

The emergence and characterization of macrophage-tropic SIV/HIV chimeric viruses (SHIVs) present in CD4⁺ T cell-depleted rhesus monkeys

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Abstract: Highly pathogenic simian immunodeficiency virus/human immunodeficiency virus type 1 chimeric viruses (SHIVs) induce an extremely rapid, systemic, and irreversible depletion of CD4⁺ T lymphocytes following their inoculation into rhesus macaques. Confocal fluorescence microscopy was used to demonstrate that high levels of viremia in infected animals were sustained by virus-producing tissue macrophage (mφ) following the irreversible elimination of CD4⁺ T lymphocytes by highly pathogenic SHIV_{DH12R}. The envelope glycoproteins carried by plasma virus in CD4-depleted animals were found to contain specific alterations affecting the V2 region of gp120; similar V2 changes were observed during independent monkey infections. The altered V2 loops contained double amino acid deletions and the loss of a highly conserved N-linked glycosylation site. In contrast to the starting highly pathogenic SHIV, which is exclusively T cell-tropic, some mφ-phase SHIVs, bearing altered V2 regions, were able to establish spreading infections of cultured alveolar mφ. *J. Leukoc. Biol.* 74: 772–780; 2003.

Key Words: immunodeficiency · *in situ* hybridization · viral load · lymphocyte depletion · HIV *env* · V2 loop

INTRODUCTION

The principal target of human immunodeficiency virus type 1 (HIV-1) in seropositive individuals is the CD4⁺ T lymphocyte. Virus-induced depletion of this T cell subset eventually results in symptomatic disease, most prominently, in the appearance of opportunistic infections. Like all other lentiviruses, HIV-1 has retained the capacity to infect macrophage (mφ), a property appreciated early in the epidemic when virus-producing multinucleated giant cells were detected in the brains of infected persons [1, 2]. *In vivo*, infected mφ are considered to be a significant reservoir of HIV-1 because of their reported resistance to the cytopathic effects of the virus and their intrinsically long lifespan.

Unlike circulating CD4⁺ T cells, HIV-1-producing tissue mφ have been difficult to study by noninvasive techniques. Consequently, very little is known about the dynamics of virus production in cells of this lineage *in vivo* and whether circulating monocytes or more differentiated tissue mφ are the initial targets of infection. As a result, investigators have turned to an *in vitro* surrogate, the monocyte-derived mφ (MDM), to investigate parameters affecting the susceptibility of mφ to HIV-1.

We and others have reported that highly pathogenic simian immunodeficiency virus (SIV)/HIV-1 chimeric viruses (SHIVs) cause an extremely rapid (3–5 weeks), irreversible, and systemic depletion of CD4⁺ T lymphocytes following intravenous (*i.v.*) inoculation of rhesus monkeys [3–5]. Despite the absence of significant numbers of CD4⁺ T cells, SHIV-infected animals survive for an additional 4–6 months and continue to generate high levels of plasma virus (>1×10⁶ RNA copies/ml) [6]. *In situ* hybridization (ISH) coupled with confocal immunofluorescence have demonstrated that mφ in a variety of tissues sustain high plasma virus loads in the absence of CD4⁺ T lymphocytes [7]. Thus, the SHIV/macaque system has the potential of serving as a useful *in vivo* model of mφ infections, permitting a further understanding of the processes of virus entry and dissemination, disease induction, and sensitivity to antiretroviral agents when the main source of progeny virus production is tissue mφ.

MATERIALS AND METHODS

Virus, animal inoculations, virus load measurements, and tissue histochemical analyses

The tissue culture-derived SHIV_{DH12} and SHIV_{DH12R} stocks have been described earlier [3, 8]. Animal inoculations, lymphocyte subset analyses, plasma viral load determinations, and ISH/immunohistochemical (IHC) analyses of fixed tissue were performed as previously reported [6, 9].

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Env gene polymerase chain reaction (PCR)

Extraction of viral RNA from rhesus monkey plasma and reverse transcription (RT) were conducted as described [10] using 9493(-) 5'-GCGAGTATC-CATCTTCCACCTCTC-3' as cDNA primer. A 3-kbp fragment, encompassing the entire HIV-1_{DH12} Env coding region, was PCR-amplified using the following set of nested primers: 6428(+) 5'-GAGCCAGTAGATCCTAGACTA-GAGCC-3' and 9493(-) as outer primers and the previously designed primers 6317(+) and 9190(-) as inner primers as previously reported [3].

V2 length analysis

A 181-bp fragment, encompassing the V2 region of gp120, was amplified by nested PCR using one of the primers in the second round of PCR labeled with the 6-carboxy-fluorescein (6-FAM) fluorophore at its 5' end [11]. The fluorescently labeled PCR-amplified products were separated on an ABI 377 DNA sequencer (PE Applied Biosystems, Foster City, CA) and were sized with GeneScan 3.1 software (PE Applied Biosystems). The amino acid (aa) length of the V2 loop was calculated by subtracting 61 bp, representing amplified constant regions outside of the V2 loop, from the measured nucleotide fragment length obtained from the gel analysis.

Recombinant viruses

Specific V2 changes were introduced into pSHIV_{DH12R-CL-7} [12] by site-directed mutagenesis using the QuickChange protocol (Stratagene, La Jolla, CA) as described [13]. Virus stocks were prepared by transfecting plasmids into RD cells as previously reported [13].

Virus replication in monkey alveolar mφ (AM)

Rhesus macaque AM were collected from uninfected rhesus macaques by bronchoalveolar lavage and were cultivated as described previously [14].

Approximately 4×10^5 AM were resuspended in Dulbecco's minimum essential medium (400 μ l) and plated in LabTek II glass chamber slides (Nalge Nunc International, Rochester, NY). Following medium changes on days 1 and 2, the adherent cells were immediately infected with virus [normalized by RT activity (1×10^7 32 P cpm)] as indicated in the text. In each case, the infected cultures were monitored for up to 25 days with complete medium changes every other day. Progeny virus production was assessed by the RT activity in the culture supernatants as described previously [3].

RESULTS

HIV-1 can establish infection in humans and chimpanzees but induces disease only in humans. As SIV, the close primate lentivirus relative of HIV-1, does induce an AIDS-like disease in Asian macaques and possesses a genomic organization similar to that of HIV-1 [15], investigators began constructing SHIVs to assess the pathogenic potential of HIV-1-encoded proteins and as a challenge virus for viral envelope glycoprotein-based vaccines [16]. Using a molecular clone of SIV_{mac239} [17] as the genetic backbone, SHIV_{DH12} was constructed containing the *vpr*, *tat*, *rev*, *vpu*, and *env* genes from the dual-tropic, primary HIV-1_{DH12} isolate (Fig. 1) [8, 18]. In contrast to SIV, but similar to other first-generation SHIVs, SHIV_{DH12} did not replicate to high titers following inoculation of rhesus monkeys, did not cause a depletion of CD4⁺ T lymphocytes, and did not induce disease (not shown). However, treatment of a naïve

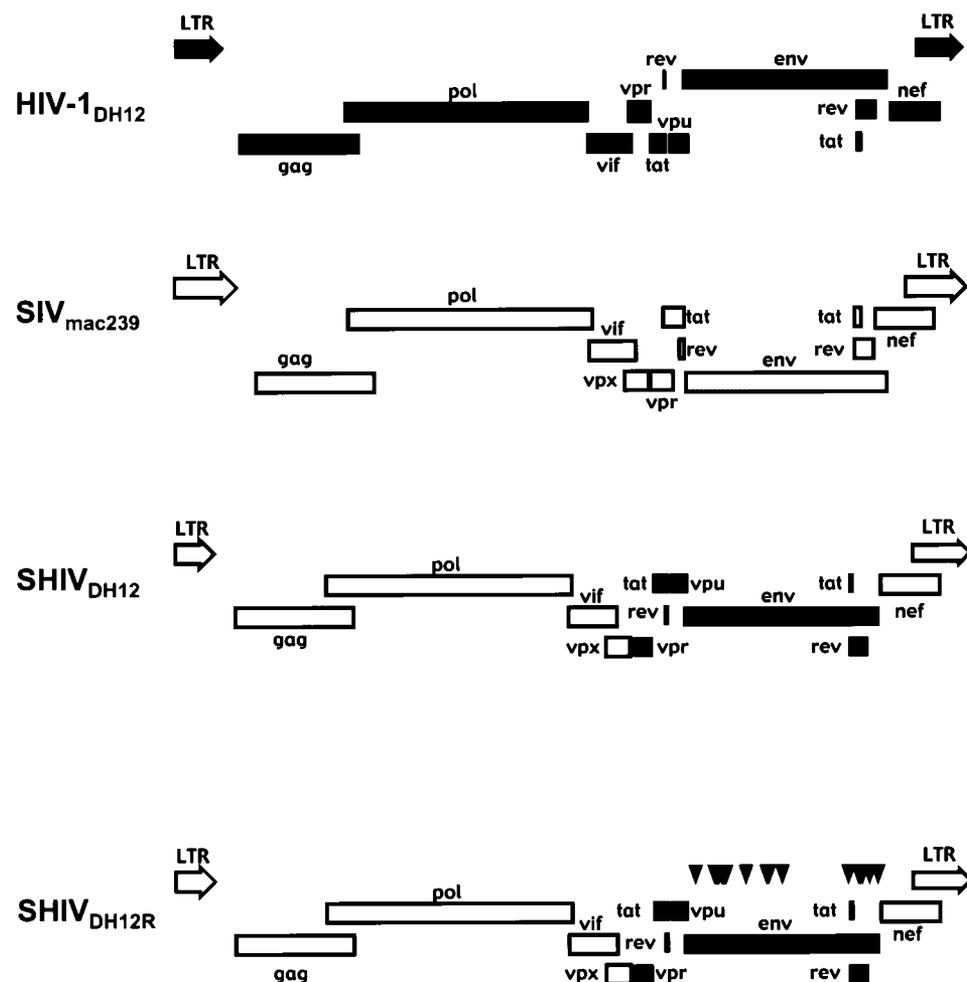


Fig. 1. The genomic organizations of HIV-1, SIV, and SIV/HIV chimeric viruses. The parental, dual-tropic, primary isolate HIV-1_{DH12} (filled bars) and the pathogenic molecular clone of SIV_{mac239} (open bars) are shown at the top. The arrowheads on the SHIV_{DH12R} genome indicate *env* gene changes accompanying the acquisition of the highly pathogenic phenotype, which appeared during a single passage in rhesus monkey Rh565Z. LTR, Long-terminal repeat.

macaque (#565Z) with anti-human CD8 monoclonal antibody (mAb) to suppress the immune response to a primary infection with the nonpathogenic SHIV_{DH12} resulted in high levels of peak viremia, a transient but profound loss of CD4⁺ T cells, and the development of immunodeficiency requiring euthanasia at week 68 (Fig. 2) [3, 19]. The virus recovered from blood and tissue suspensions from monkey 565Z at the time of necropsy was designated SHIV_{DH12R} and subsequently shown to reproducibly generate high levels of viral RNA in plasma, induce a rapid, complete, and systemic depletion of CD4⁺ T lymphocytes, and cause death from immunodeficiency within 3–6 months of inoculation (Fig. 3) [3]. To ascertain when the highly pathogenic SHIV_{DH12R} variant emerged in animal 565Z, virus was isolated at weeks 39 and 52 postinfection and inoculated into naïve monkeys; the SHIV, recovered at week 52 but not 39, caused the signature rapid loss of CD4⁺ T cells in

recipient animals. The virus recovered at week 52 was designated SHIV_{DH12R-PS1} [20] (Fig. 2).

It should be emphasized that although the aggressive, CD4⁺ T cell-depleting phenotype exhibited by the highly pathogenic SHIVs generates a readily recognizable syndrome and the rapid onset of disease, this clinical course is profoundly different from that commonly associated with SIV and HIV-1 infections. The latter are characterized by the gradual depletion of CD4⁺ T cells and the development of immunodeficiency over much longer time frames (1–2 years for SIV and 10 years for HIV-1) [21–24]. As will be shown below, the SHIV/macaque system only models the acute and terminal stages of primate lentiviral infections in vivo, not the long intermediate asymptomatic phase seen in SIV and HIV-1 infections.

The extremely rapid SHIV-induced depletion of CD4⁺ T lymphocytes and virus production in tissues was examined

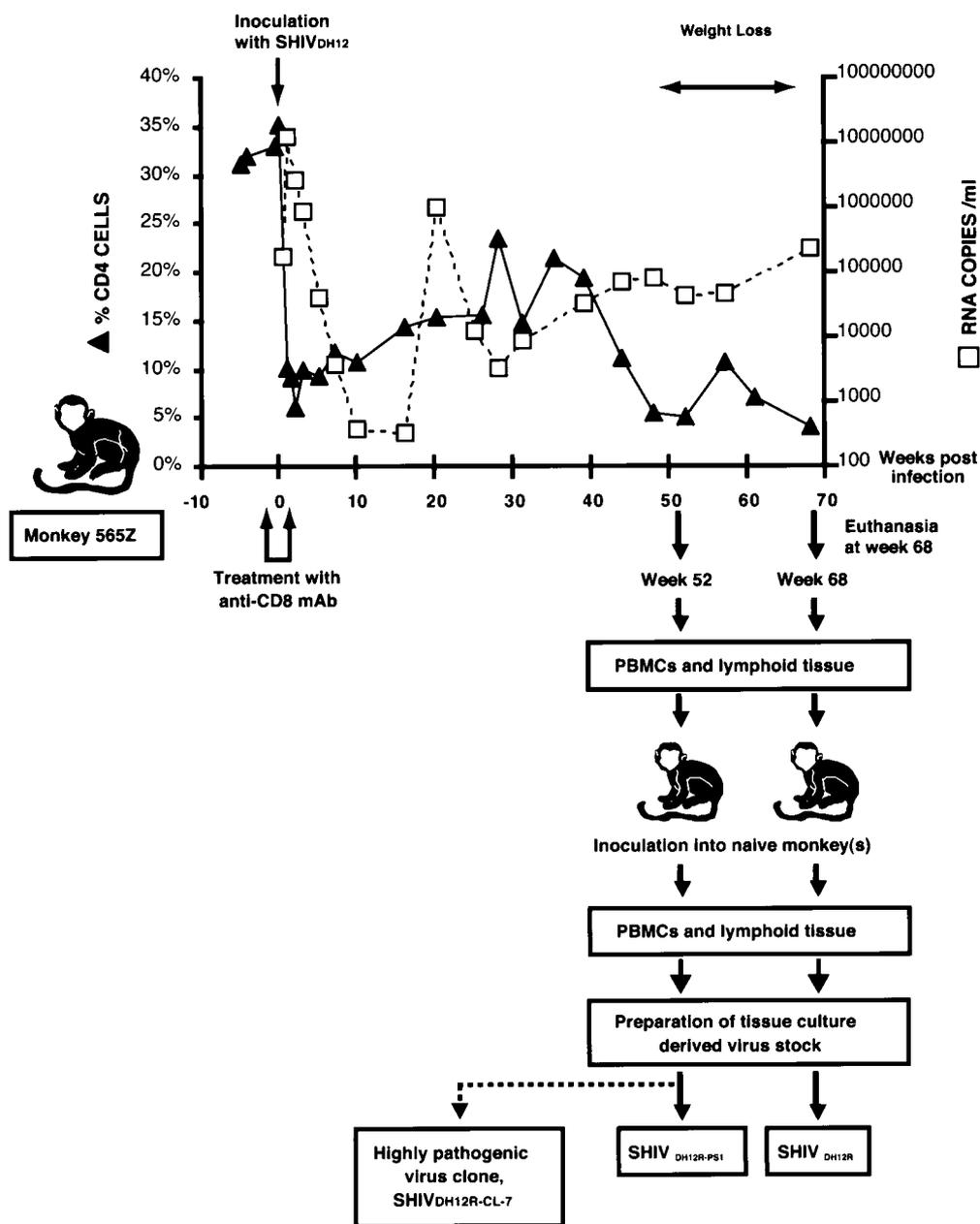


Fig. 2. The emergence of highly pathogenic SHIV_{DH12R} and SHIV_{DH12R-PS1} during a single passage of the nonpathogenic SHIV_{DH12} in rhesus monkey 565Z. The times of anti-human CD8 antibody administrations and virus inoculation are indicated. SHIV_{DH12R} and SHIV_{DH12R-PS1} were recovered from peripheral blood mononuclear cells (PBMCs) and lymphoid tissue suspension collected at weeks 68 and 52, respectively. The SHIV_{DH12R-CL-7} molecular clone was generated from the cells infected with SHIV_{DH12R-PS1}.

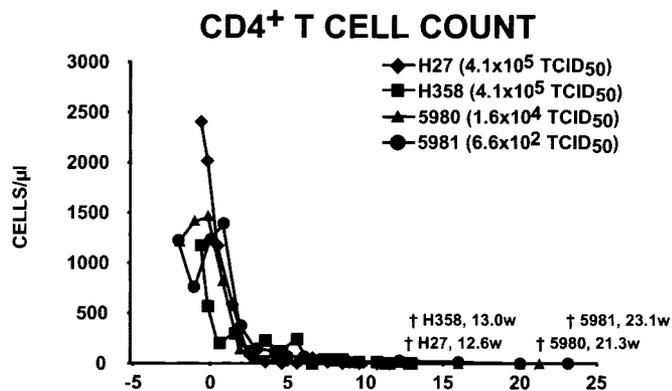
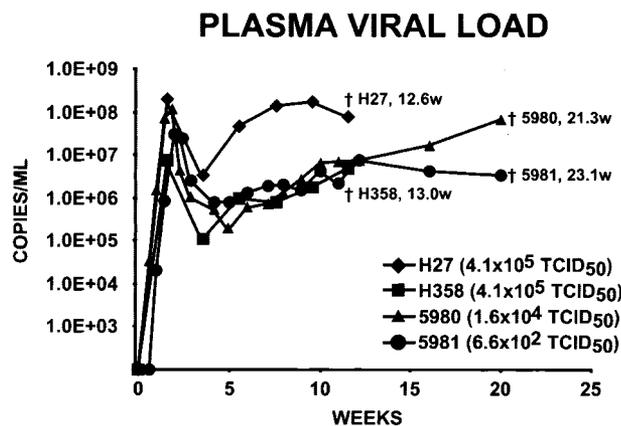


Fig. 3. Plasma viral load and peripheral blood CD4⁺ T-lymphocyte profiles of SHIV_{DH12R}-infected animals. The four macaques were i.v. inoculated with the indicated amounts of SHIV_{DH12R}. †, Times of euthanasia. TCID₅₀, Tissue culture infectious dose(50).

systemically by serially sacrificing SHIV_{DH12R}-infected rhesus monkeys between days 3 and 21 postinfection. DNA PCR analyses demonstrated proviral DNA in lymph nodes on day 3 postinfection; by day 14, 2–5% of lymph node cells were producing SHIV DNA [9]. CD4⁺ T cells, located in paracortical areas of lymph nodes and detected by IHC staining of formalin-fixed tissue, rapidly declined between days 10 and 14 postinoculation (**Fig. 4, a and b**). Confocal microscopic analysis of a day-10 specimen, using a riboprobe recognizing SHIV sequences and an anti-CD3 mAb, revealed that the virus-producing cells were, in fact, T lymphocytes (**Fig. 4, c–e**). No reactivity with the mφ-specific HAM56 mAb was detectable by ISH in samples collected during the first 3 weeks of infection (not shown). Virus production during the acute infection, monitored by DNA PCR and ISH increased systemically, peaking somewhat earlier in lymphoid tissues (days 10–14) than in PBMCs (days 14–21).

The systemic and irreversible loss of CD4⁺ T cells was accompanied by a 30- to 200-fold reduction of SHIV RNA levels in the plasma (**Fig. 3**). However, despite the absence of significant numbers of CD4⁺ T lymphocytes in the blood or tissues, plasma viremia remained at fairly high levels (>10⁵ RNA copies/ml) and gradually increased 20- to 50-fold over the next several months. Tissue, collected from CD4⁺ T cell-depleted monkeys at the time of necropsy, was examined by

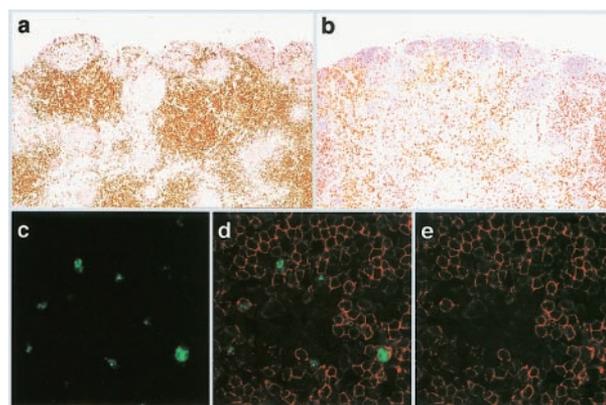


Fig. 4. Infection and depletion of CD4⁺ lymphocytes in the mesenteric lymph nodes of SHIV_{DH12R}-infected animals. CD4 IHC staining of the lymph node samples collected from animals killed on days 10 (a) and 14 (b) postinfection. Confocal fluorescence microscopy of mesenteric lymph node samples from a monkey killed on day 10 postinfection using a virus-specific riboprobe (c) and an anti-CD3 mAb (e). A merged image is shown in d. Original magnifications: a, ×5; b, ×4; c–e, ×63.

ISH and IHC techniques to identify the source of the sustained high virus loads. As shown in **Figure 5a**, multinucleated, virus-producing cells with a morphology typical of mφ were present throughout a mesenteric lymph node specimen. These cells were significantly larger than the background nonreactive cells, presumably of lymphocyte lineage. The precise identification of the lymph node cell type actively producing SHIV RNA was determined by confocal immunofluorescence microscopy, using a riboprobe specific for viral RNA sequences and mAb reactive with mφ or T lymphocytes. The results of this analysis indicated that virtually all of the ISH-positive cells stained with the mφ-specific mAb (**Fig. 6, a–c**), whereas minimal colocalization was observed when the anti-CD3 mAb was used (**Fig. 6, d–f**). Quantitation of this and similar confocal analyses indicated that >95% of virus-positive cells were

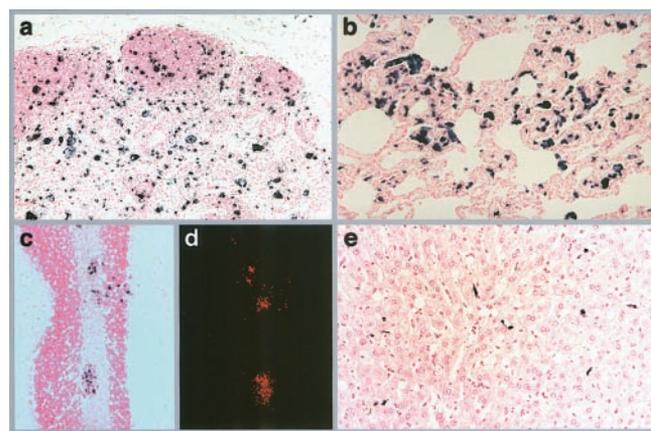


Fig. 5. Viral RNA production during the mφ phase of SHIV_{DH12R} infection. In situ RNA hybridizations were performed on tissues collected during the mφ phase of infection. a, Mesenteric lymph node (original magnification, ×10); b, lung (×10); c, cerebellum (×20); and e, liver (×20). (d) Histosection adjacent to c, stained with the mφ-specific mAb, HAM56.

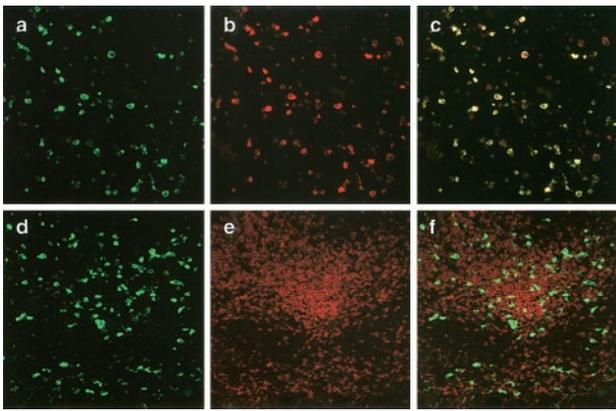


Fig. 6. Tissue mφ are the major virus-producing cells during the mφ phase of SHIV_{DH12R} infection. The mesenteric lymph from a late-stage animal node was subjected to confocal microscopy-based in situ RNA hybridization/immunohistochemistry. (a and d) Viral riboprobe hybridization; b, mφ (HAM56) immunohistochemistry; e, T lymphocyte (CD3) immunohistochemistry; c, merged image of a and b. Note that colocalization of the signals detected in a and b merge to yellow in c; f, merged image of d and e. Original magnification, ×40.

tissue mφ. In some lymph node specimens, up to 25% of the HAM56-reactive cells were producing SHIV RNA (not shown).

Examination of nonlymphoid tissues also revealed large numbers of virus-positive mφ. Multinucleated cells, with a morphology characteristic of AM and producing viral RNA, were present in histosections of the lung (Fig. 5b). Viral RNA was detected in cells having morphological features of mφ/microglia in various parts of the central nervous system (CNS). These virus-positive cells were scattered throughout the brain parenchyma and were not necessarily located in perivascular regions. A cerebellum sample, collected at week 22 postinfection, contained cells that stained with the SHIV riboprobe and the HAM56 mAb (Fig. 5, c and d). Flat cells, lining sinusoids of the liver and exhibiting the morphology of Kupffer cells, were also virus-positive (Fig. 5e).

As noted earlier, SHIV-positive tissue mφ were not detectable by ISH during the peak of virus production between weeks 1 and 3. However, when it became apparent that plasma viremia gradually increased after weeks 4–5 postinfection (Fig. 3), lymph node specimens were re-examined by confocal fluorescence microscopy to ascertain the types of virus-producing cells maintaining the high virus loads. As shown in **Figure 7**, SHIV-positive T lymphocytes and mφ were present in the sample collected at week 6 postinfection. Taken together, these results indicate that the initial burst of virus production in inoculated rhesus monkeys occurs in CD4⁺ T lymphocytes (T cell phase of infection), and the high levels of viremia measured following the systemic depletion of CD4⁺ T cells are sustained by tissue mφ (the mφ phase of infection). It is likely that some mφ also become infected during the T cell phase but only become detectable as the number of CD4⁺ T lymphocytes markedly declines.

As a switch in the virus-producing cell type occurred in SHIV-infected macaques, viral envelope glycoproteins were examined to ascertain whether specific alterations had accompanied the transition from the T cell to the mφ phase [12]. For

this purpose, a total of seven rhesus monkeys were infected with virus: five with SHIV_{DH12R} and two with SHIV_{DH12R-PS1} (Fig. 2). All seven animals produced high levels of plasma viral RNA (>10⁷ copies/ml) within 2 weeks of infection and experienced irreversible depletion of their CD4⁺ T cells (data not shown). Complete *env* gene segments (3 kbp in size) were RT-PCR-amplified from plasma samples obtained at week 2 (the T cell phase) and following the systemic loss of CD4⁺ T lymphocytes (the mφ phase) between weeks 12 and 35, depending on the animal. Independent PCR clones from the two time points were sequenced for each of the infected macaques. Although both of the uncloned virus stocks used for animal inoculations were genetically heterogeneous, sequence analysis indicated that at the time of peak plasma viremia (week 2 postinfection), they had become quite homogeneous (data not shown). The consensus *env* gene sequence at week 2 was similar to a recently obtained molecular clone of SHIV_{DH12R}, designated SHIV_{DH12R-CL-7} [12], which also induces rapid and irreversible CD4⁺ T cell loss in rhesus monkeys.

RT-PCR and sequence analyses of plasma viral RNA, collected during the mφ phase of independent SHIV infections in seven animals, were remarkable in that the only consistent changes identified within the entire Env coding region were located in the V2 domain of gp120. The aa sequence of some of these altered V2 loops are shown in **Figure 8** (upper) compared with the consensus SHIV_{DH12R} V2 sequence present in plasma virus during the T cell phase of infection. The predominant and unexpected finding was the presence of double aa deletions mapping to two specific regions of the V2 loop (residues 164–165 and 186–187). Among this “M” (mφ) series of V2 regions, one (M-4) contained both sets of double aa deletions. Several of the mφ-phase V2 segments had also lost a potential N-linked glycosylation site (at position 197) in addition to the 2 or 4 aa deletion.

The emergence of SHIVs bearing deleted V2 regions was independently confirmed using a length polymorphism assay capable of detecting minor variants present in the entire virus

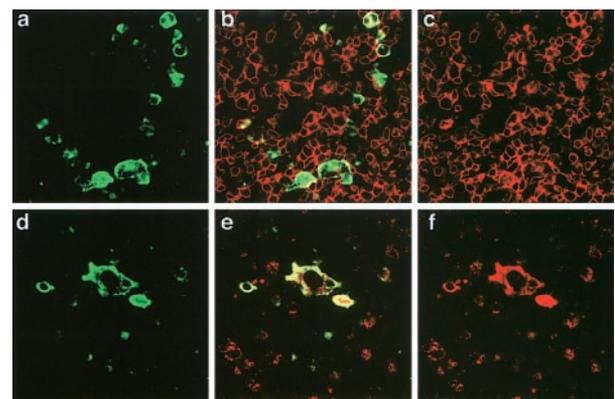


Fig. 7. The transition from the T cell to the mφ phase of the SHIV_{DH12R} infection. An inguinal lymph node, biopsied from a SHIV_{DH12R}-infected animal at 6 weeks postinfection, was subjected to confocal fluorescence microscopy using a riboprobe recognizing SHIV sequences (a and d, visualized in green) and immunostaining specific for CD3⁺ T lymphocytes (c, visualized in red) or mφ (f, visualized in red). Merged images of a and c (b), or d and f (e). Original magnification, ×63.

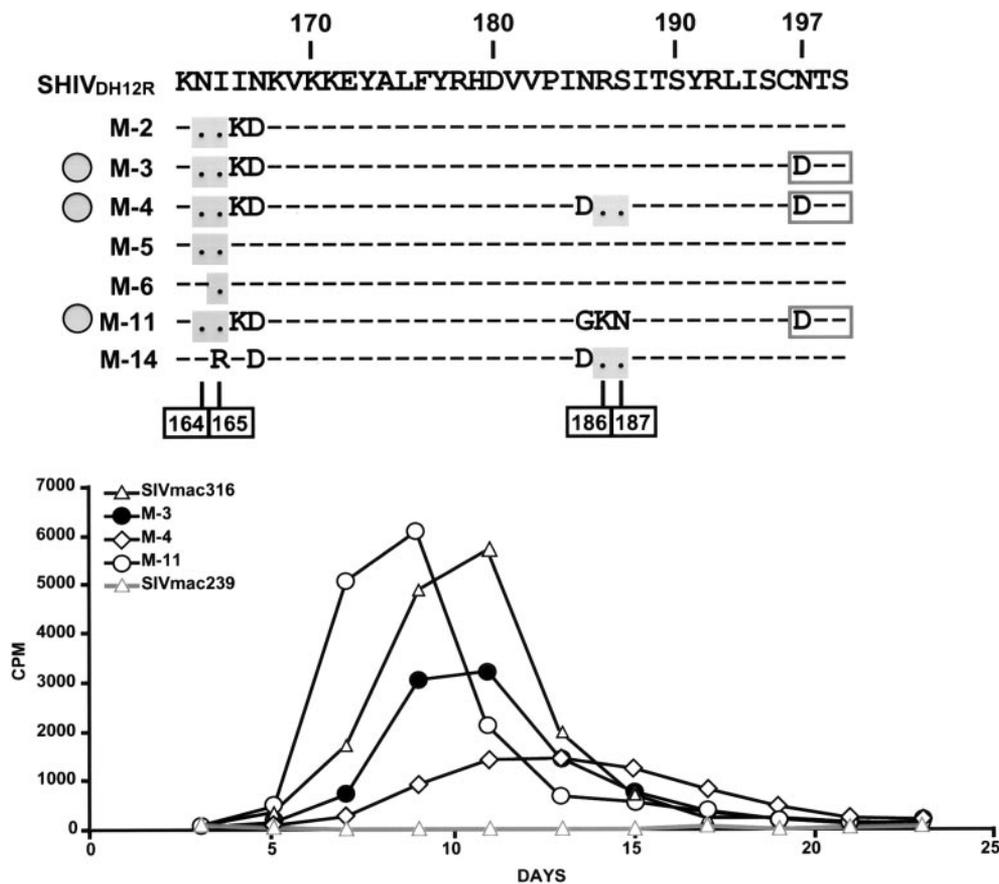


Fig. 8. Replication of mφ phase-derived viruses in monkey AM. The aa sequence of the V2 regions from representative, independent RT-PCR, full-length, mφ-phase *env* genes is shown (upper). Dashes indicate aa identity; dots denote deleted residues. The positions of deleted aa are indicated (lower); aa changes resulting in a loss of the N-glycosylation site at residue 197 are boxed. Site-directed mutagenesis of SHIV_{DH12R-CL-7} was used to prepare the M series of mφ-phase SHIVs containing the indicated V2 changes. The infectivity of three of these SHIVs (indicated by shaded circles) was tested in rhesus AM (lower). Rhesus monkey AM (5×10^5 cells) were infected with ~ 8000 TCID₅₀ of each virus stock, and progeny virus production was monitored for 23 days by the RT activity released into the culture fluid. CPM, Counts per minute.

population. Briefly, during the second round of nested PCR, the primer used for amplification was labeled with the 6-FAM fluorophore at its 5' end. Fluorescently labeled V2 PCR products were separated on a DNA sequencing gel and their sizes determined. As shown in **Figure 9** (left panels), a single population of gp120, corresponding to the full-length 40-aa V2 loop, was present in the plasma samples of six of the seven SHIV-infected monkeys during the T cell phase of infection. In contrast, the V2 loops associated with virus present in all seven of the animals during the mφ phase were smaller in size (Fig. 9, right panels). In three of the monkeys (181A, 5980, 96N093), the 2-aa deleted V2 variant was the only species detected. Together with the sequence data presented in Figure 8, these results confirm the emergence of mφ-phase SHIVs with shortened V2 loops.

The replicative properties of SHIVs bearing altered gp120 V2 regions were examined by first introducing the aa changes/deletions into the cloned SHIV_{DH12R-CL-7} viral DNA by site-directed mutagenesis. SHIVs carrying the seven mφ-phase gp120 V2 regions shown in Figure 8 (upper) were able to infect cultured monkey PBMCs, generating peak levels of p27 ranging from 30 (M-11) to 300 (M-14) ng/ml by day 8 postinfection. The possible acquisition of mφ tropism by SHIV variants emerging in monkeys with markedly depleted numbers of CD4⁺ T cells was examined by measuring their infectivity in rhesus AM, collected by bronchoalveolar lavage from uninfected macaques. As controls for this experiment, the mφ-tropic SIV strain, SIV_{mac316} [25], exhibited robust replication

kinetics in AM, whereas the T cell-tropic SIV_{mac239} failed to generate detectable progeny virions (Fig. 8, lower). The highly pathogenic SHIV_{DH12R} was also unable to infect macaque AM (not shown). In contrast, three of the mφ-phase SHIVs (M-3, M-4, and M-11) readily established infections in rhesus monkey AM; in a similar experiment, SHIV M-14 failed to replicate in AM (data not shown).

DISCUSSION

The signature property of SHIV_{DH12R} and other highly pathogenic SHIVs is their capacity to reproducibly induce systemic and nearly complete depletion of CD4⁺ T lymphocytes within weeks of inoculation [3–5]. In the absence of CD4⁺ T cells, virus production *in vivo* is sustained by tissue mφ [7]. During the primary infection, virus production occurs almost exclusively within CD4⁺ T cells. As this lymphocyte subset is eliminated, a second wave of viral progeny is generated, this time in tissue mφ. In one animal, at least, virus-producing T lymphocytes and mφ could be demonstrated in an inguinal lymph node biopsied at 6 weeks postinoculation (Fig. 7), marking the transition to the mφ phase of infection. At later times, tissue mφ become the predominant source of viremia, releasing large amounts of progeny virions into the plasma that reach levels of 10^6 – 10^7 RNA copies/ml (Fig. 3).

A surprising feature of the SHIV/rhesus monkey model was the emergence, during the transition from the T cell to the mφ

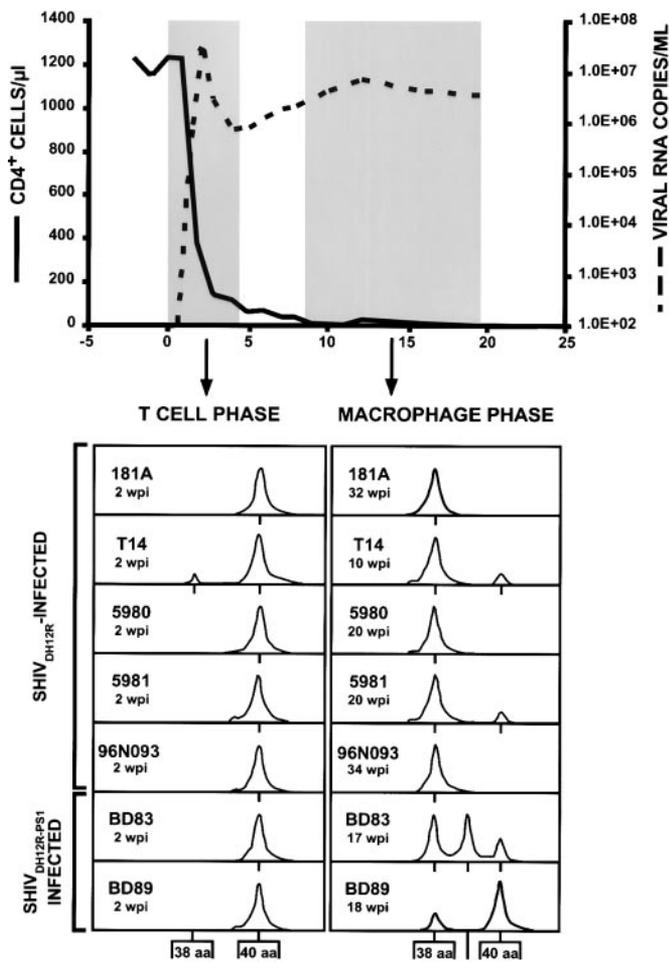


Fig. 9. V2 loop-length polymorphism analyses of plasma virus present during the T cell and m ϕ phases of SHIV_{DH12R} infections. The CD4⁺ T lymphocyte counts (solid) and plasma viral RNA (dashed) profile from a representative infection are depicted (upper). The gp120 V2 regions from five SHIV_{DH12R} and two SHIV_{DH12R-PS1}-infected animals were subjected to the length polymorphism analysis. Note that the V2 region during the T cell phase of infection in the SHIV_{DH12R} stock and during the T cell phase is 40 aa in length.

phase of infection, of viral variants bearing similarly deleted gp120 V2 regions. The same double aa V2 deletions appeared during independent infections of seven macaques (Figs. 8 and 9) [10]. It is quite likely that in the absence of CD4⁺ T lymphocytes, the capacity to infect tissue m ϕ is strongly selected for in vivo. Although it is not presently clear why specific alterations of V2 would facilitate entry into m ϕ , it is known that the V1 and V2 loops of HIV-1 gp120, situated on the surface of the trimeric envelope complex, partially occlude the CD4 and chemokine coreceptor-binding sites of gp120 [26]. The conformational changes affecting the viral envelope glycoprotein during the binding of virions to CD4 on the surface of target cells repositions the common V1/V2 stem and exposes the high-affinity gp120-binding site for the coreceptor [26–30]. It is therefore possible that a deletion in V2, in combination with the loss of the highly conserved glycosylation site at residue 197 (Fig. 8), creates a SHIV_{DH12R} gp120 conformation with greater affinity for CD4 and/or the chemokine coreceptor.

It is now known that the expression of CD4 and chemokine receptor is modulated, depending on the activation or differentiation status of the cell. The low levels of CD4 on the surface of freshly isolated human monocytes increase significantly during their differentiation in culture into MDM [31, 32]. The expression of CD4 on the surface of human and rhesus monkey AM has been reported to be extremely low-to-undetectable and does not change during in vitro cultivation [33, 34]. The capacity to infect AM may therefore be limited to virions carrying unique gp120s, which are able to mediate fusion with cells expressing very little surface CD4 and chemokine receptor. The use of AM, rather than MDM, may represent a more rigorous measure of m ϕ tropism, possibly identifying SHIV *env* gene variants with high affinities for CD4. Nonetheless, SIV_{mac316} and three of four m ϕ -phase SHIVs replicated in AM, whereas SHIV_{DH12R}, which induced rapid and irreversible elimination of CD4⁺ T cells, did not. SHIV_{DH12R} appears to be exclusively T cell-tropic.

Studies are currently in progress to assess which gp120 determinants confer the m ϕ -tropic properties to late-stage SHIVs. This is an important issue, as such viral envelope elements could direct the incoming virus into specialized body compartments such as the CNS. The nucleotide sequence and infectivity data obtained thus far suggest that the double aa deletion affecting residues 164/165 but not 186/187 is critical for m ϕ tropism, as SHIVs M-3, M-4, and M-11 were able to infect AM, whereas SHIV M-14 was not (see Fig. 8). The three m ϕ -tropic SHIVs, in addition, have lost the highly conserved glycan at position 197 compared with the exclusively T-tropic SHIV_{DH12R}. Additional experiments, examining, for example, SHIV M-5, which is deleted at residues 164/165 but has retained the downstream glycosylation site (see Fig. 8), could reveal the relative contributions of each of these V2 motifs for m ϕ tropism. The functional implications of gp120s with aa deletions or the loss of an N-linked glycosylation site affecting V2 are presently unknown. The receptor- and coreceptor-binding properties of gp120s containing specifically altered V2 regions are in the process of being evaluated.

Preliminary results have recently been obtained indicating that the starting CD4⁺ T cell-depleting SHIV as well as the m ϕ -tropic SHIVs, which emerge later, use CXCR4 not CCR5 for infection of rhesus macaque PBMCs (T. Igarashi et al., in preparation). In those experiments, small molecule competitors that target specific chemokine receptors were used to block spreading virus infections. For HIV-1, infection of MDM is usually associated with CCR5 use. Nonetheless, some dual-tropic HIV-1 strains have been shown to enter MDM by using CXCR4, and a recent isolate from a CCR5 Δ 32 homozygote has been reported to use CXCR4 during MDM infections [35–38]. Thus, similar to SIV, SHIV_{DH12R} does not change coreceptor use as a consequence of acquiring tropism for m ϕ [39, 40]. The T-tropic SIV_{mac239} and m ϕ -tropic SIV use CCR5, and the T-tropic SHIV_{DH12R} and the m ϕ -tropic SHIVs M-3, M-4, and M-11 use CXCR4 during productive infections of rhesus PBMCs. Analogous-blocking experiments are planned to assess coreceptor use of the m ϕ -tropic SHIVs during infections of AM.

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