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Human Immunodeficiency Virus Type 1 Entry into T Cells: More-Rapid Escape from an Anti-V3 Loop Than from an Antireceptor Antibody

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The entry of human immunodeficiency virus type 1 into two T-cell lines has been analyzed to determine the relative time courses with which virus entry can be blocked (i) by washing, (ii) by adding a monoclonal antibody to the V3 loop of gp120 that neutralizes without blocking CD4 binding (0.5β), or (iii) by adding an antireceptor monoclonal antibody that competes for virus binding (leu3a). During entry into C8166 cells, 50% escape from the wash as well as the anti-V3 loop antibody required 20 min, whereas 50% escape from the leu3a block required 45 minutes. In contrast, during entry into H9 cells, 50% escape from the wash block required 50 min, 50% escape from the anti-V3 loop antibody required 110 min, and 50% escape from the antireceptor antibody required 190 min. These results demonstrate that the times required for entering virus to escape each of the blocks were cell type specific. They also demonstrate that V3 loop-dependent steps occur relatively early in entry and suggest that binding of gp120 to CD4 is important for late as well as early steps in human immunodeficiency virus type 1 entry.

Entry of human immunodeficiency virus type 1 (HIV-1) into T cells is mediated by the viral envelope glycoproteins gp120 and gp41 and the CD4 host cell protein, which serves as a receptor (for reviews, see references 7, 13, and 21). HIV-1 envelope glycoproteins form the surface spikes visualized in electron micrographs of virions. Each spike represents an oligomer of gp120 and gp41 molecules (3). The gp41 transmembrane proteins anchor the oligomer in the viral membrane (5). Viral entry is mediated by gp120 binding to CD4 (for recent articles on this subject, see references 22 and 26), putative postbinding conformational changes that expose a fusion domain at the N terminus of gp41 (2, 8, 9, 14, 19), and fusion of viral and host cell membranes. Entry is pH independent and can occur by fusion through the cytoplasmic membrane or the membrane of an endocytic vesicle (6, 18, 20).

Recently, we used single-cycle analyses to demonstrate that differences in the permissiveness of T-cell lines for HIV-1 NL4-3 infections are determined by differences in the permissiveness of the cells for virus entry (25). In the highly permissive C8166 cells, 50% of the infectious units of NL4-3 escaped an antireceptor antibody block (leu3a block) within approximately 30 min of infection. In contrast, in three less permissive cell lines, H9, A3.01, and Jurkat, 50% entry required an average of 4 h. Entry efficiency correlated with entry rate, with stocks of NL4-3 having about three-times-higher titers of infectious units on C8166 cells than on H9 cells. In each of the cell types, entry required CD4. However, the rate and efficiency of entry did not correlate with surface levels of CD4.

The current study was undertaken to further characterize steps that determine the rates with which infectious units of NL4-3 enter cells. In particular, we wished to determine the relative time courses with which infectious units of NL4-3 escaped the ability to be blocked by the antireceptor monoclonal antibody leu3a and by a neutralizing monoclonal antibody to the third variable (V3) loop of gp120 (for a recent article on this subject, see reference 15). Neutralizing antibody to the V3 loop does not block gp120-CD4 binding (23) and is thought to act by inhibiting postbinding conformational changes that are required for entry. Studies of the time course of NL4-3 escape from an anti-V3 loop antibody were undertaken with C8166 and H9 cells. These two cell lines have substantial differences in their permissiveness for NL4-3 entry (25). A well-characterized neutralizing monoclonal antibody to the V3 loop, 0.5β, was used to block V3 loop activity (17).

To establish conditions that would allow us to use 0.5β as an entry block, 0.5β concentrations of 0.02 to 20 μg/ml were tested for their ability to block NL4-3 entry into C8166 and H9 cells. Neutralization assays were conducted for NL4-3 that had been preincubated with 0.5β for 30 min at 37°C prior to its addition to cells as well as for virus that was not exposed to 0.5β until the time at which it was added to cells. Several concentrations of leu3a were also tested for their ability to block entry into C8166 and H9 cells. In these tests, leu3a was added to cells 10 min before the addition of virus. Both antibodies successfully blocked NL4-3 infections on C8166 and H9 cells (Fig. 1). The concentration of 0.5β required to block entry was 100 to 1,000 times higher than that of leu3a. The efficiency of the 0.5β block was not affected by preincubating virus with antibody. Further blocking experiments were done with 20 μg of 0.5β per ml and 250 ng of leu3a per ml. These levels of the two different antibodies achieved ≥80% blocking of entry within 10 min (Fig. 1). By working with levels of antibody that gave similar levels of blocking, we minimized effects of differences in affinity or binding kinetics on escape times.

The next series of experiments compared the time courses

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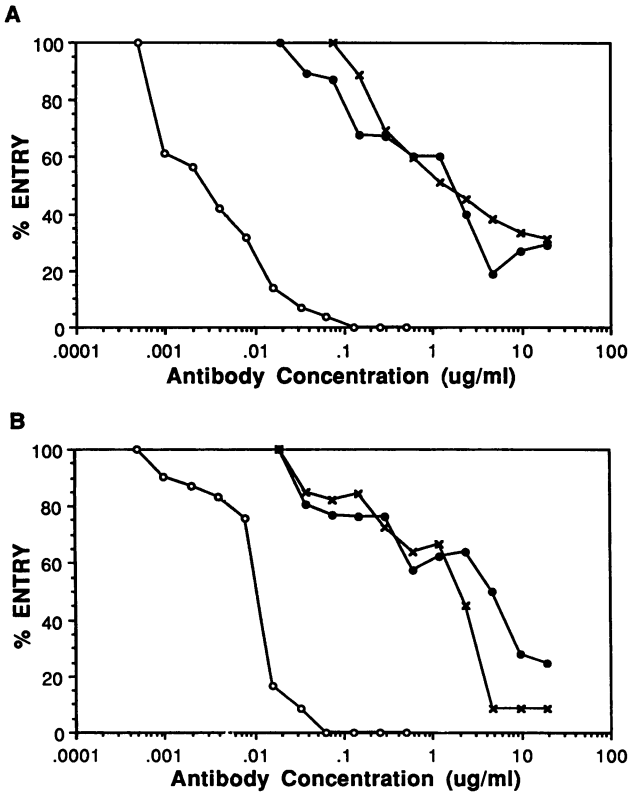

FIG. 1. Concentrations of the leu3a and 0.5β monoclonal antibodies required to block entry of NL4-3 into C8166 (A) and H9 (B) cells. The indicated concentrations of leu3a (○) were added to cells 10 min before the initiation of infections. The indicated concentrations of 0.5β were added to virus 30 min before infection (●) or to cells 10 min before infection (×). Percent entry is 100 times the number of cells that underwent infection in the presence of the blocking antibody divided by the number of cells that underwent infection in the absence of the blocking antibody. See text for details.

with which entry could be blocked by washing cells, by adding the antireceptor antibody (leu3a block), or by adding the anti-V3 loop neutralizing antibody (0.5β block) (Fig. 2 and Table 1). Infections were initiated with prewarmed cells and virus in the presence of 2 μg of Polybrene per ml at multiplicities of 0.25 C8166 infectious units per cell. At various times postinfection, aliquots of infected cultures underwent one of the blocking treatments (wash, addition of 250 ng of leu3a per ml, or addition of 20 μg of 0.5β per ml). At 12 to 15 h postinfection for H9 cells and 6 h for C8166 cells, leu3a (250 ng/ml) and dideoxycytidine (2 μM) were added to the cultures to prevent the spread of progeny virus or syncytium formation, which would confuse the quantitation of the first round of infection (25). Successful entry was scored by determining plateau levels of virus-expressing cells by indirect immunofluorescence (assays were done ≥30 h after infection of C8166 cells and ≥65 h after infection of H9 cells) (25).

The time courses for escape from each of the blocks were cell line specific (Fig. 2 and Table 1). The wash block, which measures the rate of virus adsorption to cells, was the first block to be passed, with infectious units requiring about three times longer to adsorb to H9 than to C8166 cells. Interestingly, in C8166 infections, escapes from the wash and anti-V3 loop blocks had indistinguishable time courses. This suggests that V3 loop-dependent steps in entry take place essentially simultaneously with binding in C8166 cells. In contrast, during entry into H9 cells, 50% escape from 0.5β required more than twice as long as escape from the wash. Unexpectedly, in both C8166 and H9 cells, the leu3a escape followed the 0.5β escape. About 25 min elapsed between escape from 0.5β and escape from leu3a in C8166 cells, whereas about 80 min elapsed between these escapes in H9 cells.

To test whether the blocking pattern observed for 0.5β was a general pattern for anti-V3 loop antibodies, a polyclonal serum raised against a V3 loop peptide (RP135) in goats (10) was tested for its blocking activity. A 1:40 dilution of this serum neutralized >95% of NL4-3 within 10 min. The patterns of escape from the anti-RP135 serum were very similar to those observed for 0.5β on both C8166 cells and H9 cells (data not shown). As observed with 0.5β, each of the escapes preceded the leu3a escape. Thus, relatively rapid escape from anti-V3 loop antibodies appears to be a general characteristic of NL4-3 infections.

We had not anticipated that escape from the anti-V3 loop antibodies would precede escape from leu3a. The relatively rapid escape from the V3 loop blocks suggests that V3 loop function in entry is early and short-lived. This could be consistent with the hypothesis that proteolytic cleavage of the V3 loop facilitates the exposure and function of the gp41 fusion domain (2, 9, 11, 12). In contrast, the relatively long periods required for leu3a escape suggest that late as well as early steps in entry require binding of gp120 to CD4. Quantitative analyses of the ability of synthetic soluble CD4 to block infectivity have demonstrated that multiple gp120-CD4 interactions are required for the entry of a single infectious unit (16). Our results suggest that these multiple interactions constitute a rate-limiting step in infection. One function of multiple gp120-CD4 binding events might be the triggering of conformational changes necessary for the fusion step of entry. The enhancement of the infectivity of the African green monkey simian immunodeficiency virus by soluble CD4 would be consistent with this possibility (1). A second explanation might be that fusion requires the recruitment of the gp41 proteins present in several spikes into a single multimeric structure to form a fusion pore (24). Such a multimeric structure would be consistent with the identification of a *trans*-dominant mutation in the fusion domain of gp41 (4).

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TABLE 1. Times required for infectious units of NL4-3 to escape various entry blocks in C8166 and H9 infections

Block	Time required for 50% escape (min) in[a]:	
	C8166 cells	H9 cells
Wash	20	50
0.5β	20	110
leu3a	45	190

[a] Data are taken from the time courses presented in Fig. 2. See text for details.

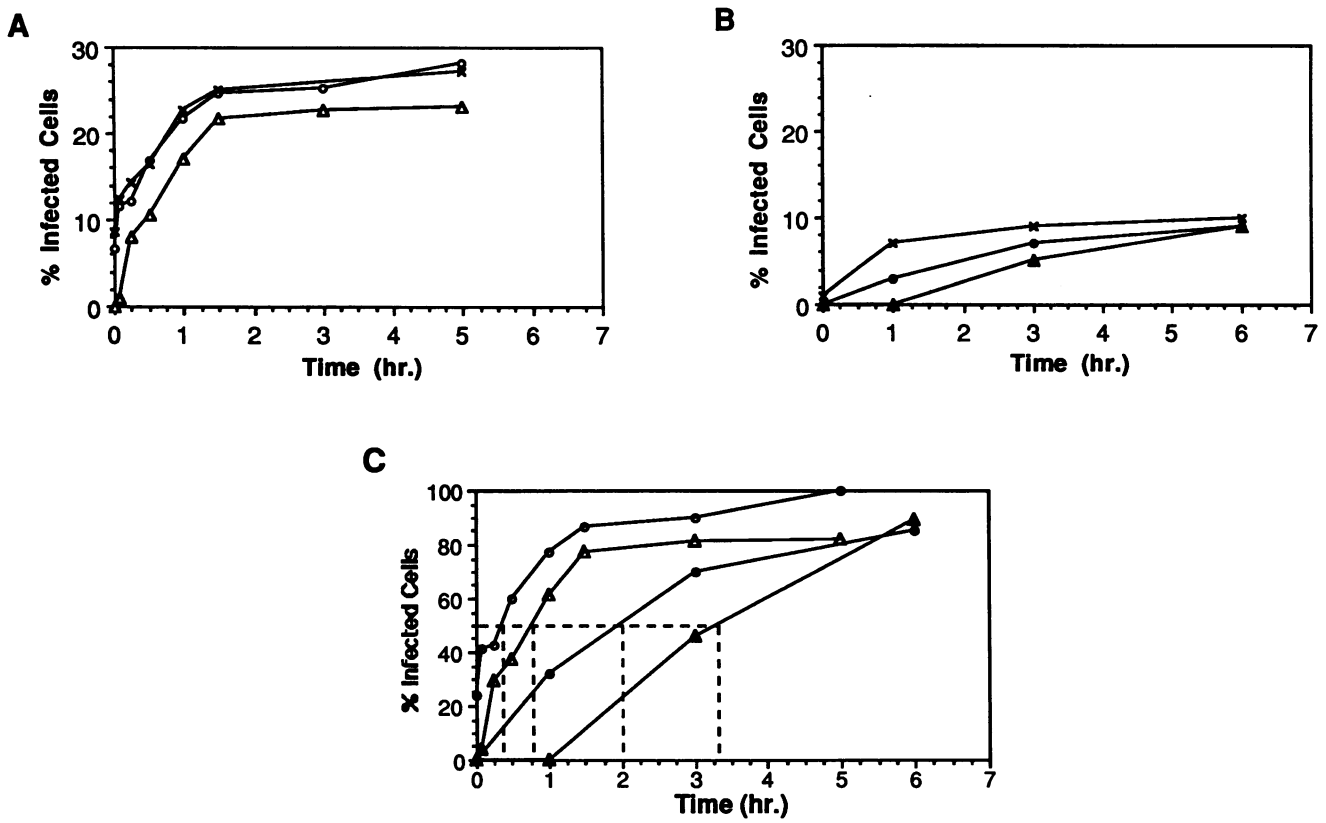

FIG. 2. Time courses with which infectious units of NL4-3 escaped various treatments. Shown are time courses for escape from the wash block (×), the 0.5β block (○ and ●), and the leu3a block (△ and ▲) in C8166 cells (A) and H9 cells (B). (C) Data from panels A and B are normalized for percent entry. The dashed lines indicate how the 50% escape times presented in Table 1 were estimated.

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