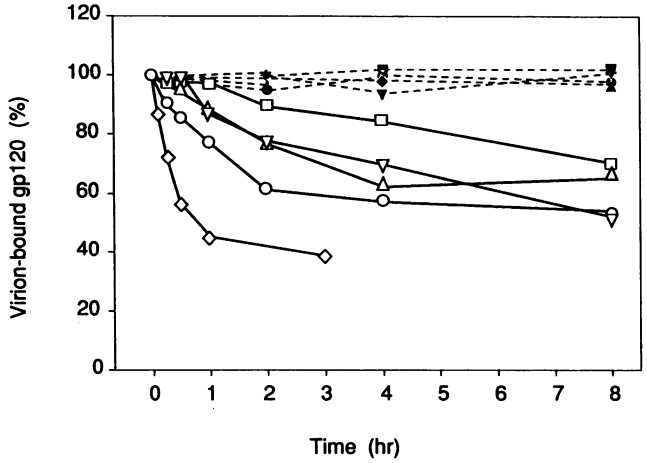

FIG. 3. Rate of loss of gp120 from virions at 37°C. Virions (100 μl) were incubated at 37°C with (open symbols) or without (closed symbols) 1,100 nM sCD4 (220 nM for RF) for the times indicated before gel filtration. Symbols: ○ and ●, LS; □ and ■, WM; △ and ▲, JSH2; ▽ and ▼, MDP1; ◇ and ◆, RF.

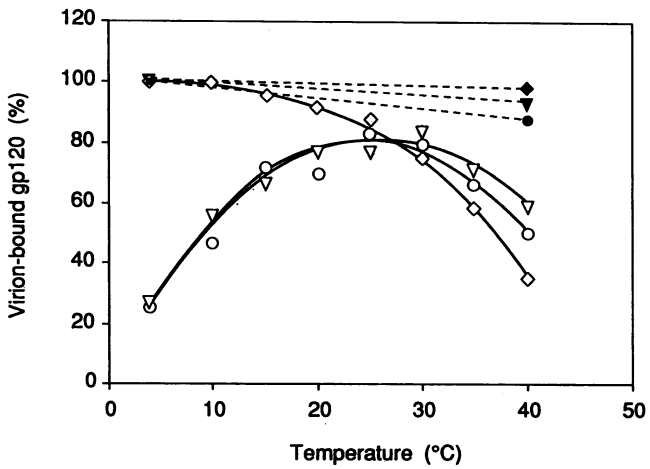

FIG. 4. Temperature dependence of gp120 loss from virions. Virions (100 μl) were incubated at the temperatures indicated for 2 h with (open symbols) or without (closed symbols) 2,200 nM sCD4 (550 nM for RF) before gel filtration. Symbols: ○ and ●, LS; ▽ and ▼, MDP1; ◇ and ◆, RF.

nM) sCD4 concentrations (Fig. 3). Similar data were obtained when loss of virion-sCD4 complexes was measured by ELISA (data not shown). The rate of gp120 shedding varied between isolates but was always much slower than the rapid shedding of gp120 from RF in response to a saturating sCD4 concentration (220 nM). The rates of gp120 loss were estimated from the slopes of the decay curves and were, in arbitrary units, as follows: RF, 100; LS, 20; MDP1, 10; JSH2, 10; and WM, 4. ACP1 was not tested. In the absence of sCD4, there was no significant loss of gp120 from any of the primary isolates within 8 h at 37°C or from RF within 4 h (Fig. 3).

The resistance of the primary isolates to gp120 shedding is therefore manifested by both a large increase in the sCD4 concentration required to induce shedding and a decrease in the rate of shedding in response to saturating sCD4 concentrations. The concentration factor is at least partially due to the reduced binding of sCD4 to the virions, but some isolates (e.g., WM) retain their gp120 for prolonged periods even when saturated with sCD4.

We noted above that three of the four primary isolates incubated for 1 h at either 4 or 37°C lost more gp120 at the lower temperature than at the higher. This contrasted markedly with RF, which sheds gp120 after sCD4 binding at 37°C but not at 4°C (Fig. 2) (44, 46). The temperature dependence of the shedding reaction was therefore investigated for isolates LS, MDP1, and RF (Fig. 4). There was no significant shedding of gp120 from RF at temperatures below about 25°C, but in the range between 25 and 40°C, shedding was proportional to the reaction temperature. Similar data were obtained with IIIB (data not shown). For LS and MDP1, however, the temperature dependence was different in that gp120 was lost from the viruses at 4°C but the extent of shedding then decreased as the temperature was raised from 4 to 25°C. Above about 30°C, there was a further increase in gp120 loss, similar to that seen for RF (Fig. 4).

DISCUSSION

We have demonstrated that intact virions of five primary HIV-1 isolates that are relatively resistant to sCD4 and CD4-IgG neutralization (13) bind the soluble receptor molecules with apparently reduced affinity compared with RF and IIIB, isolates adapted to growth in transformed T cells in vitro. Furthermore, the primary isolate virions are refractory to sCD4- and CD4-IgG-induced gp120 shedding. Since competitive inhibition due to sCD4 binding and irreversible inhibition due to gp120 shedding both contribute to neutralization of HIV-1 by sCD4 (7, 20, 25, 29, 30, 46, 48), reductions in the efficiency of either neutralization mechanism (or, more probably, both) can account for the neutralization resistance of these primary isolates. We do not, however, suggest that all primary isolates will behave exactly as those studied above: two additional isolates poorly neutralized by sCD4 readily shed gp120 after sCD4 binding but are spread in culture predominantly via cell-cell transfer, which is resistant to sCD4 inhibition (38a).

While the variation in sCD4- and CD4-IgG-binding affinities between soluble gp120 from RF and IIIB and from the primary isolates was very small (1.5- to 3-fold), a much greater variation (10- to 30-fold) between intact virions of these isolates was observed at 37°C (Fig. 1 and Table 1). This difference between soluble gp120 and intact virions indicates that most of the variation in sCD4 binding is related to differences in the tertiary and/or quaternary structure of gp120 on the intact virions of the different isolates. For example, the quaternary structure of a glycoprotein spike may modulate the ability of individual gp120 subunits to bind sCD4 and this may vary between isolates. It is also possible that the presence of gp41 influences the structure of the CD4-binding site on virions (44, 46) and that the reduced binding of sCD4 to primary isolate virions is directly related to their increased retention of gp120 at 37°C.

Our data are consistent with a model which proposes that virion-bound gp120 can bind sCD4 with either low or high affinity (44). The high-affinity state is exemplified by the binding of sCD4 to RF or IIIB virions at 37°C; the low-affinity state is exemplified by the binding of sCD4 to RF or IIIB virions at 4°C or to the primary isolate virions at 37°C. The high-affinity interaction between virions and sCD4 may or may not be identical in mechanism to that between soluble gp120 and sCD4 (the differences in the sCD4-binding curves

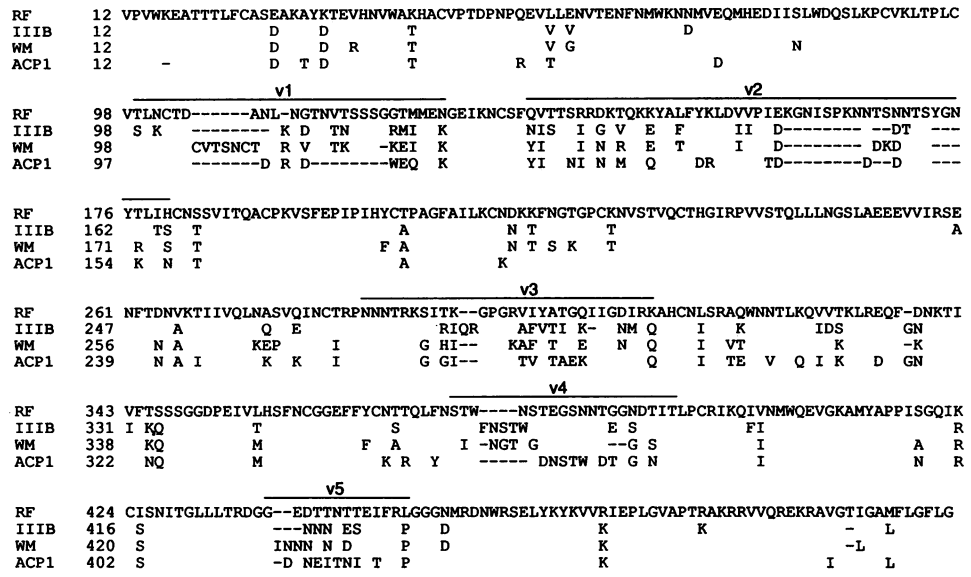

FIG. 5. Amino acid sequence comparison of gp120 glycoproteins from primary and cell culture-adapted HIV-1 isolates. Sequences are derived from references 5 and 49. Only amino acids differing from those of RF are shown for the other isolates. The sequences shown begin at residue 12 of the mature gp120 glycoprotein. For IIIB, the sequence of the PV22 clone is shown (49). Hypervariable domains V1 through V5 are indicated.

for RF virions at 37°C and soluble RF gp120 could be attributable in part to dissociation of sCD4 from virions during gel filtration). The low-affinity reaction is mechanistically distinct. For RF and IIIB, the transition between the low- and high-affinity binding states is favored at temperatures above approximately 20 to 25°C (44), but we suggest that for the primary isolates the transition to the high-affinity state is much less probable and the CD4-binding sites are predominantly of low affinity, even at 37°C. The interaction between sCD4 and the primary isolates at 37°C may therefore resemble that between sCD4 and RF at 4°C.

We propose that shedding of gp120 from virions can take place only after formation of the high-affinity interaction between gp120 and sCD4 and that a conformational change in the envelope glycoproteins that creates the high-affinity CD4-binding site simultaneously weakens the association of gp120 with gp41. For RF, this takes place rapidly at temperatures above 20 to 25°C (44); for the primary isolates, it occurs only slowly at 37°C. A possible mechanism for the proposed conformational change would be if a region of gp120 that is normally in contact with gp41 were induced to dissociate from that molecule in response to the initial contact of gp120 with sCD4 and instead to associate with a separate region of sCD4. It should be emphasized, however, that this is only speculation, and other explanations are possible.

There is genetic evidence that certain gp120 mutations, notably in the C3 and C4 domains, affect binding of gp120 to both gp41 and CD4 (22, 50). Alignment of the gp120 amino acid sequences of primary isolates WM and ACP1 (5) with those of laboratory-adapted isolates RF and IIIB (49) (Fig. 5) shows that the sequence variation conforms to the pattern of hypervariable and conserved regions reported previously for gp120 (42). There are few amino acid changes in the conserved regions between the primary and laboratory-adapted isolates. Comparison of the amino acid residues whose mutation in the IIIB sequence influences CD4 binding and/or gp41 association (22, 50) with the same residues in the primary isolate sequences revealed no obvious explanation for the functional differences between the isolates. Relation of the phenotypic variation we observed to subtle changes in the viral genotype will require greater knowledge of structure-function relationships in the HIV envelope than we now possess.

However, there is an additional complexity, indicated by the unusual temperature dependence of the shedding reaction for primary isolates. For two primary isolates (LS and MDP1), the gp120-gp41 interaction was destabilized by sCD4 at low temperatures whereas an enthalpically driven conformational change may counteract this at higher temperatures. For RF, the converse was observed. Thus, different isolates change the conformations of their envelope glycoproteins in different ways after binding sCD4. The importance of these changes for HIV infectivity remains to be evaluated, but the mechanism of the virion-sCD4 interaction is evidently not identical for all HIV-1 isolates. Conformational changes induced in the HIV envelope after binding to CD4 on the cell surface may also vary between isolates and influence the efficiency of virus entry; anti-CD4 monoclonal antibodies that block HIV infection post-CD4 binding have inhibitory effects on primary HIV-1 isolates different from those of cell culture-adapted isolates (38a).

It is intriguing that the cell culture-adapted HIV-1 isolates we studied (IIIB, RF, and MN) shed gp120 very easily (39, 48) but the primary isolates do not. Destabilization of the gp120-gp41 interaction may be one of the mechanisms by which some HIV-1 isolates adapt to growth in transformed T-cell lines in vitro. Alternatively, there may be selection pressure for virions with a higher affinity for CD4, which could coincidentally select for an unstable gp120-gp41 interaction if these phenotypes were directly related. It is not known whether there is positive selection for growth in transformed T cells or negative selection due to removal of the virus from the influence of the immune system, but it is clear from this study that the envelope glycoproteins of primary and tissue culture-adapted isolates behave in subtly

different ways. Concentration only on the latter may lead to misconceptions of the way HIV interacts with its host in vivo and to flawed drug and vaccine strategies. More attention should be focused on primary viruses. The increased stability of the envelopes of the primary isolates analyzed in this study suggests that the potential pathological consequences of spontaneous gp120 shedding postulated on the basis of in vitro experiments with the HIV-1 isolate LAV-1/IIIB or recombinant gp120 (9, 16, 18, 27, 32, 34, 41, 57) may be less serious in vivo than hitherto supposed.

It is also clear that, providing sCD4 and cellular CD4 behave equivalently, gp120 shedding is not essential for viral infectivity, as the primary isolates are infectious. This has been discussed extensively elsewhere (1, 47). We do, however, believe that sCD4-activated changes in the conformation of the virus envelope are crucial components of the virus-cell fusion reaction (54), and such changes have been demonstrated to occur both dependent on and independently of shedding (12, 20, 54).

Finally, while our results suggest that the primary HIV-1 isolates we studied are relatively resistant to sCD4 because of lower binding affinity and greater envelope stability, it is important to note that these isolates are neutralized by high concentrations of sCD4 (5, 13). Thus, the lack of clinical efficacy of sCD4 in previous trials (24, 55) may be explained by inability to achieve sufficient concentrations of the drug in plasma. Further development of sCD4-based therapies will potentially benefit, therefore, from the design of molecules that persist in serum at high concentrations and have increased affinity and avidity for gp120 (4, 6, 8, 61, 62).

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