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Kishore K. Srivastava

Roberto Fernandez-Larsson

Donna M. Zinkus

University of Massachusetts Medical Center

See next page for additional authors

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Human immunodeficiency virus type 1 NL4-3 replication in four T-cell lines: rate and efficiency of entry, a major determinant of permissiveness

Authors

Kishore K. Srivastava, Roberto Fernandez-Larsson, Donna M. Zinkus, and Harriet L. Robinson

Human Immunodeficiency Virus Type 1 NL4-3 Replication in Four T-Cell Lines: Rate and Efficiency of Entry, a Major Determinant of Permissiveness

KISHORE K. SRIVASTAVA, ROBERTO FERNANDEZ-LARSSON, DONNA M. ZINKUS,
AND HARRIET L. ROBINSON*

Department of Pathology, University of Massachusetts Medical Center, Worcester, Massachusetts 01655

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Single-cycle infections have been used to study the human immunodeficiency virus type 1 (HIV-1) life cycle in CD4⁺ T-cell lines that differ in their permissiveness for infection. In single-cycle infections of highly permissive C8166 cells, 50% of the infectious units escaped being blocked by a monoclonal antibody against the virus binding site on CD4 (leu3a) within 30 min. In contrast, 50% of the infectious units for three less permissive cell lines (H9, A3.01, and Jurkat) required 4 h to escape the leu3a block. Entry was also more efficient in the highly permissive cells, with NL4-3 stocks having three times more infectious units for C8166 cells than for H9, A3.01, or Jurkat cells. Postentry steps up through reverse transcription required ~3.5 h in each of the cell lines. The times lapsing between reverse transcription and the expression of reverse transcripts ranged from 17 to 25 h in the different cell lines. Virus production per cell was also similar in the different cell lines (within 1.5-fold of each other). These results indicate that a major determinant of the permissiveness of growing T cells for HIV-1 is the rate and efficiency of virus entry.

CD4⁺ cell lines vary in their susceptibility to human immunodeficiency virus type 1 (HIV-1) infection, with independent isolates of HIV-1 displaying characteristic potentials for replication on different cell lines (3, 4, 6, 9, 16). To gain insight into which step or steps of the virus life cycle determine these differences, we have analyzed single-cycle infections of a laboratory strain of HIV-1 (HIV-1 NL4-3) in four T-cell lines that differ in their permissiveness for infection. These lines included highly permissive C8166 cells (17) and less permissive H9 (14), A3.01 (5), and Jurkat (21) cells. Infections were initiated with HIV-1 NL4-3 which had been recovered from pNL4-3, a DNA constructed from 5' HIV-1-NY5 and 3' HIV-1-LAV-1 sequences (1). NL4-3 encodes all known HIV-1 gene products. Most infections were initiated at a multiplicity of ~0.25 C8166-infectious units (IU), with the spread of progeny virus being restricted by the addition of inhibitors during the first cycle of infection (see below).

Virus entry was followed with timed additions of leu3a, a monoclonal antibody with high affinity ($K_d = 4.4 \times 10^{-10}$) for the virus-binding site on CD4 (13, 17). Addition of 240 ng of leu3a per ml 10 min prior to the addition of virus completely blocked infection in each of the lines. The addition of leu3a did not affect the growth of the lines. Reverse transcription was followed by timed additions of an inhibitor of reverse transcriptase, dideoxycytidine (ddC). The addition of 2 μ M ddC to cultures prior to the addition of virus completely prevented infection while causing only modest reductions in cell growth (<30% reductions in cell numbers occurred in treated cultures) (11). Leu3a was also added to cultures receiving ddC to prevent syncytium formation. Escape from the leu3a or ddC blocks was scored by culturing cells for a time sufficient for the infection to have undergone full expression and then assay-

ing for the percentage of cells expressing HIV-1 by indirect immunofluorescence (15). For C8166 cells, such assays were performed ≥ 26 h after infection. For the less permissive cell lines, assays were performed ≥ 45 h after infection. In initial experiments, assays were performed at two times (such as 48 and 72 h after infection) to ensure that the number of virus-expressing cells had reached plateau levels.

The time required for expression of a completed reverse transcript was determined by adding ddC and leu3a during an empirically determined window of time between the completion of reverse transcription and the expression of progeny virus. Aliquots of these cultures were assayed for virus-expressing cells at regular intervals, beginning 18 to 20 h postinfection. The presence of ddC and leu3a in these cultures prevented the spread of progeny virus and ensured analysis of a single-cycle infection.

Interestingly, NL4-3 escaped the leu3a block eight times more rapidly and three times more efficiently in infections of the highly permissive C8166 cells than in infections of the less permissive cells (Fig. 1 and Table 1). Whereas only 30 min were required for 50% of an infection to escape the leu3a block in C8166 infections, 4 h were required for 50% escape in the H9, A3.01, and Jurkat cultures. The more rapid escape from the leu3a block in C8166 cells was also accompanied by more efficient infection, with stocks infecting about three times more C8166 than H9, A3.01, or Jurkat cells.

Entry of virus into cells requires virus binding, putative conformational changes in the viral glycoprotein that expose the fusion domain, and the fusion of viral and host cell membranes. We do not know which of these steps determines the differences in the time course of leu3a escape. Leu3a is very effective at blocking virus binding to CD4. Leu3a may also be able to displace virus that has undergone binding but not penetration. The apparent surface densities of CD4 on the four cell lines did not correlate with the rate or efficiency of leu3a escape (Table 1). Thus, differences in the

* Corresponding author.

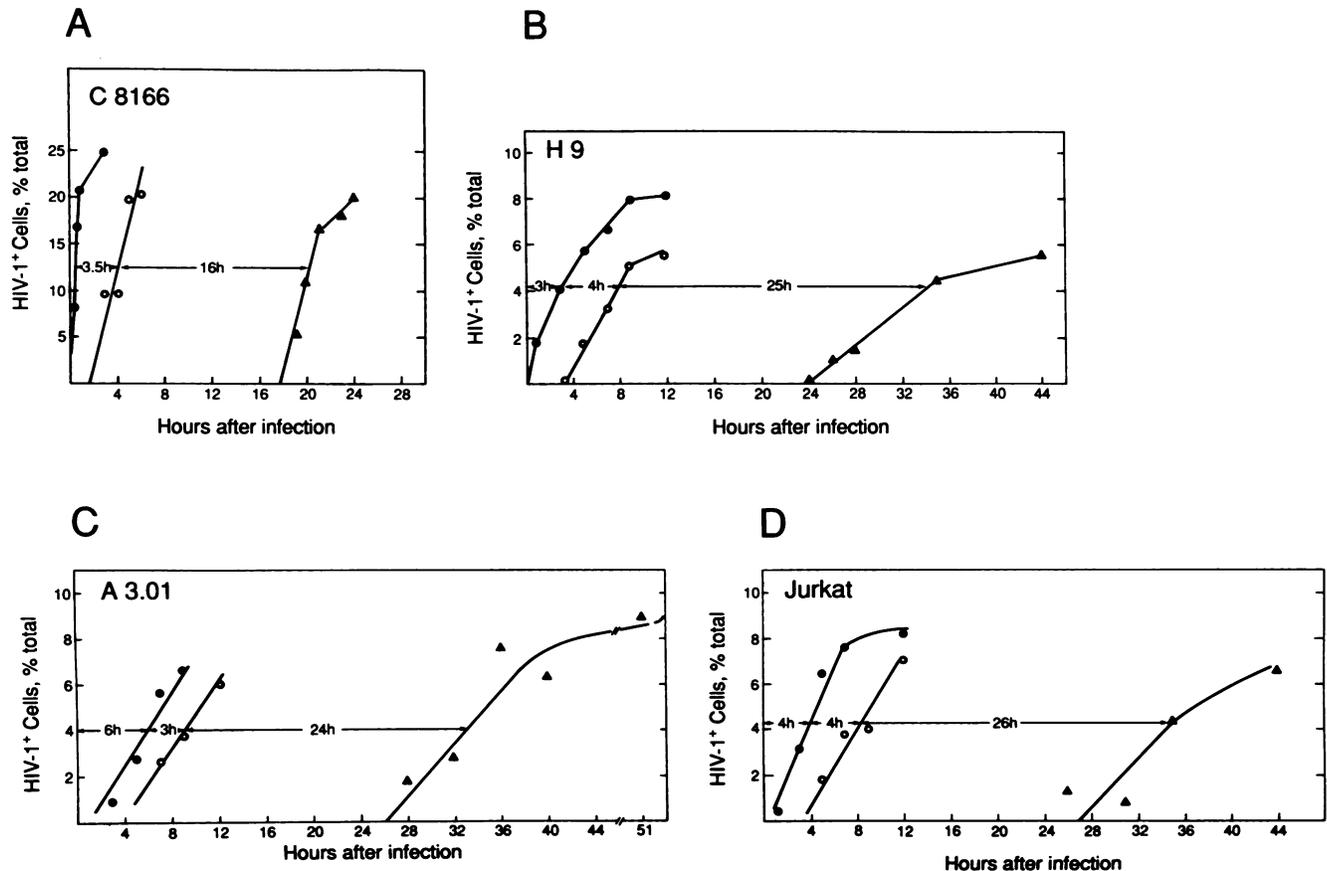


FIG. 1. Single-cycle infections of NL4-3 in C8166, H9, A3.01, and Jurkat cells. See text for details. Cells were infected at time zero. The times of addition of leu3a (●) or leu3a and ddC (○) to cultures and the times at which cultures were assayed for virus expression (△, ▲) are shown.

time course of leu3a escape are determined by host cell parameters other than mere surface density of CD4. These could involve susceptibility to syncytium formation. C8166 cells are unusually susceptible to NL4-3-induced syncytia, whereas the less permissive cells are either less susceptible or resistant.

Each of the infections required about 3.5 h to progress from the leu3a block past the ddC block. The similar length of this time period in each of the infections suggests that penetration, uncoating, and reverse transcription follow similar tempos in each of the infected cell lines. This 3.5-h

period is slightly shorter than would be predicted for the completion of a 9.7-kb reverse transcript by the polymerases of avian and murine C-type viruses, which incorporate ~30 nucleotides per minute (22). Thus, most of the time between the escape from the leu3a block and the escape from the ddC block may have been taken up by reverse transcription, with both penetration and uncoating requiring relatively little time. Relatively rapid penetration would be consistent with the results of studies in which trypsin treatments and fluorescence dequenching have demonstrated 50% entry of prebound virus in ≤ 15 min (10, 12).

The time required for expression of reverse transcripts exhibited a 1.5-fold difference among the cell lines, with an average of 22 to 25 h elapsing between the ddC escape and the appearance of virus-expressing cells in H9, A3.01, and Jurkat cells and only 17 h elapsing in C8166 cells. The shorter time required for reverse transcript expression in C8166 cells could reflect Tax expression in these human T-cell-lymphotropic virus type 1-immortalized cells (7): Tax could shorten the eclipse phase by activating the expression of the HIV-1 long terminal repeat (19). The time to expression did not correlate with short doubling time, as C8166 cells had the longest doubling time of the four cell lines tested.

The relative levels of NL4-3 production also varied about 1.5-fold among the cell lines. During the 27 to 28 h immediately following the appearance of virus-expressing cells, C8166 cells produced ~0.3 pg of p24 per infected cell,

TABLE 1. Single-cycle infection of NL4-3 in growing T cells^a

Cell line	Relative CD4 level	Time (h) needed for entry ^b	Time (h) needed for reverse transcription ^c	Time (h) needed for expression ^d
C8166	0.6	0.5	3.5 (3-4)	17 (16-18)
H9	0.3	4 (3-6)	3.5 (3-4)	22 (20-25)
A3.01	1.0	4 (2-6)	3.5 (3-4)	25 (22-29)
Jurkat	0.1	5 (4-5)	3.5 (3-4)	25 (23-26)

^a For details, see the text. Values represent the average number of hours (range) required for specific steps in the HIV-1 life cycle in three independent experiments. Relative CD4 levels were determined as described in reference 20.

^b Hours needed for 50% of the infectious virus to escape the leu3a block.

^c Hours between leu3a escape and leu3a plus ddC escape.

^d Hours between leu3a plus ddC escape and expression of virus proteins.

whereas A3.01 and H9 cells produced ~0.2 pg of p24 per infected cell.

These results clearly demonstrate that the rate and efficiency with which HIV-1 escapes the leu3a block are the major determinants of the relative permissiveness of T cells for infection. We think this finding will be of general importance in determining the pathogenicity and tropism of HIV-1 isolates. Kim et al. (8) have reported that the relative tropism of HIV-1-W13 for H9 and U937 (a monocyte-macrophage line) correlates with the level of virus binding as measured by cell-associated RNA at 4 h after infection. Entry playing a key role in permissiveness is also consistent with transfection overcoming T-cell restrictions on the replication of a patient isolate (2).

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