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Equal Levels of gp120 Retention and Neutralization Resistance of Phenotypically Distinct Primary Human Immunodeficiency Virus Type 1 Variants upon Soluble CD4 Treatment

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Human immunodeficiency virus type 1 (HIV-1) variants passaged in T-cell lines, often called laboratory isolates, are potently neutralized by soluble CD4 (sCD4), whereas primary HIV-1 variants are highly resistant to sCD4 neutralization. Previously, it was demonstrated that the domain from V1 to V3 of the HIV-1 gp120 molecule contains one of the major determinants of sCD4 neutralization sensitivity, and the same region has also been implicated as influencing syncytium-inducing (SI) capacity and T-cell-line tropism. To determine possible differences in sCD4 neutralization sensitivity between phenotypically distinct primary HIV-1 variants, a panel of non-syncytium-inducing (NSI) and SI HIV-1 variants was studied. Primary NSI and SI HIV-1 variants appeared to be equally resistant to sCD4 neutralization. Consistent with this observation, sCD4 did not induce gp120 shedding from either primary NSI or SI HIV-1 variants at 37°C. Thus, it is not the potential of certain primary HIV-1 variants to infect T-cell lines but rather their adaptation to T-cell lines that is reflected in specific properties of the viral envelope which influence sCD4 neutralization sensitivity.

The biological variability of human immunodeficiency virus type 1 (HIV-1) plays a role in the pathogenesis of AIDS (5, 7, 13, 26, 45, 46). In the asymptomatic stage of HIV-1 infection, non-syncytium-inducing (NSI), preferentially macrophage-tropic HIV-1 variants predominate (40), and these variants remain present throughout the infection (7, 41). In contrast, syncytium-inducing (SI) HIV-1 variants, which can replicate efficiently in T-cell lines (27), emerge during the course of HIV-1 infection in 50 to 60% of infected individuals (12, 26, 46). The emergence of more cytopathic, SI HIV-1 variants precedes an accelerated CD4 T-cell depletion and progression to AIDS (4, 7, 26).

The CD4 molecule serves as the principal receptor for HIV-1 (9, 24, 28, 29). The interaction of the HIV-1 external envelope glycoprotein gp120 with the CD4 molecule ultimately mediates a pH-independent fusion of virus and cell membrane that is required for HIV-1 entry (44). Consequently, the gp120-CD4 interaction has provided a target for therapeutic intervention in HIV-1 infection (33). One experimental approach was to compete for binding of HIV-1 to the CD4 receptor on the cell with soluble forms of the CD4 molecule. In vitro experiments demonstrated that soluble CD4 (sCD4) potently neutralizes HIV-1 variants that have been passaged extensively in T-cell lines (so-called laboratory HIV-1 variants) (10, 14, 20, 43, 49). Studies with such laboratory HIV-1 variants revealed that the binding of sCD4 to gp120 on virions and HIV-1-infected cells (19, 32, 37), as well as sCD4 binding to gp120 on cells stably expressing HIV-1 envelope glycoproteins (15, 32), resulted in the dissociation of gp120 from its complex with gp41. The dissociation of gp120 may contribute to the sCD4-induced neutralization. However, upon therapeutic application of sCD4, no significant antiviral effect could be detected, as assessed by p24 antigen levels in serum (23, 39) and infectious HIV-1 titers in blood (8). The clinical failure of sCD4, although some short-term beneficial effects of high-dose sCD4 treatment have been reported (38), could be explained by the observation that, compared with laboratory HIV-1 variants, primary HIV-1 variants which had been passaged only in peripheral blood mononuclear cells (PBMC) were highly resistant to neutralization by sCD4 in vitro (8, 23, 36, 39). This relative resistance of primary HIV-1 variants correlated with reduced sCD4 binding to virions and increased virion gp120 retention (31).

Studies with recombinant viruses generated from laboratory HIV-1 variants and primary NSI, macrophage-tropic HIV-1 variants revealed that the region from the V1 domain to the V3 domain of the gp120 molecule contains a major determinant of sCD4 neutralization sensitivity of HIV-1 (22, 25, 34). The same region of gp120 has also been delineated as important for differences in SI capacity and cytotropism (2, 6, 11, 17, 25, 35, 42). Thus, the available data suggest that sensitivity to sCD4 neutralization segregates with SI capacity.

To explore possible differences between primary NSI and primary SI HIV-1 variants in their interactions with sCD4, a panel of four primary NSI and two primary SI HIV-1 variants was chosen (Table 1) (16, 18). All HIV-1 variants were recovered from cryopreserved PBMC from participants of the Amsterdam cohort studies. PBMC from the infected individuals were cocultivated with 3-day-old phytohemagglutinin-prestimulated PBMC from seronegative blood donors (45). For comparison, the SI HIV-1 variant ACH-320.3.1, which was molecularly cloned after a single passage through the H9 cell line, and two HIV-1 variants extensively passaged in T-cell lines (IIIB and the recombinant NL4-3 [1] that contains the 3′ half of IIIB) were used.

To determine the sCD4 neutralization sensitivity of each HIV-1 variant, an inoculum of fifty 50% tissue culture infective

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TABLE 1. sCD4 IC_{50}s for neutralization of HIV-1 variants with distinct biological phenotypes

HIV-1 isolate	1st day of p24 detection	PBMC maximum p24 production (ng/ml)	CPE[a]	MT-2 CPE[a]	sCD4 IC_{50} (μg/ml) at:	
					37°C	4°C
IIIB	4	520	+	++	0.2	0.3
ACH-320.3.1	5	330	+	++	51.1	33.6
ACH-320.2A.1.2	4	530	+	++	45.9	20.4
Ams-16.2	5	250	++	+++	29.9	22.4
ACH-320.2A.2.1	6	205	−	−	45.2	36.3
ACH-239.11	5	310	−	−	73.6	9.4
Ams-180	4	270	−	−	63.1	31.6
Ams-181	5	320	−	−	36.7	26.9

[a] CPE, cytopathic effect (i.e., SI capacity). −, no syncytia; +, 1 to 10 syncytia; ++, 11 to 50 syncytia; +++, >50 syncytia per 100× field.

dose per ml was incubated for 2 h with 0, 0.12, 0.24, 0.49, 0.98, 1.95, 3.91, 7.81, 15.63, 31.25, 62.50, 125, or 250 μg of recombinant sCD4 (Genentech Inc.) per ml. For use in the experiments, all viral stocks had been grown and their titers had been determined on 3-day-old phytohemagglutinin-prestimulated PBMC. Since it was previously shown that, in contrast to laboratory-adapted HIV-1 variants, primary viruses shed gp120 to a greater extent after sCD4 treatment at 4°C than at 37°C (30, 31), the sCD4 neutralization sensitivity was determined at preincubation temperatures of both 4 and 37°C before the addition of the virus-sCD4 mixture to the target cells at 37°C. These were 1.5×10^5 3-day phytohemagglutinin-prestimulated PBMC from seronegative blood donors. The cells were cultured in the presence of interleukin 2 (10 U/ml), and on days 7 and 14 the culture supernatants were analyzed for virus production as reflected by the presence of HIV-1 p24 (47). Each datum point represents quadruplicate cultures (Table 1).

The sCD4 concentrations that reduced the virus titer by 50% (50% inhibitory concentrations [IC_{50}s]) at 4 and 37°C are shown in Table 1. The sCD4 IC_{50}s did not vary between days 7 and 14 (data not shown). As was shown previously (10, 14, 20, 43, 49), laboratory HIV-1 variant HIV-1 IIIB was highly sensitive to neutralization by sCD4 at 37°C (IC_{50} of 0.2 μg/ml). Despite its single earlier passage in H9 cells, the PBMC-grown molecular clone ACH-320.3.1 was highly resistant to sCD4 neutralization. In comparison with HIV-1 IIIB, the primary HIV-1 variants required a 150- to 368-fold (IC_{50} of 29.9 to 73.6 μg/ml) larger amount of sCD4 for 50% neutralization at 37°C. However, no profound distinction between the sCD4 neutralization sensitivities of the NSI and SI primary HIV-1 variants was observed. The sCD4 IC_{50}s at 37°C of the primary HIV-1 variants were somewhat lower than those at 4°C. However, as found at 37°C, no major difference between the sCD4 neutralization sensitivities of NSI and SI primary HIV-1 variants was observed when the virus-sCD4 preincubation temperature was reduced to 4°C. Furthermore, PBMC-grown HIV-1 IIIB remained highly sensitive to neutralization by sCD4 at 4°C (IC_{50} of 0.3 μg/ml), whereas the H9-passaged PBMC-grown HIV-1 variant ACH-320.3.1 was as resistant to sCD4 neutralization as the primary HIV-1 variants. In comparison with HIV-1 IIIB, the primary HIV-1 variants required a 31- to 121-fold (IC_{50} of 9.4 to 36.3 μg/ml) larger amount of sCD4 for 50% neutralization at 4°C.

To investigate the effect of sCD4 on gp120 retention of primary HIV-1 variants, 700-μl aliquots of HIV-1-containing culture supernatants of the NSI primary HIV-1 variants ACH-320.2A.2.1 and ACH-239.11 and the SI primary HIV-1 variants ACH-320.2A.1.2 and Ams-16.2 were incubated with or without 10 μg of sCD4 per ml at 4 and 37°C. For comparison, the single H9-passaged PBMC-grown HIV-1 variant ACH-320.3.1 and the frequently T-cell-line-passaged HIV-1 recombinant NL4-3 were used. One hundred-microliter samples were assayed for gp120 retention at 0, 0.5, 1, 2, 4, and 8 h. The fractionation of untreated controls and the HIV-1-sCD4 mixtures was performed as described before, by a combination of gel filtration on Sephacryl S-1000 and enzyme-linked immunosorbent assay (31, 32).

In the absence of sCD4, SI primary HIV-1 variant ACH-320.2A.1.2 showed some spontaneous gp120 dissociation after 4 h at 4°C (Fig. 1C), whereas none of the other HIV-1 variants lost significant amounts of gp120 within 8 h at 4 or 37°C (Fig. 1B and D to F). In the presence of sCD4, the primary HIV-1 variants and the single-H9-passaged HIV-1 variant ACH-320.3.1 did not differ in their gp120 loss at 37°C. Thus, sCD4 did not cause significant gp120 shedding from either the NSI or the SI primary HIV-1 variants within 8 h at 37°C (Fig. 1B to F). The observation that sCD4 induced gp120 shedding from NL4-3 (Fig. 1A) at both 4 and 37°C is consistent with results obtained previously with other laboratory HIV-1 variants (19, 31, 32).

In contrast to the results obtained at 37°C, the primary HIV-1 variants demonstrated a clear gp120 loss within 2 h in the presence of sCD4 at 4°C (Fig. 1B to E). No distinction between gp120 losses of NSI and SI primary HIV-1 variants was observed. The rate of sCD4-induced gp120 loss from the H9-passaged HIV-1 variant ACH-320.3.1 at 4°C was identical to that observed at 37°C (Fig. 1B).

Previously, it was demonstrated that a segment of gp120 spanning the V1 domain up to the V3 domain harbors major determinants of sCD4 neutralization sensitivity (22, 25, 34), SI capacity (2, 11, 17), and cytotropism (6, 21, 25, 35, 42). We show here that the SI phenotype, with the ability to enter a T-cell line, does not segregate with sCD4 sensitivity at 37°C. Dissociation of the gp120 molecule from its complex with the gp41 molecule may not be required for syncytium formation and HIV-1 entry in T cells (48), but our data do not unequivocally resolve this issue. This is in agreement with the finding that envelope glycoprotein mutants of the laboratory HIV-1 variant HXBc2, which exhibited marked decreases in gp120 loss in the presence of sCD4, could still efficiently form syncytia (48). Moreover, it has been demonstrated with the laboratory HIV-1 variant BH10 that syncytium formation can occur under conditions in which sCD4 does not induce gp120 dissociation (15). These findings imply that upon passage in T-cell lines a selection for additional mutations in the region from the V2 to the V3 domain or elsewhere in the envelope of certain viruses of primary SI, T-cell-line-tropic HIV-1 variants may occur. These additional mutations may increase the capacity of HIV-1 to replicate in T-cell lines, which may be concurrent with the acquisition of sCD4 sensitivity at 37°C (50, 51). The HIV-1 variant ACH-320.3.1, which was obtained after only a single passage in H9 cells, did not differ for sCD4-induced gp120 shedding and was as resistant to sCD4 neutralization as the primary HIV-1 variants. The sCD4 neutralization sensitivity and gp120 shedding observed for NL4-3 suggest that multiple passages in T-cell lines, which will lead to an accumulation of mutations, are required to create an HIV-1 variant fully adapted to the T-cell line (laboratory HIV-1 variant) (3, 50, 51).

In conclusion, we have demonstrated that primary SI, potentially T-cell-line-tropic, and NSI HIV-1 variants can be equally resistant to neutralization by sCD4 and do not differ in

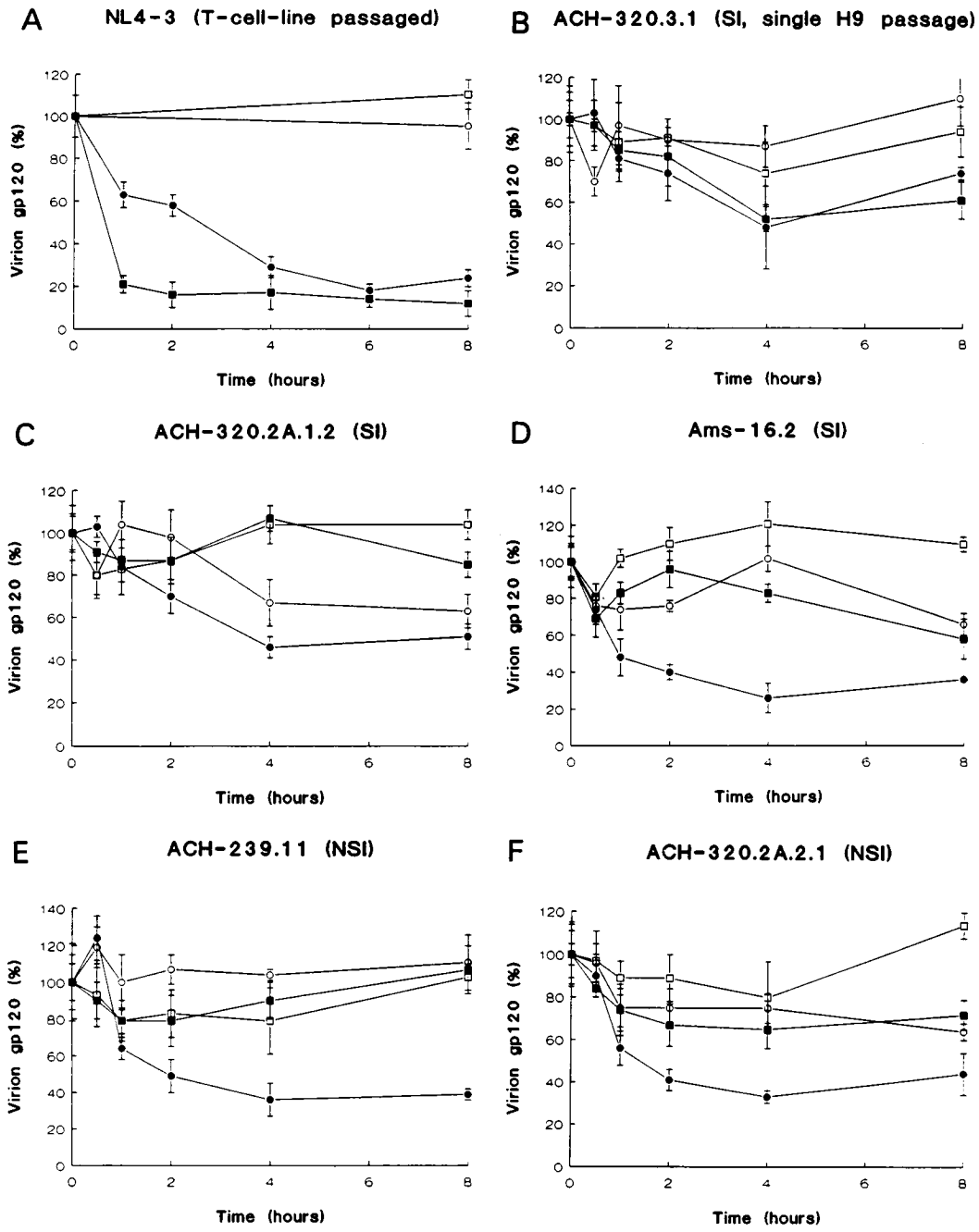

FIG. 1. gp120 retention by phenotypically distinct primary HIV-1 variants. HIV-1 variants were incubated for the times indicated with (●, ■) or without (○, □) 10 μg of sCD4 per ml at 4°C (○, ●) and 37°C (□, ■). The extent of gp120 shedding was determined by measuring the amounts of virion-bound (V) and soluble (S) gp120 present in each sample and then using the formula V/(V + S) × 100% to give a measure of the proportion of total gp120 that was virion associated. This value was defined as 100% for the samples obtained at time zero, and the values obtained at the later time points were normalized relative to this 100% value.

their gp120 retention in the presence of sCD4 at 4 and 37°C. Thus, although SI HIV-1 variants have the potential to replicate in T-cell lines, the properties of the viral envelope that are involved in sCD4 sensitivity are gained only after viral adaptation to T-cell lines. Hence, we emphasize that primary SI variants are not the equivalent of HIV-1 variants extensively passaged in T-cell lines.

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