Accumulation of Human Immunodeficiency Virus Type 1 DNA in T Cells: Result of Multiple Infection Events

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Human immunodeficiency virus type 1 DNA synthesis was followed in a CD4$^+$ line of T cells (C8166) grown in the presence or absence of a monoclonal antibody to CD4 that blocks infection. By 48 h after infection, cultures grown in the presence of the antibody contained ~4 copies of human immunodeficiency virus type 1 DNA per cell, whereas those grown in the absence of the antibody contained ~80 copies of viral DNA per cell. Most of the viral DNA in cultures grown in the absence of the antibody was present in a broad smear of apparently incomplete viral sequences. In cultures grown in the presence or absence of the antibody, the 9.6-kilobase linear duplex of viral DNA appeared to undergo integration within 24 h of its appearance. These results demonstrate that T cells accumulate unintegrated human immunodeficiency virus type 1 DNA as a result of multiple virions entering cells.

Reverse transcription of virion RNA to a duplex linear DNA and the integration of this linear duplex into chromosomal DNA to form a provirus are early steps in the life cycle of retroviruses (2, 7, 29; for a review, see reference 37). In most type C virus-infected fibroblasts, the synthesis of viral DNA is limited to a few copies of viral DNA per cell. Each entering virion has the potential for producing one provirus, with virion RNA being degraded by the RNase H activity of reverse transcriptase during second-strand DNA synthesis. Reverse transcription of progeny RNA requires assembly of the RNA with capsid and polymerase proteins, a process that occurs as virions bud from cells. With the onset of virus production, reinfection (and concomitant reverse transcription) is blocked by newly synthesized envelope glycoproteins competing for receptor activity (38).

In contrast to most type C virus infections, cultures of human immunodeficiency virus type 1 (HIV-1)-infected T cells tend to undergo the persistent synthesis of viral DNA, with moderate to high levels of unintegrated viral DNA (20 to 100 copies per cell) being reported in recent infections and lower levels of unintegrated DNA being present in chronic infections (16, 21). Unintegrated DNA has also been reported in lymph nodes from patients with acquired immunodeficiency syndrome (AIDS) (33).

Precedence for the accumulation of high levels of unintegrated retroviral DNA is found in certain type C virus- and visna virus-infected cultures. In these, the accumulation of DNA reflects reinfection due to the inefficient establishment of envelope glycoprotein-mediated superinfection resistance (12, 15, 39). In vitro infections that accumulate unintegrated DNA are cytopathic (12, 15, 39). In vivo infections that persistently produce unintegrated DNA cause disease, as exemplified by avian leukosis virus-induced osteopetrosis (28) and feline leukemia virus- and equine infectious anemia virus-induced anemias (22, 27).

The goal of the current study was to determine whether infection accounted for the production of high levels of unintegrated DNA in HIV-1-infected T cells. C8166 cells were chosen for the study because these cells are unusually permissive for HIV-1 expression; most infected cells pro-

duce viral structural proteins by 24 h after infection (35). Our results clearly demonstrate that infection accounts for the accumulation of high copy numbers of unintegrated viral DNA in HIV-1-infected T cells.

MATERIALS AND METHODS

Cells. C8166 cells (30), H9 cells (25), and 8E5 cells (6) were grown at densities of 1 × 10⁶ to 2 × 10⁶ cells per ml in RPMI 1640 supplemented with 10 to 15% fetal bovine serum, penicillin, and streptomycin. C8166 cells are a line of human T-cell leukemia virus type I-immortalized cord blood lymphocytes. The line does not express detectable levels of human T-cell leukemia virus type I capsid protein but does express the *tax* gene product (10). C8166 cells express moderately high levels of CD4 (~80% of that observed on mitogen stimulated CD4$^+$ peripheral blood lymphocytes [34]). H9 cells are a subline of HuT 78 cells (ATCC TIB161). When obtained, the H9 culture contained cells with two different densities of CD4 (34). This mixed population was sorted for cells expressing the higher level of CD4 (a level similar to that present on C8166 cells). The sorted cells have maintained a uniform level of CD4 expression during further culture. 8E5 cells are a line of A3.01 cells that contain a single *pol*-defective provirus (contributed by T. Folks to the AIDS Repository; no. 95). Only cultures in which >80% of the 8E5 cells scored positive in an indirect immunofluorescence assay (IFA) (see below) were used in studies. Southern blot analyses of *Eco*RI-digested 8E5 DNA detected the two expected junction fragments of viral and cellular DNA (5.0 and 5.8 kilobases [kb]).

Plasmids. pHXB-2 was used as a source of HIV-1-IIIb sequences (5). pS4 is a subclone of the 9.2-kb *Sac*I fragment of pHXB-2 in the *Sac*I site of Bluescript (Stratagene, La Jolla, Calif.).

Antibody. A type-specific neutralizing serum for HIV-1-IIIb, goat anti-PB1 serum (26) was obtained from Repligen, Cambridge, Mass. leu3a, a mouse monoclonal antibody to CD4 that blocks syncytium formation and infection (31), was obtained from Becton Dickinson and Co. (Mountain View, Calif.). Because preparations of leu3a contain azide, leu3a was dialyzed against RPMI 1640 and sterilized by filtration through a 0.45-μm-pore-size filter. The effective concentration of leu3a in dialyzed preparations was determined by

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testing twofold dilution series for the inhibition of syncytium formation in HIV-1-IIIb-infected C8166 cells. Since experiments with known amounts of leu3a had demonstrated complete inhibition of syncytium formation with 120 but not 60 ng of leu3a per ml, a concentration of 120 ng/ml was assigned to the highest dilution giving complete inhibition of syncytium formation.

Virus production and IFA. Stocks of HIV-1-IIIb (8) were produced by infecting exponentially growing H9 cells for 6 to 12 h in the presence of polybrene (10 μg/ml). Infected cultures were seeded daily into fresh culture medium at 10^6 cells per ml. Starting at the third day after infection, culture medium was saved and cultures were tested for the fraction of cells expressing viral antigens by using an indirect IFA. For the IFA, 2.5×10^5 cells were washed with phosphate-buffered saline (PBS), suspended in 20 μl of PBS, and allowed to settle on 10-well slides (Cell Line Associates, Newfield, N.J.) by sequentially placing the 20-μl droplet over a series of wells. Settled cells were air dried, stored at room temperature, and assayed within a few days for the presence of HIV-1 antigens. HIV-1 antigens were detected by fixing the air-dried cells for 5 to 10 min at room temperature in methanol, washing with PBS, incubating for 30 min at 37°C with an appropriately diluted heat-inactivated (57°C, 30 min) serum from an HIV-1-seropositive human, washing three times with PBS, incubating for 30 min at 37°C with an appropriately diluted fluorescein-treated goat anti-human serum (H and L chain specific; Organon Teknika-Cappel, Durham, N.C.), washing three times with PBS, and mounting in 90% glycerin. Virus-positive and -negative cells were counted with a fluorescence microscope with a reticle serving as a counting grid. Stained slides could be stored at 4°C for several weeks. Titers (infectious units) of virus stocks were determined by infecting C8166 cells with 1:2, 1:8, and 1:32 dilutions of the stock in the presence of Polybrene (2 μg/ml). At 3 h after infection, leu3a (240 ng/ml) was added to cultures. At 24 h after infection, cells were assayed for the fraction expressing viral antigen by the IFA. Stock titers were calculated as (percentage of virus-expressing cells) × (concentration of C8166 cells at the time of infection) × (test dilution). Titers obtained by this method agreed well with those obtained by endpoint dilution on C8166 cells. The highest-titered stocks (~10^6 infectious units per ml) were obtained from cultures undergoing a spreading infection. All multiplicities of infection represent infectious units per cell.

C8166 cell infection. Exponentially growing C8166 cells were suspended at 10^6 cells per ml in a 1:2 dilution of a high-titered stock of HIV-1 in the presence of Polybrene (10 μg/ml). At 3 h after infection, cells were collected by centrifugation and suspended in fresh culture medium in the presence or absence of leu3a. Infections were monitored for HIV-1-expressing cells by the IFA and for dead cells by the failure to exclude trypan blue. Cultures grown in the absence of leu3a formed syncytia.

Analyses for viral DNA. DNA was extracted from a minimum of 5×10^6 cells by disrupting cells in 10 mM Tris hydrochloride (pH 7.8)–10 mM EDTA–1% sodium dodecyl sulfate, digesting with 2 μg of proteinase K per ml at 37°C for 1 to 2 h, extracting with phenol and then chloroform, and scooping out DNA that aggregated alter the addition of 0.1 M NaCl and 2 volumes of 95% ethanol. Unintegrated viral DNA was recovered as efficiently by this procedure as from precipitates collected by centrifugation at 10,000 rpm for 20 min. Precipitated DNA was dissolved in 100 μl of T1E.1 (1 mM Tris hydrochloride [pH 7.8], 0.1 mM EDTA) for the DNA from 10^7 cells. Extractions for unintegrated DNA were

performed by the method of Hirt (13). Hirt supernatant DNA was also dissolved in 100 μl of T1E.1 for 10^7 initial cells. Quantitation of DNA sequences was done by alkali denaturing approximately 4 μg of test DNA in 0.3 M NaOH at 60 to 70°C for 1 h, neutralizing the denatured DNA by the addition of 0.3 M HCl–0.2 M Tris hydrochloride (pH 8.0), and collecting serial dilutions of the denatured DNA on duplicate nylon filters in the presence of 10× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate). Serial dilutions (from 65 to 1 pg) of CsCl-purified pS4 DNA were denatured and collected onto one of the filters to serve as a standard for HIV-1 DNA. Serial dilutions (from 2 to 0.03 μg) of purified and denatured human DNA were collected onto the second filter to serve as a standard for human DNA. DNA from 8E5 cells was included as a known positive in all tests. Uninfected cell DNAs were included to determine background levels of hybridization for the HIV-1 probe. DNAs were cross-linked to filters by UV irradiation. Filters were blocked by prehybridization with blotto (14) and hybridized in the presence of 6× SSC at 67°C with nick-translated pS4 DNA (to quantitate HIV-1 sequences) or nick-translated human DNA (to quantitate human sequences). The amount of hybridized ^{32}P-labeled HIV-1 or human DNA was determined by counting washed papers with a Betascope (Betagen, Waltham, Mass.). Standard curves were constructed from data for known amounts of HIV-1 or human DNA, and the amount of HIV-1 or human DNA in test samples was determined from the standard curve. The number of copies of pS4 per picogram of DNA was calculated as (Avogadro's number)/ [(the sum of the number of nucleotides in the SacI fragment of HTLV-IIIb DNA and Bluescript) × (the average gram molecular weight of one nucleotide) × (the conversion factor for grams to picograms)] or $(6.023 \times 10^{23})/[(18.4 \times 10^3 + 6.2 \times 10^3)(330)(1 \times 10^{12})]$. Copies of HIV-1 DNA per cell were then normalized by dividing the copies of HIV-1 DNA per picogram of cell DNA in a test sample by the copies of HIV-1 DNA per picogram of DNA in the 8E5 sample. Since 8E5 cells contain a single provirus, this calculation provides an estimate of the copies per cell in the test sample. This calculation assumes that C8166 and 8E5 cells have similar amounts of DNA and normalizes the copy number of HIV-1 DNA in cultures containing syncytia to copies per 8E5 cell equivalent of DNA.

DNA blot analyses. Southern blot analyses were done on DNA fractionated by electrophoresis through 0.8 to 1.0% agarose gels in the presence of a high-salt buffer (0.04 M Tris hydrochloride, 0.001 M EDTA, 0.5 M sodium acetate [pH 7.9]). Gels were monitored for the efficiency of transfer by ethidium bromide staining. Under our conditions of transfer, the transfer of DNA fragments greater than 12 kb in size is incomplete. Experiments in which 2 to 5 μg of undigested DNA was mixed with up to 65 pg of linearized or covalently closed circular HIV-1 plasmid DNA did not reveal trapping of the plasmid DNA in high-molecular-weight DNA (data not shown). Estimates of the proportions of HIV-1 DNA present in integrated and unintegrated forms were done by using betascope analyses. Since transfer of high-molecular-weight DNA was incomplete, estimates for the amount of integrated DNA are low. In these estimates, the background of hybridization observed for high-molecular-weight uninfected cell DNA was subtracted from the band representing integrated HIV-1 DNA.

RESULTS

leu3a retards infection. To identify conditions that prevented HIV-1 infection, a type-specific neutralizing serum

TABLE 1. leu3a retards the spread of HIV-1-IIIb
in C8166 cultures[a]

C8166 cells in cocultivation (no.)	leu3a (300 ng/ml)	% HIV-1-IIIb-expressing cells after cocultivation for:		
		0 h	28 h	52 h
Infected (10^5) Uninfected (10^6)	+	10	20	79
Infected (10^5) Uninfected (10^6)	−	9	100	

[a] Exponentially growing C8166 cells were infected with 0.25 to 0.5 infectious unit of HIV-1-IIIb per cell and grown in the presence of 300 ng of leu3a per ml for 48 h. At 48 h, infected cells were cocultivated with uninfected C8166 cells that had been grown in the presence or absence of leu3a for the preceding 48 h. At the indicated times after cocultivation, HIV-1-expressing cells were quantitated by the IFA.
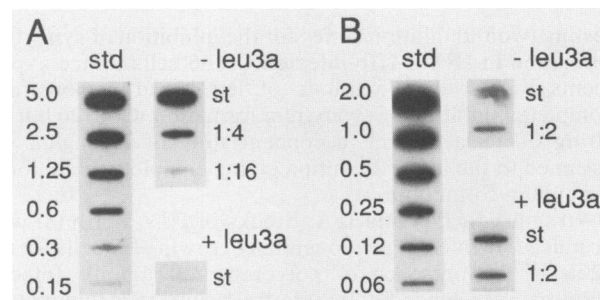

FIG. 1. Example of data used for estimation of copy numbers of HIV-1 DNA per cell. (A) Copy number of HIV-1 sequences. The standard (std) represents known copies of HIV-1 DNA × 10^6. (B) Micrograms of cell DNA. The standard represents known quantities (micrograms) of human DNA. Designations for test samples: st, undiluted sample; 1:4, etc., diluted samples.

and a receptor-blocking antibody were evaluated for their ability to prevent HIV-1-IIIb infection of C8166 cells. The neutralizing serum was a polyclonal goat antibody raised against the PB1 peptide of the gp120 envelope glycoprotein of HIV-1-IIIb (26). The receptor-blocking antibody was leu3a, a monoclonal antibody against CD4 that blocks infection and syncytium formation (31). The anti-PB1 serum did not detectably reduce the titer of the ~0.5 × 10^6 infectious units present in the stock of HIV-1-IIIb. In contrast, 240 ng of leu3a per ml completely blocked infection (data not shown).

Since HIV-1 can undergo cell-to-cell transmission (11) and C8166 cells grow in clumps, the ability of leu3a to block HIV-1 spread within an infected culture was evaluated by cocultivating 10^6 uninfected C8166 cells with 10^5 infected cells in the presence and absence of leu3a (Table 1). At various times after cocultivation, cultures were assayed for the fraction of cells expressing HIV-1 antigens. Cocultivation in the absence of leu3a resulted in all of the cells expressing HIV-1 antigens within 28 h. In cultures cocultivated in the presence of leu3a, a substantially slower spread of the infection was observed; only 10% of the uninfected cells became positive in the IFA by 28 h after infection. Thus, the presence of 300 ng of leu3a per ml substantially retarded the spread of HIV-1 in C8166 cell cultures.

HIV-1 DNA accumulation in the presence and absence of leu3a. To assess the role of infection in the accumulation of viral DNA in C8166 cells, cultures were infected with 0.25 to 0.5 infectious units of HIV-1-IIIb per cell. At 3 h after infection, cells were collected and seeded in fresh medium in the presence or absence of 300 ng of leu3a per ml. Samples of these cultures were harvested at various times after infection and analyzed for virus-expressing cells, dead cells, and the amount and forms of viral DNA in cells (Table 2, Fig. 1 and 2).

The presence of leu3a in cultures had little effect on the amount of viral DNA at 12 h after infection but a substantial and increasing effect on the accumulation of viral DNA at later times after infection (Table 2). By 24 h, cultures grown in the presence of leu3a contained <2 copies of viral DNA per cell, whereas those grown in the absence of leu3a contained ~20 copies per cell. By 48 h after infection, cells grown in the presence of leu3a contained ~4 copies of viral DNA, whereas cells grown in the absence of leu3a contained ~80. Thus, retardation of infection by leu3a resulted in a 20-fold reduction in the amount of viral DNA per cell.

Forms of viral DNA in infected cells. Undigested DNAs from various times after infection were analyzed on South-

ern blots for the presence of integrated viral DNA (present in high-molecular-weight chromosomal DNA), unintegrated duplex linear DNA (present as a 9.6-kb species), and covalently closed circular DNA (migrating as a doublet at ~5 kb) (Fig. 2A and B). In cultures grown in the presence of leu3a, much of the viral DNA underwent integration (Fig. 2A). By 24 h, most of the 9.6-kb band present at 12 h appeared to have integrated. At this time 20% of the cells expressed viral antigens. As the first round or progeny virus spread to uninfected cells, more 9.6-kb DNA appeared; this round of 9.6-kb DNA synthesis also appeared to integrate within 24 h of its appearance.

In cultures grown in the absence of leu3a (Fig. 2A and B), patterns of relative band intensities were again consistent with the 9.6-kb linear duplex of viral DNA integrating within 24 h of its appearance (see relative intensities of the 9.6-kb band at 24 h after infection and the high-molecular-weight band at 48 h after infection in Fig. 2A). By 48 h, the most abundant form of viral DNA was a broad smear of viral sequences (most clearly seen in Fig. 2B). The appearance of this smear was not accompanied by obvious degradation of high-molecular-weight chromosomal DNA (Fig. 2B).

To further analyze the forms of unintegrated viral DNA, DNA harvested at various times after infection was digested with EcoRI and analyzed on Southern blots for fragments characteristic of linear and covalently closed circular viral DNA (Fig. 2C). The EcoRI-digested DNA clearly demonstrated the appearance of fragments unique to unintegrated linear DNA (4.6 and 3.9 kb) before the appearance of bands unique to the presumed one-long-terminal-repeat (7.9 kb) and two-long-terminal-repeat forms (8.5 kb) of covalently closed circular DNA. Fragments representing closed circular DNA always migrated as a doublet, and the more rapidly migrating form of the doublet (presumed one-long-terminal-repeat form) was present at higher levels than the more slowly migrating form (most clearly exhibited in Fig. 2A and C). Analyses of EcoRI fragments of Hirt supernatant DNA from cultures grown in the absence of leu3a revealed bands representing the linear and closed circular forms, the 1.1-kb fragment common to all forms, and a smear of fragments migrating as approximately 3 kb or shorter linear species (presumably representing the smear observed in undigested DNA).

DISCUSSION

Accumulation of HIV-1 DNA. Our results demonstrate that the accumulation of HIV-1 DNA in T cells is due to multiple
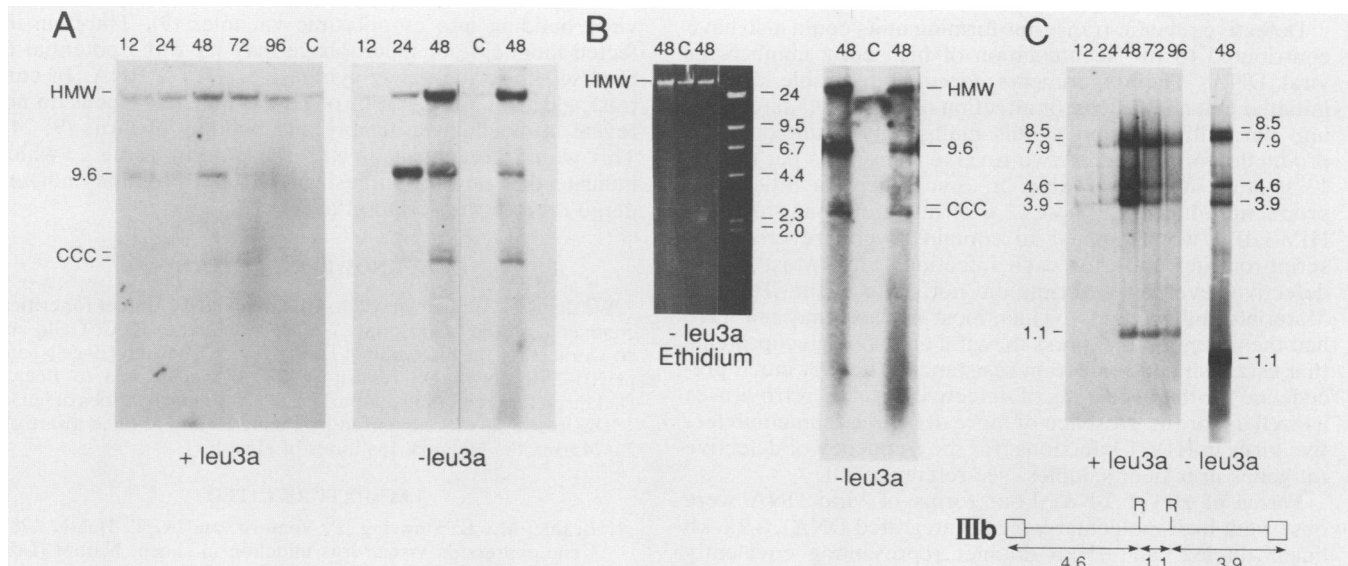
FIG. 2. Forms of HIV-1-IIIb DNA in C8166 cells at various times after infection. (A and B) Blot analyses of undigested total cell DNA. Panels A and B present independent blots of the same 48-h samples. (C) Blot analysis of EcoRI-digested total cell DNA (+leu3a samples) and Hirt supernatant DNA (-leu3a sample). The schematic diagram shows the positions of EcoRI sites (R) in HIV-1-IIIb. DNA blots were hybridized with nick-translated pS4 sequences. Numbers at the tops of lanes indicate hours after infection. Abbreviations: HMW, high-molecular-weight chromosomal DNA; CCC, covalently closed circular DNA. The sizes of fragments are indicated in kilobases. Designations below data indicate whether DNAs were from cultures grown in the presence or absence of leu3a. Ethidium indicates an ethidium bromide-stained gel. A lane containing HindIII marker fragments is presented for the ethidium bromide-stained gel.

infection events (Table 2). This was demonstrated by infecting cultures and then growing the infected cultures in the presence or absence of an antibody to CD4 that blocks HIV-1 infection (Table 1). During the first 48 h of infection, cultures grown in the presence of the antibody accumulated ~4 copies of HIV-1 DNA per cell, whereas those grown in the absence of antibody accumulated ~80 copies of viral DNA per cell (Table 2). In the latter cultures, most of the 80 copies were incomplete viral sequences that migrated as a broad smear (Fig. 2B).

The accumulation of high copy numbers of HIV-1 DNA in C8166 cultures correlated with the appearance of virus-expressing cells. Thus, the production of high copy numbers of viral DNA was most likely due to progeny virus infecting

cells that had not established superinfection resistance. HIV-1 envelope glycoproteins are able to establish superinfection resistance (4, 36). However, the time between the initiation of an infection and the establishment of superinfection resistance may be prolonged by differential gene expression (16) and the dependence of HIV-1 envelope glycoprotein synthesis on the expression of tat and rev proteins (17, 18; for a review, see reference 3). Electron micrographs reveal HIV-1-producing cells releasing high levels of budding virions (24, 36). Thus, high levels of virion production coupled with relatively slow establishment of superinfection resistance could contribute to the copy numbers of viral DNA present in cultures grown in the absence of leu3a.

TABLE 2. HIV-1-IIIb DNA copies per cell[a]

Treatment[b]	h after infection	% IFA+ cells	% Dead cells[c]	DNA copies/cell[d]		% Integrated DNA[e]	PV/cell[f]
				Expt 1	Expt 2		
With leu3a	12	<1	1	2.6, 1		19	0.3
	24	20	1	1.6, 1.5		37	0.6
	48	70	2	5, 3.5	5.6, 2	34	1.4
	72	90	10	6, 4.5		48	2.5
	96	95	50	7.3, 4		39	2.2
Without leu3a	12	<1	1	2.3, 1		<10	<0.2
	24	(Syncytia)		24, 15		15	3.0
	48	(Syncytia)		88, 58	75, 100	15	12.0

[a] For experimental details, see the text.
[b] C8166 cells infected with HIV-1-IIIb were tested with and without leu3a.
[c] Dead cells were not quantitated in cultures undergoing syncytium formation.
[d] Copy numbers for cultures containing syncytia are normalized for single-cell equivalents of DNA.
[e] Percentage of integrated viral DNA was estimated by dividing counts hybridized to high-molecular-weight DNA by counts hybridized to all forms of viral DNA. These represent minimum estimates, because transfer of high-molecular-weight DNA was incomplete.
[f] Proviruses (PV) (integrated viral DNA) per cell were estimated by multiplying the average number of viral DNA copies per cell by the estimated percent integrated.

Defective reverse-transcript-forming units could also have contributed to the accumulation of high copy numbers of viral DNA. The experiments reported in Table 2 were initiated at a multiplicity of infection of 0.25 to 0.5 infectious unit per cell. Infection at this multiplicity resulted in the production of just under two reverse transcripts per cell by 12 h after infection (Table 2). Since there is little virus production during the first 12 h of infection, our stocks of HIV-1-IIIb would appear to contain several reverse-transcript-forming units for each infectious unit. Most of the defective reverse transcripts did not score in the IFA (see Materials and Methods). Thus, most of these may not have had the potential to express the viral envelope glycoproteins that establish superinfection resistance. There is much precedence for the packaging of defective virus by retroviruses as well as for the presence of more defective than nondefective virus in HIV-1 infections (for the frequency of defective *tat* genes in patient samples, see reference 20).

Forms of HIV-1 DNA. Four forms of viral DNA were observed: high-molecular-weight (integrated DNA), a 9.6-kb linear duplex, an ~5-kb doublet representing covalently closed circular DNA, and a broad smear of incomplete viral sequences. In cultures grown in the presence or absence of leu3a, the relative hybridization intensities of the 9.6-kb linear duplex and high-molecular-weight sequences were consistent with the 9.6-kb species being the precursor to integrated DNA. Irrespective of whether a culture was grown in the presence or absence of leu3a, the 9.6-kb species appeared to undergo integration within 24 h of its appearance. The broad smear of viral sequences was only detected in cultures undergoing active reinfection. This smear could represent nascent transcripts, since it appeared at at time when the culture was producing high copy numbers of HIV-1 DNA and since smears of viral DNA occur in cultures undergoing active synthesis of hepatitis B virus DNA (Table 2) (19). Alternatively, this smear could represent degradation of DNA as cultures underwent cell death (note that the smear includes sequences longer than full-length viral DNA), defective-deleted forms of viral DNA, or a combination of the above possibilities. The appearance of the smear did not accompany widespread degradation of chromosomal DNA, since ethidium bromide stains of gels containing undigested DNA did not reveal smears of chromosomal DNA or nucleosome ladders (Fig. 2B).

Generality of results. Our experiments clearly demonstrate that T cells accumulate unintegrated HIV-1 DNA as a result of multiple virions entering cells and not by intracellular reverse transcription of newly synthesized HIV-1 RNA. This result is consistent with polymerase chain reaction analyses of the copy number of HIV-1 DNA in peripheral blood lymphocytes from patients with AIDS. These tests suggest that the rare infected T cells (~1% of the CD4$^+$ cells) contain a single copy of viral DNA (32). This single copy of DNA presumably arises from reverse transcription of the RNA of an infecting virus.

The mechanism for DNA accumulation in HIV-1-infected T cells may not necessarily represent the sole mechanism for DNA accumulation in other cell types. In visna virus-infected sheep, ~1% of the choroid plexus cells and monocytes contain multiple copies of viral DNA (1, 23). The presence of multiple reverse transcripts in the infrequent positive cell suggests that a single infectious event can generate multiple copies of viral DNA. This may correlate with whether virions undergo intracellular assembly. Electron micrographs of HIV-1-infected monocytes or macrophages reveal intracellular assembly, with the assembling

virus budding into cytoplasmic vacuoles (9). Thus, an infected monocyte or macrophage may have the potential to reverse transcribe newly synthesized HIV-1 RNA. In contrast, electron micrographs of HIV-1-infected T cells do not reveal intracellular assembly and budding of virus (9, 24). This would be consistent with the data in Table 2, which indicate that newly synthesized HIV-1 RNA does not undergo reverse transcription in T cells.

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