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Macrophage Tropism Determinants of Human Immunodeficiency Virus Type 1 In Vivo

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Strains of human immunodeficiency virus type 1 differ in their abilities to infect and replicate in primary human macrophages. Chimeric clones were constructed from a provirus unable to infect macrophages (NLHX) and envelope sequences (V3 loop) of viruses derived without cultivation from brain (YU2 and w1-1c1) or spleen (w2-1b4) tissues. The substituted V3 loop sequences in each case were sufficient to confer upon NLHX the ability to infect macrophages. Furthermore, an envelope domain immediately N terminal to the V3 loop also was found to modulate the level of replication in macrophages. These results demonstrate that an envelope determinant derived directly from patients with AIDS confers HIV-1 tropism for macrophages.

The primary cellular targets for human immunodeficiency virus type 1 (HIV-1) are CD4$^+$ lymphocytes and monocyte-derived macrophages (7). Whereas the lymphocyte is the major infected cell type in the blood, the macrophage is the predominant infected cell type in the brain and spinal cord (5, 9, 19, 36). Infected macrophages have also been identified in lymph nodes and lungs, and infected monocytes have been isolated from the blood of patients (1, 10, 20). The critical role of macrophages as a reservoir for visna virus infection further emphasizes the importance of this cell type in lentivirus pathogenesis (12, 28). The macrophage is also a target for infection by caprine arthritis-encephalitis virus and equine infectious anemia virus, as well as simian immunodeficiency viruses and HIV-2 (4, 15, 22, 27).

Lentiviruses, such as HIV-1, display a remarkable degree of sequence heterogeneity, resulting in differences in the capacity of diverse strains to infect macrophages (8, 11, 13, 18, 23, 25, 29, 31, 33, 35). Nevertheless, most or all strains of HIV-1 are capable of infecting primary CD4$^+$ lymphocytes (16). The molecular basis for variations in tropism for macrophages is incompletely defined.

To define the viral determinants necessary for macrophage infection, in previous studies chimeric clones were constructed with sequences derived from cultured HIV-1 isolates capable of infecting macrophages (HIV-1jrfl, HIV-1bal, HIV-1ada, and HIV-1sf162) and isolates incapable of infecting macrophages (HIV-1nl4-3, HIV-1hxb3, HIV-1hxb2, and HIV-1sf2) (18, 29, 32, 35). In each case, a region including the V3 principal neutralizing determinant of the gp120 surface envelope protein of the macrophagetropic viruses (26) conferred upon the non-macrophagetropic isolate the ability to infect macrophages. However, these studies all utilized molecular clones derived from cultured isolates.

Several studies have suggested that cultured HIV-1 isolates may misrepresent the properties of viral strains found in vivo. Virus cultivation may affect the range of sequences identified, the CD4-binding capabilities of the virus, and the tropism of isolates for macrophages or other cell types (3, 10, 24). In light of these concerns, this study investigates the nature of host cell tropism by using native sequences obtained directly from infected patient tissues, without prior passage of virus in vitro through tissue culture.

Proviral clones NL4-3 (NL) and NLHX were utilized for construction of chimeric clones in which HIV-1 envelope sequences derived directly from uncultured patient material were exchanged (Fig. 1). NLHX is derived from NL by replacing a *SalI-Bam*HI fragment containing *vpu* and partial *env*, *tat*, and *rev* sequences of clone HXB2. Virus derived from NL and NLHX was incapable of infecting macrophages, though still capable of productively infecting peripheral blood mononuclear cells (PBMCs). YU2 represents a replication-competent provirus which was cloned directly from brain tissue of an adult patient with HIV-associated encephalopathy without in vitro cultivation (21). Transfection of this clone into COS-7 cells gave rise to virus capable of productively infecting both PBMCs and macrophages. A chimeric clone designated NLYU2ex, in which *vpu*, *env*, and partial *vpr*, *tat*, *rev*, and *nef* sequences derived from YU2 were substituted for the corresponding sequences of NL, was also capable of productively infecting macrophages. YU2 sequences encoding both the V3 loop and the CD4 binding region of gp120 (NLHXYU2gg) or only the V3 loop domain (NLHXYU2gp) when placed within the NLHX clone were capable of infecting macrophages. Surprisingly, when the same sequences were placed into the NL clone (NLYU2gg), productive infection was not demonstrated. Similar findings were obtained with the homologous envelope fragment of isolate ADA, which was derived from blood cultured directly on primary macrophages. The corresponding envelope fragment with the V3 loop and the CD4 binding region of gp120 was capable of productive infection of macrophages when placed into clone NLHX (NLHX ADAgg) but not when placed into clone NL (NLADAgg).

The V3 envelope domain was also found to be capable of conferring macrophage infection upon NLHX when derived from uncultured polymerase chain reaction clones from brain [NLHXw1-1c1(B)gp] or spleen [NLHXw2-1b4(S)gp]

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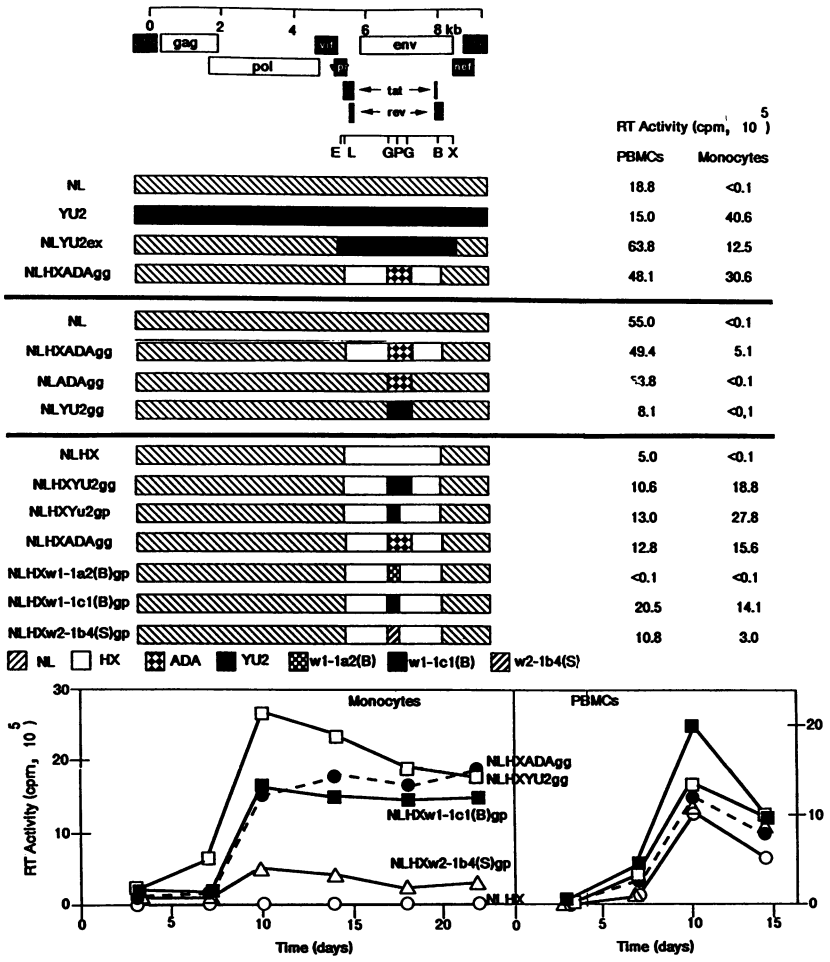

FIG. 1. Monocytetropic envelope determinants of HIV-1 strains from uncultured brain and spleen tissues. The top of the figure shows a schematic diagram of the HIV-1 genetic organization, with a scale for the nucleotide sequence positions. Restriction sites in the 3′ portion of the genome used for chimeric clone constructions are abbreviated as follows: B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; L, *Sal*I; P, *Ppu*MI; and X, *Xho*I. The structure of each clone was confirmed by restriction enzyme and nucleotide sequence analysis. The contributions to each chimeric clone are indicated by the key in the lower portion of the figure. The designation for each clone is shown to the left. Results are shown for infection experiments with PBMCs or primary monocytes performed as previously described and represent culture supernatant reverse transcriptase (RT) activity per milliliter at day 18 for monocytes and day 10 for PBMCs (35). The three panels represent different experiments. Similar results were obtained in 2 to 12 replicate experiments, and data from 3 to 5 other time points during each infection experiment gave similar findings. Kinetics of virus replication in the third experiment are shown at the bottom. Clones w1-1a2(B), w1-1c1(B), and w2-1b4(S) were obtained by polymerase chain reaction amplification of DNA extracted from brain (B) or spleen (S) tissues obtained at autopsy (6) and were cloned between *Bgl*II and *Bsu*36i sequences of pSVSX2, a modified form of pSVSX1 (14). The *Bgl*II fragment of the resultant clones was then cloned in place of the corresponding sequences of NLHX. LTR, long terminal repeat.

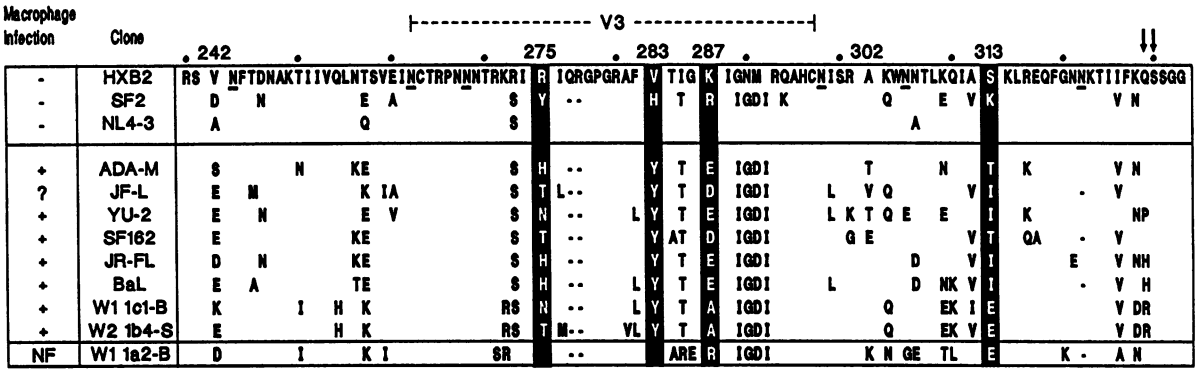

FIG. 2. Sequence alignment of tropism determinants of HIV-1 strains which differ in their ability to infect macrophages. Amino acid sequences for residues 240 to 333 (numbered according to ADA strain, as described by Westervelt et al. [35]) are shown for clone HXB2, and differences in the other isolates are indicated. Dashes indicate deletions. The first three clones listed do not replicate in macrophages, and the next eight clones listed are capable of infection and replication in both PBMCs and macrophages (18, 29, 32, 35). The last clone listed is incapable of macrophage or PBMC infection and is designated nonfunctional (NF). Cloned sequences from strain HIV-1jfl have not been tested for macrophage infection, though the virus isolate from which they were obtained is macrophage tropic (23). The black boxes at residues 275, 283, 287, and 313 indicate amino acids which consistently differ between macrophagetropic and non-macrophagetropic isolates. Dots indicate every 10th amino acid residue, and the arrows above residues 329 and 330 indicate positions previously shown to influence CD4 binding (35).

tissue of a pediatric patient with AIDS (6) (Fig. 1). Despite the marked differences in the ability of these chimeric viruses to productively infect macrophages, no significant differences in their ability to infect PBMCs were found. The biological characteristics of molecularly cloned virus strains were not altered by replication in macrophages or cocultivation with lymphocytes over the time course of these experiments. One clone [NLHXw1-1a2(B)gp] was incapable of replication on macrophages or PBMCs. The biological basis for this phenotype is currently under study.

Sequence comparisons of the macrophage tropism domain of the envelope are portrayed in Fig. 2. Numerous differences between isolates which do not infect macrophages (HXB2, SF2, and NL4-3) and those which infect macrophages (ADA-M, JF-L, YU-2, SF162, JR-FL, BaL, W1-1c1-B, and W1-1b4-S) are noted. However, at only four positions are sequences consistently different among isolates which differ in their ability to infect macrophages (black boxes in Fig. 2). Most interesting is the finding that at position 283, a tyrosine residue is found in all isolates capable of macrophage infection. At position 287, an acidic amino acid or an alanine residue is present in macrophagetropic isolates, whereas basic amino acids are identified at this position in the three isolates incapable of macrophage infection. These sequences may modify the function of this tropism domain, which may contribute to CD4 binding activity, regulate conformational changes in gp120 after CD4 binding, and/or affect proteolytic cleavage after CD4 binding (2). One or more of these properties may be required for cell-type-specific infectivity properties.

An additional set of chimeric clones was constructed to determine why the V3 loop sequences were active in the context of the NLHX clone but not in the NL clone. For this purpose, different NL-derived portions of the *vpu* and partial *vpr*, *env*, *tat*, and *rev* sequences between the *Sal*I and *Bam*HI sites of NLADAgg were replaced with HXB2 sequences (Fig. 3). Whereas all clones gave rise to virus that could productively infect PBMCs, only clones NLHXsbAD Agg, NLHXkbADAgg, and NLHXkg_1ADAgg were capable of productively infecting macrophages. These clones have in common HXB2 sequences between the *Kpn*I and 5′ *Bgl*II sites, which encode only gp120 sequences. Virus strains derived from clones lacking this domain (NLHXlsADAgg, NLHXskADAgg, and NLHXg_2bADAgg) were incapable of productive infection of macrophages. However, in each case, virus could be rescued from infected macrophages after 24 days of culture by cocultivation with PBMCs (34). Thus, the additional gp120 determinant is not required for macrophage infection but enhances some aspect of the virus replication cycle. Therefore, we have designated this region an efficiency domain. However, no significant differences in the ability of these isolates to replicate in PBMCs were noted. The upstream (kg) determinants of gp120 alone from HXB2 or ADA do not result in virus that can productively infect monocytes or virus that can be rescued from monocytes by cocultivation with lymphocytes (NLHX clone in Fig. 1 and reference 35).

A similar finding was noted by Shioda and colleagues, who demonstrated that the V3 loop domain, while necessary for infection of macrophages, was not sufficient for high levels of virus production in macrophages (32). An additional determinant which mapped between a *Dra*III site and a *Stu*I site (residues 97 to 171; Fig. 4) was found to be required for efficient replication in macrophages. In the current study, the only amino acid differences between NL4-3 and HXB2 sequences in this region are between residues 134 and 162 and at residue 236. Figure 4 depicts an alignment of sequences within this region, which demonstrates differences between clones which do and clones which do not confer efficient replication in macrophages in the presence of the required V3 domain. These sequences include a portion of the variable domain V2 found within a disulfide-linked loop (17). Differences between the clones in potential N-glycosylation sites in this region are noted. No single amino acid position is consistently different in the clones which exhibit inefficient versus efficient macrophage replication. Site-specific mutagenesis will be required to determine the importance of residues in this domain for HIV-1 replication in macrophages. Though previous work has demonstrated that sequences in C2 and V1 may regulate HIV-1 infectivity without modulating CD4 binding (38), V2 residues have not yet been examined for their effects on virus replication. It is

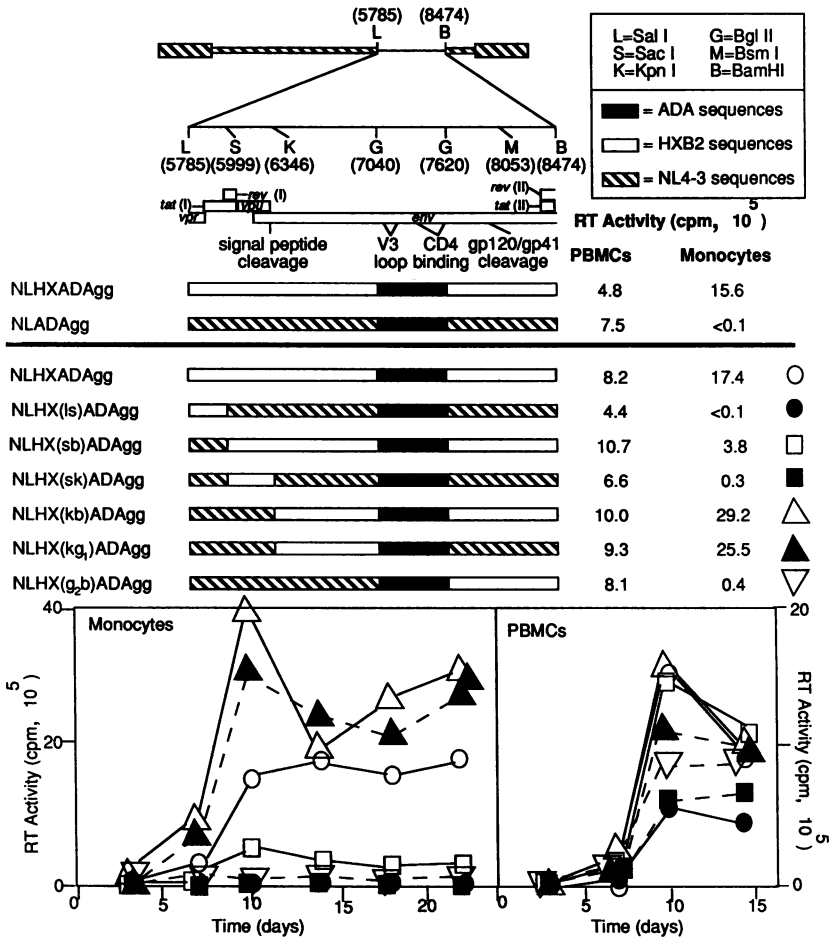

FIG. 3. Effect of sequences flanking the tropism determinant on HIV-1 replication in monocytes. A schematic of the HIV-1 genome is shown at the top, with sequences expanded between the *Sal*I and *Bam*HI sites at positions 5785 and 8474, respectively (30). The relative positions of *tat*, *vpr*, *vpu*, *rev*, and *env* are indicated, as well as the sequences in *env* encoding the signal peptide cleavage site, the V3 loop, the CD4 binding domain, and the gp120-gp41 cleavage site. Chimeric clones are diagrammatically represented below to show the contributions of ADA, HXB2, and NL4-3 sequences within each clone. Results of studies of replication in monocytes or PBMCs are shown to the right of each clone as the reverse transcriptase (RT) activity per milliliter obtained on day 18 for the first two clones or day 14 for the remainder of the clones examined in a separate experiment. Similar results were obtained in 2 to 12 replicate experiments, and data from 3 to 5 other time points during each infection experiment gave similar findings. Kinetics of virus replication are shown at the bottom.

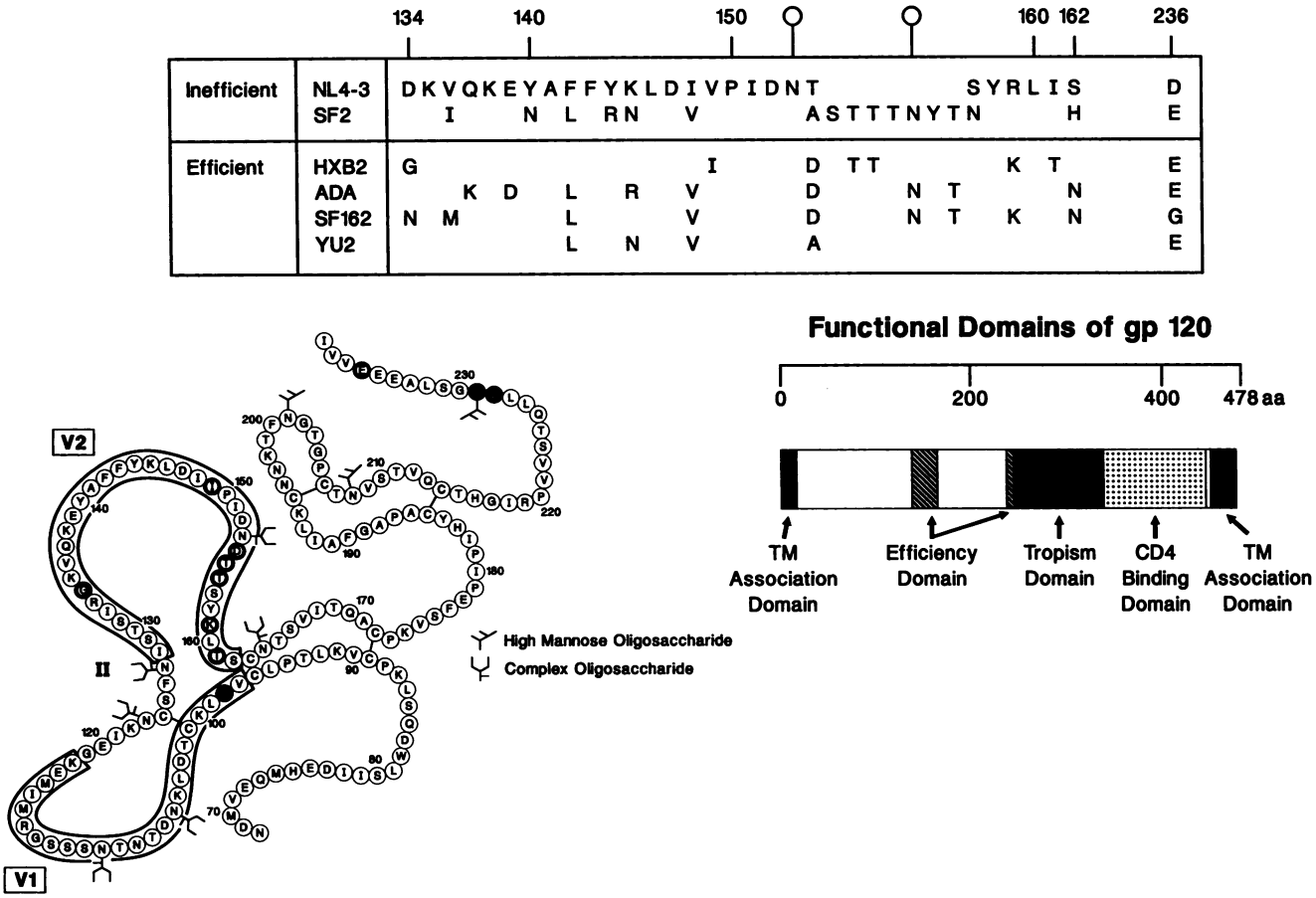

FIG. 4. Sequence alignment of an envelope domain N terminal to V3 which modulates the replication of HIV-1 in monocytes. Predicted amino acid residues 134 to 162 and 236 of clone NL4-3 gp120 are shown, and differences relative to clones SF2, HXB2, ADA, SF162, and YU2 are indicated. Clones NL4-3 and SF2 replicate inefficiently in monocytes, whereas clones HXB2, ADA, SF162, and YU2 result in viruses which are macrophagetropic. Nonconserved (O) potential N-linked glycosylation sites are indicated at the top. Residues are numbered according to reference 35. In the bottom left-hand portion of the figure, a schematic shows the predicted disulfide bonds and N-glycosylation site utilization in the domain encoded by sequences between the *Kpn*I and *Bgl*II sites of isolate HXB2. Eight partly shaded residues (134, 149, 154, 155, 156, 159, 161, and 236) are those which differ from NL4-3. Residues 98 and 229, which are completely shaded, have been implicated in virus uptake (38). The darkly shaded residue 228 has been shown to be important for export of the envelope protein from the endoplasmic reticulum (37). In the bottom right-hand portion of the figure, the position of the efficiency domain within the mature gp120 protein is shown relative to the positions of the tropism domain, the CD4 binding domain, and the TM association domains.

possible that the efficiency domain may enhance the activity of the V3 domain to increase the level of HIV-1 infection of macrophages (29, 34). Alternatively, this domain may influence a distinct aspect of the virus replication cycle in macrophages.

In conclusion, this work confirms the identification of an envelope determinant for macrophage tropism by using sequences from three additional viral genomes. Importantly, this determinant is present in viruses that have not been subjected to in vitro selection procedures. In addition, a second region of envelope which modifies the efficiency of virus infection or replication in macrophages has been identified. Sequence comparisons identified specific residues that may be exploited by site-directed mutagenesis to further define the biological activity of this second domain.

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