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Human immunodeficiency virus 1 reservoir in CD4$^+$ T cells is restricted to certain Vβ subsets

DANA DOBRESCU*, SHARA KABAK*, KAMINI MEHTA*, CHANG H. SUH*, ADAM ASCH*, PAUL U. CAMERON†, ANDREW S. HODTSEV*, AND DAVID N. POSNETT*‡

*Department of Medicine, Cornell University Medical College, and ‡Laboratory of Human Molecular Immunobiology, Immunology Program, Graduate School of Medical Sciences, Cornell University, New York, NY 10021; and †The Rockefeller University, New York, NY 10021

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ABSTRACT **The human immunodeficiency virus 1 (HIV-1) replicates more efficiently in T-cell lines expressing T-cell receptors derived from certain Vβ genes, Vβ12 in particular, suggesting the effects of a superantigen. The targeted Vβ12 subset was not deleted in HIV-1-infected patients. It was therefore possible that it might represent an *in vivo* viral reservoir. Viral load was assessed by quantitative PCR with *gag* primers and with an infectivity assay to measure competent virus. It was shown that the tiny Vβ12 subset (1–2% of T cells) often has a higher viral load than other Vβ subsets in infected patients. Selective HIV-1 replication in Vβ12 cells was also observed 6–8 days after *in vitro* infection of peripheral blood lymphocytes from normal, HIV-1 negative donors. Viral replication in targeted Vβ subsets may serve to promote a biologically relevant viral reservoir.**

Typically superantigens (SAGs) activate T-cell subsets depending on expression of T-cell receptors using selected Vβ genes. A previous study demonstrated that human immunodeficiency virus 1 (HIV-1) replicates 10- to 100-fold more efficiently in cultured CD4$^+$ Vβ12 cells compared with control Vβ subsets, regardless of the HLA type of the donor (1). However, the control subsets did support efficient high-level viral replication if they were activated by exogenous mitogens or SAGs (1, 2). This suggested that Vβ12 cells were being activated during culture, possibly by a SAG. This interpretation was supported by experiments in which non-T cells, obtained *ex vivo* from HIV-1-infected donors, specifically activated Vβ12 cells to proliferate (1).

Vβ12 cells were not deleted in HIV-1-infected patients (1, 3, 4). In the prototypic model for a viral SAG, the role of the mouse mammary tumor virus SAG is to assist in establishing a viral reservoir (5, 6). Herein we test the hypothesis that the Vβ12 subset represents a favored HIV-1 viral reservoir (2).

MATERIALS AND METHODS

Patients. Adult seropositive hemophiliac patients were recruited from the Hemophilia Clinic at The New York Hospital. In addition, two seropositive patients with a history of i.v. drug abuse (006, M3) and one patient without apparent risk exposure (M1) were studied. Two of these were women (M1, M3). Informed consent was obtained from all patients. Normal volunteers negative for HIV-1 *gag* by PCR were also studied.

Immunofluorescence. A series of monoclonal antibodies specific for various Vβ gene products were used for two-color immunofluorescence as described (3).

Isolation of Vβ Subsets. Peripheral blood lymphocytes (PBL) were isolated by Ficoll/Hypaque centrifugation. T cells and non-T cells were isolated by rosetting with neuraminidase-treated sheep red blood cells. Vβ subsets were isolated by incubating T cells (usually 3×10^6) with a Vβ-specific monoclonal antibody for 45 min, followed by three washes with phosphate-buffered saline (PBS) and a second incubation with goat anti-mouse Ig-coated magnetic beads (Dynal, Great Neck, NY) at 20 beads per target cell for 30 min on ice. Cells adherent to the beads were then separated by using a magnet and washed with PBS. The T cells remaining after removal of the magnetic beads were stained with Vβ-specific monoclonal antibody demonstrating 75–100% efficiency of the positive selection. The positively selected subset can also be analyzed after prolonged culture to disengage cells from the beads and expand cell numbers. Such experiments have previously demonstrated the high degree of specificity of this type of positive selection (1). Once isolated, these subsets were immediately lysed to prepare DNA for PCR, avoiding *in vitro* cell culture—i.e., approximately 3–4 h after the blood was drawn.

PCR. Positively selected Vβ cell subsets, approximately 10^4 cells per subset, were lysed while still attached to the magnetic beads in 50 μl of 10 mM Tris·HCl, pH 8.0/1 mM EDTA/0.001% Triton X-100/0.0001% SDS/600 μg of proteinase K per ml/at 56°C for 1 h followed by 95°C for 15 min. One half of the DNA lysate was used for a 50-μl PCR reaction containing 10 mM Tris·HCl, pH 9.0, 50 mM KCl, 0.01% gelatin, 0.1% Triton X-100, 3.5 mM $MgCl_2$, 0.4 mM dNTPs, 2 units *Taq* DNA polymerase (Promega), 10^6 cpm of kinased sense *gag* primer, 10^5 cpm of kinased sense HLA-DQα primer, and equal molar ratios of the corresponding unlabeled antisense primers (about 10 pmol of *gag* primers and 1 pmol of HLA-DQα primers per reaction). Four primer sequences were used in these experiments: *gag* (sense), 5′-ATAATCCACCTATCCCAGTAGGAGAAAT-3′; *gag* (antisense), 5′-TTTGGTCCTTGTCTTATGTCCAGAATG-3′; HLA-DQα (sense), 5′-GTGCTGCAGGTGTAAACTTGTACCAG-3′; and HLA-DQα (antisense), 5′-CACGGATCCGGTAGCAGCGGTAGAGTT-3′ (7, 8). PCR was performed with 28 cycles of 94°C for 30 sec, 60°C for 30 sec, and 72°C for 60 sec. PCR products were resolved on an 8% polyacrylamide gel and exposed to x-ray film. In some experiments, the HLA-DQα primer was not labeled and the product was detected separately by ethidium bromide staining of an agarose gel.

Quantitation of PCR Products. With two sets of primers in each tube the PCR yielded two bands: a *gag* band and an HLA-DQα band, the intensities of which were a function of the numbers of HIV-1 copies and the numbers of cell genome copies, respectively. Serial 1:10 dilutions of the ACH-2 cell line, containing a single integrated HIV-1 genome per cell (9), in a constant number of uninfected cells served as a control for the quality of the PCR reaction. An additional set of 1:5 dilutions of both ACH-2 and uninfected T cells (Fig. 1) served to construct standard curves from the densitometry values of the HLA-DQα and *gag* bands. The $\log_{10}$ OD values (x) were

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Abbreviations: HIV-1, human immunodeficiency virus 1; PBL, peripheral blood lymphocytes; IL-2, interleukin 2; SAG, superantigen; $TCID_{50}$, tissue culture 50% infective dose.

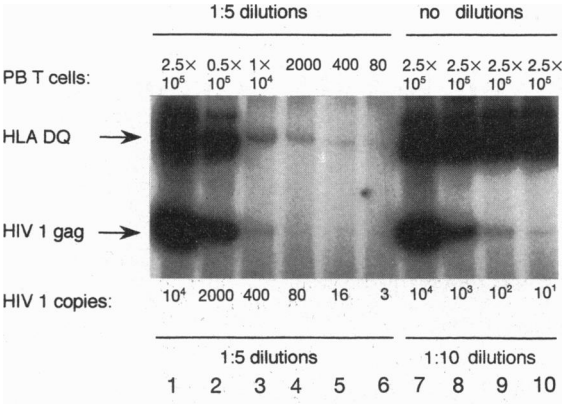

FIG. 1. Controls for quantitative PCR. In lanes 1–6 both the uninfected peripheral blood (PB) T cells and the infected ACH-2 are serially diluted together. In lanes 7–10 the uninfected PB T cells are kept constant at 2.5×10^5 and the ACH-2 cells are serially diluted 1:10.

plotted against the $\log_{10}$ of known numbers of HIV-1 (or HLA-DQ) genome copies (y). A straight line was obtained by regression analysis ($r > 0.95$) described by the function $\log y = s(\log x) + c$, where s is the slope of the curve, and c is the value at the y-axis intersection. With this equation, copy numbers of HIV-1 (or HLA-DQ) could be derived from experimental OD values to calculate copies of HIV-1 per 1000 cells. The validity of these extrapolations was confirmed by running PCR samples either undiluted or diluted 1:5 or 1:25. Known ratios of ACH-2 cells with uninfected cells were also used at various total cell numbers. The calculated ratio of HIV-1 copies to cell copies should remain the same with such controls, and in fact the variation was generally less than ±10% of the mean. The numbers of cells in the relevant Vβ subsets were also estimated from two-color immunofluorescence data published elsewhere (3). These numbers correlated well with the data obtained by densitometry of the PCR HLA-DQα bands. The CD4/CD8 ratio among Vβ subsets was similar.

Infectivity Assay. Positively selected Vβ subsets from a single blood sample were equally split for (*i*) PCR analysis and (*ii*) an infectivity assay. For the infectivity assay, Vβ subsets ($\approx 10^4$ cells) were cocultured with 10^6 indicator cells (2-day cultures of phytohemagglutinin- and alloantigen-activated PBL from an uninfected normal donor) in medium containing 10% (vol/vol) fetal calf serum and 50 units of interleukin 2 (IL-2) per ml. One half of the supernatant was collected every 4 days and exchanged with fresh medium. Supernatants were tested for p24 antigen by an antigen-capture ELISA assay (10).

***In Vitro* Infection of PBL.** A total of 3×10^6 PBL were infected with 3000 tissue culture 50% infective doses ($TCID_{50}$) of the HIV-1 TIIIB isolate and cultured for 6–8 days in medium containing 10% (vol/vol) fetal calf serum and 50 units of IL-2 per ml at a cell concentration of 10^6 cells per ml. Vβ subsets were then isolated as described above from 3×10^6 cultured cells per Vβ subset (expected yield of isolated subsets: 10^4–10^5 cells). In some experiments, the cells remaining after the positive selection—e.g., Vβ12-depleted cells—were also collected. In others, T cells were isolated by sheep red blood cell rosetting and depleted of plastic-adherent cells prior to being cultured with or without adding back non-T cells at a 1:10 ratio (non-T cells to T cells). Non-T cells were irradiated with 3000 Rads and, when indicated, treated with sodium periodate ($NaIO_4$) as described (1).

RESULTS

Quantitative PCR for HIV-1 *gag* in Vβ Subsets. To examine whether the Vβ12 subset might represent an HIV-1 viral reservoir *in vivo*, a quantitative PCR was used to measure viral burden in Vβ subsets (Figs. 1–3). Since prior studies had shown maximal HIV-1 replication in Vβ12 cells and minimal replication in Vβ6.7a cells (1), these two subsets were isolated from HIV-1 seropositive patients (Fig. 2). In 7 of 10 hemophiliac patients (006, 007, 012, 013, 016, 017, and 033) the viral load appeared to be higher in the Vβ12 cells relative to the load in Vβ6.7a cells (Fig. 2). A repeat sample from patient 012 (far right in Fig. 2) shows similar skewing, with most of the viral *gag* PCR product in the Vβ12 cells. However, the total yield of cells was markedly decreased in the second sample.

Because of possible variations in the cell numbers in each Vβ subset and variations in the efficiency of the positive selection, HIV-1 load was assessed as a function of the total number of cells in each Vβ subset. This was done by extrapolating HLA-DQα and HIV-1 *gag* bands onto standard 1:5 dilution curves (Fig. 1), as described in *Materials and Methods*. The viral burden in Vβ12 and Vβ6.7a cells in 23 seropositive patients is summarized in Table 1. The majority of the patients (16/23) have 2- to 360-fold more viral DNA in the Vβ12 cells than in the Vβ6.7a cells. Only two patients have more viral DNA in the Vβ6.7a subset than in the Vβ12 subset, and in 5 patients the burden is not heavily skewed (Vβ12 to Vβ6.7a ratio between 1 and 2). Repeat determinations showed little variation in the HIV-1 load ratio (Table 1 and Fig. 3).

The degree of skewing of the viral burden to the Vβ12 subset was not correlated with clinical stage of disease, as measured by the total CD4 cell count, with the presence of clinical signs of AIDS, with the terminal stages of AIDS, or with the pace of $CD4^+$ T-cell depletion (CD4 slope). There was also no apparent correlation with HLA alleles in these patients (data not shown).

Patient to patient variation was observed in the calculated viral copy number normalized for cell numbers, as previously noted (11). For example, the viral load in the blood Vβ12 cells differs by a factor of 10^3 between patients 025 and 041; yet they have similar ratios of HIV-1 load (Vβ12/Vβ6.7a). Viral load increases in the later stages of HIV-1 disease (11), although the intracellular load may be distributed primarily in lymph nodes rather than in the blood (13). On average, our patients with a clinical diagnosis of AIDS did have higher viral loads (HIV-1 copies per 1000 cells) in both Vβ subsets compared with

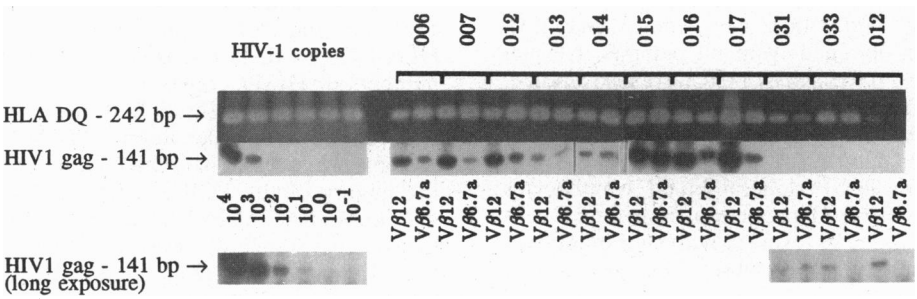

FIG. 2. HIV-1 load in Vβ12 and Vβ6.7a subsets from 10 hemophiliac patients. Two separate samples from patient 012 were analyzed. For HIV-1 *gag* bands, two exposures are shown (12 h and 48 h). The HIV-1 copies represent known numbers (1:10 dilutions) of ACH-2 cells admixed to a constant number (250,000) of uninfected CEM cells (human T-cell line).

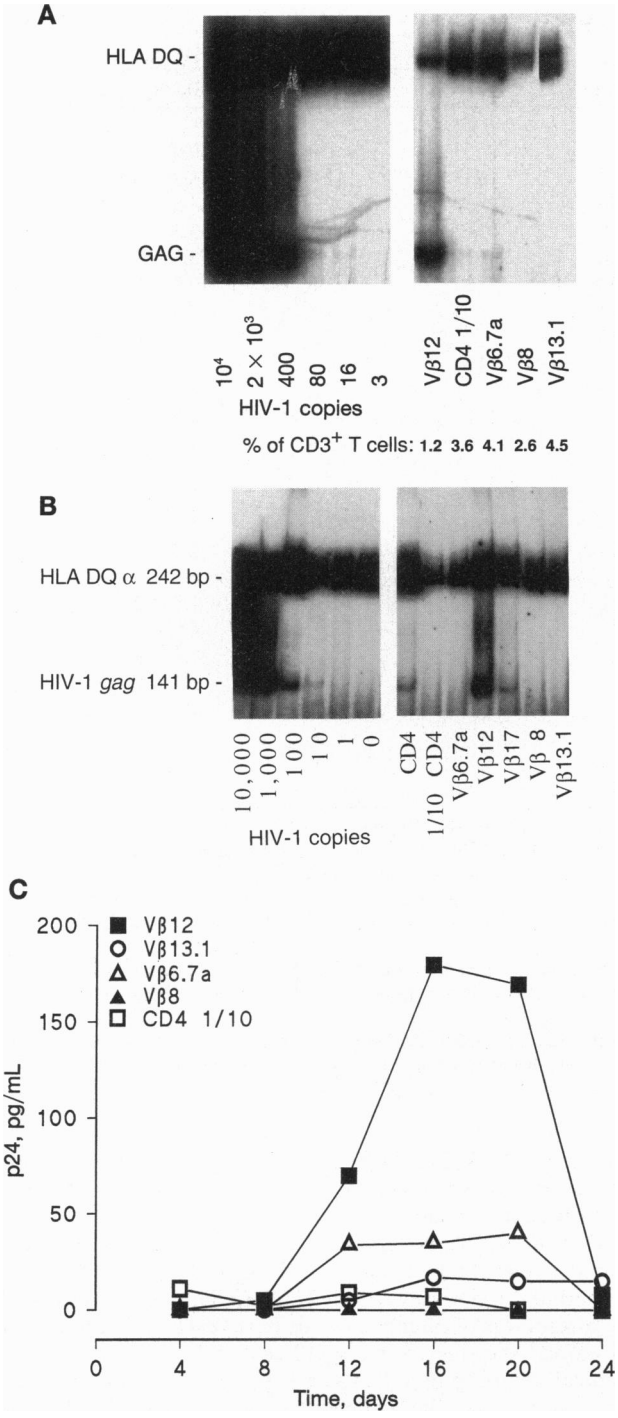

FIG. 3. Analysis of viral burden in Vβ subsets from patient M3. Positively selected Vβ subsets from a single blood sample were equally split for PCR analysis in *A* and infectivity assay in *C*. The percentages of $CD3^+$ cells expressing a given Vβ as determined by indirect immunofluorescence are indicated in panel *A*. Thirty-six percent of the $CD3^+$ cells expressed CD4. As a control containing all Vβ subsets, we used 10% of the positively selected CD4 cells, representing a similar total number of T cells to the various Vβ subsets. Vβ subsets from a second blood sample obtained 4 months later were analyzed by PCR in *B*. The total number of $CD4^+$ Vβ12 cells should in theory be the same in the $CD4^+$-selected (CD4) and the Vβ12-selected (Vβ12) samples. The decreased intensity of the *gag* band in $CD4^+$-selected vs. Vβ12-selected subsets may be due to decreased expression or accessibility of CD4 at the cell surface of HIV-1-infected cells, resulting in a decreased efficiency of positive selection.

individuals without AIDS: mean ± standard deviation of HIV-1 load in Vβ6.7a cells in patients with AIDS was 122.2 ± 142.5 versus 24.9 ± 55.5 in patients without AIDS; load in Vβ12 cells in patients with AIDS was 328.7 ± 355.0 versus 100.5 ± 220.1 in patients without AIDS.

Vβ12 Cells Represent a Reservoir for Infectious Virus. The preceding data suggest that the Vβ12 subset may function as a viral reservoir in a majority of patients. However, the presence of *gag* DNA in these cells is not sufficient to indicate competence to produce infectious virions. Recent estimates indicate that only 1 in 60,000 HIV-1 genomes in an infected individual is competent to infect new host cells (11). It is therefore necessary to compare viral burden measured by PCR with viral burden measured in an infectivity assay.

In patient M3, for example, the HIV-1 load is dramatically shifted to the Vβ12 subset in two PCR experiments performed with samples obtained 4 months apart (Fig. 3 *A* and *B*). The intensity of the HLA-DQα bands demonstrates that the number of positively selected Vβ12 cells is comparable with, if not smaller than, the other selected subsets (Fig. 3*A*), and the percentages measured by fluorescence also indicate that the Vβ12 subset is one of the smallest (Fig. 3*A*). Yet this subset contains practically all of the HIV-1 viral burden in the peripheral blood of this patient. To address whether this potential reservoir includes competent virus, each positively selected Vβ subset was divided equally for (*i*) PCR (Fig. 3*A*) and (*ii*) coculture with indicator cells (Fig. 3*C*). Infectious virus was primarily derived from the Vβ12 subset (Fig. 3*C*). As this subset produces the large majority of competent infectious virus, it represents a biologically relevant reservoir for HIV-1.

HIV-1 Replicates Preferentially in Vβ12 Cells After *in Vitro* Infection. Previous work had shown high-level viral replication in isolated Vβ12 cell lines infected *in vitro* compared with cell lines expressing other Vβ genes (1). However, it is not yet known whether initial HIV-1 replication is selective for certain Vβ subsets after *in vitro* infection of fresh bulk T cells from normal donors.

Normal PBL, obtained from donors determined to be HIV-1 negative by PCR, were infected with HIV-1 *in vitro* and cultured in medium containing 50 units of IL-2 per ml. Mitogen activation of T cells, used routinely to obtain optimal *in vitro* infection, was omitted to avoid obscuring the Vβ-selective effect of a putative SAG, since addition of phytohemagglutinin leads to high-level HIV-1 replication in all Vβ subsets (data not shown). In preliminary experiments, PCR for viral *gag* in these unstimulated cell cultures did not yield positive results until about day 6 of culture. Therefore, cultured cells were separated into Vβ subsets after 6 or 8 days and assayed for viral load by PCR (Fig. 4). In two unrelated donors HIV-1 was strongly skewed to the Vβ12 subset (Fig. 4). Similar results were obtained with seven unrelated normal adult donors. HIV-1 replication in Vβ12 cells was not observed in isolated T cells cultured with IL-2, but was reconstituted if irradiated non-T cells were added back to the T cells (data not shown). Moreover, autologous, irradiated non-T cells treated with the mitogen $NaIO_4$ (1) were often more efficient at reconstituting HIV-1 replication in Vβ12 cells.

In one donor it was possible to analyze 13 Vβ subsets simultaneously on day 7 of culture. HIV-1 *gag* was selectively present in the Vβ12 subset (Fig. 5). In other experiments Vβ12-depleted cells lacked the HIV-1 *gag* band, but control cells depleted of the Vβ17 subset still gave a strong HIV-1 *gag* band (data not shown). These data indicate that most of the HIV-1 viral replication is occurring in the Vβ12 subset. In several experiments we found that the Vβ12 subset becomes relatively more predominant among $CD3^+$ $CD4^+$ T cells in these cultures. Vβ12 cells represented 18% of $CD3^+$ $CD4^+$ T cells among HIV-1-infected T cells cultured (day 7) with autologous $NaIO_4$-treated non-T cells. By comparison, this subset measured 2% of $CD4^+$ T cells at the onset of the culture and other control Vβ subsets were not expanded after 7 days of culture. This result is consistent with the relatively intense

Table 1. Viral burden in Vβ12 and Vβ6.7a T cells

Patient	AIDS*	$CD4^+$ cells per mm^3	CD4 slope†	HIV load‡		HIV ratio§ of Vβ12 to Vβ6.7
				Vβ12	Vβ6.7a	
Group 1						
M1	−	593		3.6	<0.01	360.0
M3	−	410		833	12.2	68.3 (>100)
042	−	510	−26	69	2	34.5
006	(+)¶	240		212	16	13.3
017	−	353	−60	11	0.8	13.3 (2.9)
001	−	684	−20	36	3.6	10.0
033	+¶	4	−81	3.3	0.4	8.3
015	−	595	−73	1.53	0.25	6.1 (2.6, 3.3)
012	+¶	261	−84	193	40	5.7 (4.8)
025	−	234	+8	1	0.2	5.0
041	(+)	141	−47	1111	228	4.9
040	−	487	−78	24	5	4.8
007	+¶	15	−12	194	43	4.5
005	−	561	−53	338	96	3.5
024	−	592	−102	3	1	3.0
009	−	351	−129	2	0.9	2.2
Group 2						
018	+¶	6	−51	786	447	1.8 (1.2)
016	+	41	−19	360	240	1.5
014	(+)¶	77	−47	44	32	1.4 (0.5)
013	−	303	−39	19	18	1.1
038	(+)	153	−17	55	53	1.0
Group 3						
031	−	712	+33	1.9	3.0	0.6
039	−	500	−19	64	205	0.3

*A diagnosis of AIDS was made on the basis of opportunistic infections, AIDS-related malignancy or dementia, or a $CD4^+$ T-cell count below 200. (+) indicates a low CD4 count was the only criterion for AIDS. All patients were hemophiliacs except for M3 and 006 who were i.v. drug users and M1 whose only known risk was heterosexual activity.

†The CD4 slope (change in $CD4^+$ T-cell count per year) was calculated by regression analysis based on counts of $CD4^+$ cells per mm^3 of blood, obtained over several years at 6-month intervals. Most patients were followed for over 10 years and had documented seroconversion from 7 years to >13 years prior to the study.

‡HIV load is expressed as HIV-1 copies per 1000 cells of the indicated subset. The cell numbers in Vβ subsets were assessed by two-color immunofluorescence with Vβ-specific monoclonal antibody (3) and by densitometry of the radioactively labeled HLA-DQα bands.

§The ratio was calculated from data on HIV-1 copies per 1000 cells in each Vβ subset. Ratios from repeat samples obtained at intervals of 3–9 months are indicated in parentheses. The patients are listed in order of viral load skewing to the Vβ12 subset. Group 1 patients have ratios greater than 2; group 2 between 1 and 2; and group 3, below 1.

¶Death occurred with 1 year after study.

HLA-DQα band seen in the Vβ12 lane in Fig. 5. It is also consistent with the known effect of SAGs resulting in expansion of the targeted Vβ subsets (14–16). However, expansion, per se, of the Vβ12 CD4 cells is not the reason for the higher viral load in this subset. Subsets in Fig. 5 with equivalent HLA-DQα bands (CD4 1:10 and Vβ5.3) showed no HIV-1 *gag* bands. The Vβ12 subset of patient M3 in Fig. 3*A* contained fewer cells than control subsets judging by both the intensity of the HLA-DQα band and the percentage of cells measured by immunofluorescence. Finally, the ratios calculated in Table 1 are corrected for cell numbers. Therefore, we conclude that HIV-1 replication is more efficient in Vβ12 $CD4^+$ cells compared with other subsets both *in vivo* and *in vitro*.

DISCUSSION

$CD4^+$ T cells contain the major reservoir of HIV-1 (9, 17, 18) *in vivo*. The majority of the infected $CD4^+$ cells reside in lymph nodes (13), but viral DNA can readily be detected by PCR in $CD4^+$ cells from the peripheral blood. Within the PBL $CD4^+$ subset, only a small proportion of the cells (0.1–10%) are infected with HIV-1 (9, 19). Previous studies have characterized this reservoir of HIV-1-infected $CD4^+$ cells as $CD45RO^+$ (20), $HLA\text{-}DR^-$ (21) quiescent T cells thought to contain integrated proviral DNA. During the clinically latent phase of infection, these cells maintain a low level of productive HIV-1 replication (11, 13), possibly because of repeated T-cell activation resulting in CD25 expression (12). Continuous low-grade productive infection is likely to be relevant for viral transmission to a new host.

Herein, we show that the HIV-1 viral load in peripheral blood T cells of infected patients is skewed to the Vβ12 subset, sometimes quite dramatically (Fig. 3). This subset appears to be a reservoir of infectious virus *in vivo*. The phenomenon of viral-load skewing to the Vβ12 subset is not restricted to a clinical subgroup of patients and appears to be generally valid since it can also be seen with *in vitro* infected cells from unrelated normal donors. Viral replication in Vβ12 cells appears to be dependent on the presence of MHC class II-bearing non-T cells but is not MHC restricted, confirming previous findings (1). In addition, antibodies to class II MHC antigens were able to inhibit Vβ-selective HIV-1 replication (1). Normal Vβ12 (S511+) cells are not inherently more activated by cell-cycle analysis (22) or phenotypic analysis (2) than T cells expressing other Vβs. They do not preferentially proliferate in cultures containing IL-2 and fetal calf serum unless a Vβ12-specific SAG is present (14–16). Vβ12 cells are approximately equally distributed among $CD4^+$ and $CD8^+$ subsets in both HIV-1-negative and HIV-1-positive patients (1, 3).

Vβ12 cells are not selectively depleted in HIV-1-infected patients (1, 3), in spite of evidence that the non-T cells of the same HIV-1-infected patients specifically stimulate Vβ12 cells to proliferate (1). Perhaps this is not so surprising since T-cell

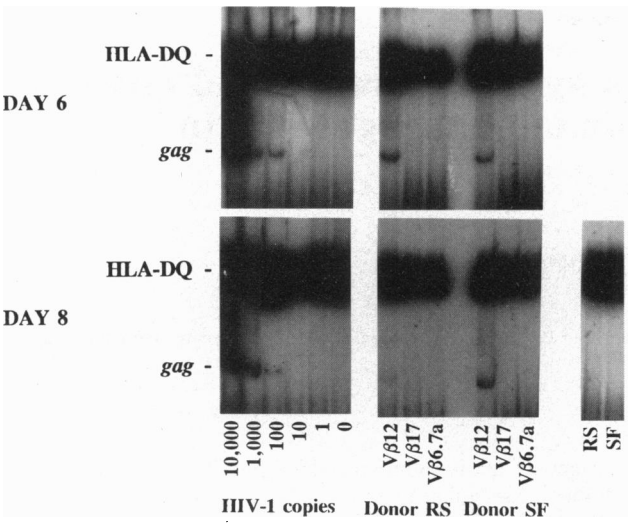

FIG. 4. Viral burden in Vβ subsets after *in vitro* infection of normal peripheral blood mononuclear cells. Shown are three selected Vβ subsets from two normal donors. The PBL were infected with 3000 $TCID_{50}$ of the TIIIB isolate and cultured for 6 or 8 days in medium containing 10% (vol/vol) fetal calf serum and 50 units of IL-2 per ml. Uninfected fresh PBL (10^6) from each donor were analyzed on the far right as a control.

subsets activated by SAGs under suboptimal conditions may not necessarily be deleted but just remain anergic for variable periods of time (23–25). In addition, Vβ deletions have not been observed in humans naturally exposed to SAGs, such as in toxic shock syndrome (26). Viral-load skewing *in vivo* is still observed many years after infection. For instance M3 (Fig. 3) seroconverted 6 years prior to this study. This suggests that the principal driving viral replication in the Vβ12 subset is continuously or intermittently present in such patients. Moreover, if this is due to a HIV-1 gene product, one would expect a highly conserved gene among different isolates with a low mutation rate, since the majority of patients analyzed had a higher viral load in Vβ12 cells than in Vβ6.7a cells. In fact, very similar findings were obtained with several different HIV-1 isolates, including street isolates freshly obtained from patients (1, 2).

An equally plausible hypothesis is that Vβ12 cells are activated by a non-HIV-1 encoded gene. This could be a product of an endogenous human gene or the product of a ubiquitous virus such as Epstein–Barr virus or cytomegalovirus (27).

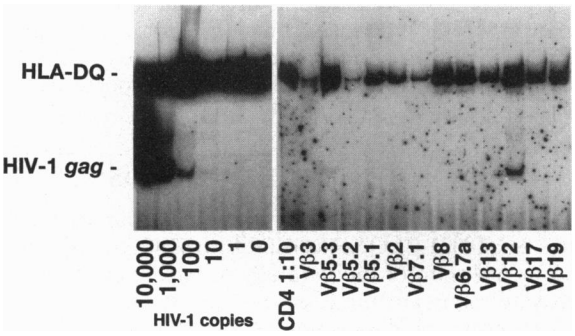

FIG. 5. HIV-1 load in 12 Vβ subsets 7 days after *in vitro* infection of normal T cells. Isolated T cells (3×10^6) infected with 3000 $TCID_{50}$ were cultured for 7 days with autologous non-T cells treated with $NaIO_4$. On day 7, Vβ subsets were isolated and PCR was performed.

Vβ-selective viral replication in Vβ12 cells could be due to a putative SAG. Alternatively, this phenomenon could be due to a promiscuous, HLA nonrestricted but Vβ-selective peptide antigen, similar to the tetanus toxoid peptide, tt830–844, described by Boitel *et al.* (28).

Regardless of its nature, a Vβ-selective element targets Vβ12 cells and presumably activates them, facilitating viral replication in this subset. This could serve to establish a viral reservoir from which HIV-1 may gradually disseminate to infect other tissues and other hosts. A role for SAGs in establishing a viral reservoir after vertical transmission of exogenous mouse mammary tumor virus in newborn mice has been well documented (5, 6). We postulate that viral SAGs in general may have evolved as useful products to promote viral replication and establishment of a reservoir.

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