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13. Characteristics of the anti-CRH (TS 6) antiserum used are as follows: titer, 1:96,000; concentration that causes 10% displacement of radiolabeled tracer (ED_{90}), 40 pg/ml; concentration that causes 50% displacement of radiolabeled tracer (ED_{50}), 170 pg/ml; cross-reactivity, less than 0.001% for growth hormone–releasing hormone, luteinizing hormone–releasing hormone, antidiuretic hormone, ACTH, luteinizing hormone, follicle-stimulating hormone, TSH, prolactin, β-endorphin, and growth hormone. Characteristics of the anti-TNFα antiserum used are as follows: titer, 1:80,000; ED_{90}, 25 pg/ml; ED_{50}, 200 pg/ml; cross-reactivity, less than 0.01% for tumor necrosis factor–β.
14. Mean (± SEM) plasma corticosterone was similar 7 hours after carrageenin administration in anti-CRH–treated animals (39 ± 9 μg/dl) and in controls (44 ± 6).
15. Anti-CRH (1.0 ml) was injected synchronously with carrageenin into the air pouch as in Fig. 1.
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18. Approximately 0.5 g of tissue was added to 10 volumes of boiling 2 M acetic acid and kept at 95°C for 10 min. Samples were sonicated for 1 min on ice and centrifuged at 15,000*g* for 30 min. Three volumes of acetone were added to the supernatant. The precipitated proteins were removed by centrifugation (15,000*g*, 30 min), and the supernatant was evaporated to dryness under vacuum. The samples were reconstituted in radioimmunoassay buffer for measurement as described (*19, 23*).
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21. The samples were reconstituted in 200 μl of a solution of 25% acetonitrile, 0.075% trifluoroacetic acid (TFA), and water and injected onto a C18 mBondapak (Waters Associates, Marlboro, MA) analytical HPLC column (3.9 mm by 300 mm). The chromatography was performed with a linear gradient from a 25% acetonitrile, 0.075% TFA, and water solution to an 80% acetonitrile, 0.02% TFA, and water solution over a 30-min period at a flow rate of 1 ml/min. Samples (1 ml) were collected, lyophilized to dryness, reconstituted with radioimmunoassay buffer, and assayed for CRH content as described (*19*).
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29. Dexamethasone (50 μg, Sigma) or somatostatin analog BIM 23014 (10 μg, IPSEN International, Paris, France) was given intraperitoneally to each rat, 1 hour before or simultaneously with carrageenin administration, respectively, as described in (*12*) and Fig. 1.
30. We thank M. Tsokos for helpful discussions.

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Quiescent T Lymphocytes as an Inducible Virus Reservoir in HIV-1 Infection

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To better understand the basis for human immunodeficiency virus type 1 (HIV-1) persistence and latency, the form in which viral DNA exists in the peripheral T lymphocyte reservoir of infected individuals was investigated. In asymptomatic individuals, HIV-1 was harbored predominantly as full-length, unintegrated complementary DNA. These extrachromosomal DNA forms retained the ability to integrate upon T cell activation in vitro. In patients with acquired immunodeficiency syndrome (AIDS), there was an increase in integrated relative to extrachromosomal DNA forms. By analysis of DNA from patient lymphocyte subpopulations depleted of human lymphocyte antigen–Dr receptor–positive cells, quiescent T cells were identified as the source of extrachromosomal HIV-1 DNA. Thus quiescent T lymphocytes may be a major and inducible HIV-1 reservoir in infected individuals.

THE MAJOR RESERVOIR FOR HIV-1 in the peripheral blood compartment of infected individuals is the $CD4^+$ T lymphocyte (*1, 2*). The high percentage of cells (1 to 0.01%) within this reservoir that contain viral DNA (*2*) is difficult to reconcile with low percentage of infected cells (0.01 to 0.001%) that express viral RNA at levels detectable by in situ hybridization (*3*). In addition, the gradual depletion of $CD4^+$ T lymphocytes during disease progression (*4*) contrasts with the acute cytocidal nature of HIV-1 infection of permissive T lymphocytes in vitro (*5*). These features suggest that a small population of infected cells is permissive for virus replication, whereas the majority of host cells harbor HIV-1 in a minimally replicative yet inducible state.

The life cycle of retroviruses can be separated into pre- and postintegration stages. Integration of HIV-1 DNA with the host cell genome, which depends on the activated state of the host cell (*6*), must occur for a

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productive virus infection (*6*, 7). Thus, although HIV-1 retains the capacity to bind and infect quiescent T lymphocytes in vitro (*6–8*), inefficient reverse transcription (7) and a block to integration of full-length HIV-1 DNA (*6*) restrict the viral life cycle in these cells to preintegration events.

Current in vitro models for HIV-1 persistence and latency have focused on agents that activate HIV-1 gene expression in cells harboring latent integrated provirus (*9*). However, initial integration requires T cell activation (*6*, 7), and, because the number of activated T cells in asymptomatic individuals is low, it has been hypothesized that quiescent T cells form a latent and inducible reservoir for HIV-1 in vivo (*6*, 7).

To identify molecular events during HIV-1 replication in vivo, we analyzed the arrangement of proviral DNA in single infected cells. This approach allowed us to determine whether the presence of extrachromosomal HIV-1 DNA within patient lymphocytes is due to restricted integration in quiescent cells or to superinfection (*10*, *11*) of permissive cells by HIV-1. By initially determining HIV-1 proviral DNA copy number and the percentage of cells harboring HIV-1 within each infected individual included in this study (Table 1), we could analyze multiple (10 to 40) cell fractions, each containing not more than one HIV-1–infected cell (*12*). DNA was extracted from multiple replicate cell fractions and separated into high molecular weight DNA (containing integrated provirus) and low molecular weight DNA (containing extrachromosomal HIV-1 DNA forms) by agarose gel electrophoresis, and HIV-1 DNA was identified in high and low molecular weight fractions by polymerase chain reaction (PCR) (*12*).

Application of our protocol to 8E5 cells (*13*), which contain one noninfectious provirus per cell and completely lack unintegrated viral DNA, demonstrated that, if there were fewer than eight infected cells in each sample analyzed, mechanical shearing did not lead to separation of detectable amounts of integrated viral DNA in the low molecular weight fraction (Fig. 1A). Because our protocol (*12*) ensured that there was one infected cell in each fraction, we were able to distinguish integrated and unintegrated forms of HIV-1 DNA. Analysis of DNA isolated from cells infected with an HIV-1 mutant which, because of deletions in the HIV-1 intergrase coding region, is unable to associate with host-cell DNA (*6*, *14*), demonstrated that trapping of low molecular weight extrachromosomal HIV-1

Table 1. Integrated and extrachromosomal viral DNA in total and purified lymphocyte populations of HIV-1–seropositive individuals. After determination of the percentage of HIV-1–infected cells for each patient (*12*), a series of replicate (10 to 40) lymphocyte fractions was prepared. Each replicate fraction contained sufficiently few cells so that there was not more than one infected cell per fraction. The number of replicates containing HIV-1 DNA and the number of fractions analyzed are listed for each patient. The number of fractions containing exclusively integrated viral DNA, exclusively unintegrated viral DNA, or both DNA forms, were calculated as a percentage of the total number of replicates harboring viral DNA. Fractions not containing viral DNA were excluded from the analysis. For examination of viral DNA in purified lymphocyte populations, the percentages of HLA-Dr–positive cells was determined by flow cytofluorimetry after affinity cell sorting with HLA-Dr antibody–coated magnetic beads (*27*). (Asym., asymptomatic; ND, not done.)

(**A**) Total lymphocyte populations

Patient	Status	CD4/CD8	Lymphocytes per fraction	Infected/analyzed	HIV-1–infected lymphocytes (%)	Integrated DNA (%)	Extrachromosomal DNA (%)	Both forms DNA (%)
E32	AIDS	0.15	250	17/21	0.40	59	35	6
B3	AIDS	<0.03	250	17/20	0.40	66	17	17
B5	AIDS	0.08	250	25/30	0.40	60	12	28
B10	AIDS	0.39	100	8/20	1.00	75	12.5	12.5
B12	AIDS	0.28	250	20/20	1.00	40	20	40
B12			100	16/20		62.5	18.75	18.75
B12			50	5/10		80	20	0
B1	Asym.	0.68	250	10/20	0.40	30	50	20
B4	Asym.	0.76	250	19/30	0.40	21	58	21
B7	Asym.	0.52	250	6/10	0.12	33	67	0
B9	Asym.	0.09	400	21/40	0.15	14	86	0
B11	Asym.	0.12	400	12/20	0.10	25	50	25
B20	Asym.	0.25	250	5/20	0.25	20	80	0
B23	Asym.	0.87	250	7/20	0.20	29	71	0
B24	Asym.	0.30	400	11/20	0.21	18	55	27
B25	Asym.	1.79	400	17/20	0.15	0	88	12
B34	Asym.	0.97	400	6/20	0.20	33	50	17
B35	Asym.	0.17	400	5/20	0.07	0	80	20

(**B**) Purified lymphocyte populations

Patient	Lymphocyte population	Cells per fraction	HLA-Dr$^+$ cells (%)	HIV-1–infected lymphocytes (%)	Integrated DNA (%)	Extra-Chromosomal DNA (%)	Both forms DNA (%)
B28*	Total	400	25	0.30	65	35	0
B28	HLA-Dr$^+$	400	ND		80	20	0
B28	HLA-Dr$^+$	400	6		0	80	20
B30*	Total	400	32	0.125	60	20	20
B30	HLA-Dr$^+$	400	<1		0	100	0
B4	Total in vitro	250	0.40	0.40	20	60	20
B4	activated	250			100	0	0

*AIDS patient.

DNA forms in the genomic DNA fraction during gel electrophoresis did not lead to detectable amounts of unintegrated HIV-1 DNA in the high molecular weight genomic fraction (Fig. 1B).

The PCR protocol we used could detect a single HIV-1 provirus. Four HIV-1–infected cells (containing one provirus per cell) were mixed with 2×10^3 uninfected lymphocytes and distributed into five fractions to be analyzed by PCR. Four of five fractions gave positive amplifications with primers specific for HIV-1 *pol*, whereas primers to the α-tubulin gene indicated the presence of equivalent amounts of genomic DNA in each fraction (Fig. 1C).

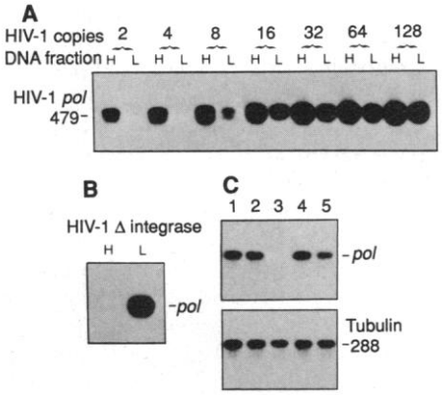

Fig. 1. Discrimination between extrachromosomal and integrated HIV-1 DNA forms. (**A**) Doubling dilutions (from 128 to 2) of 8E5 cells (containing one defective provirus per cell) were mixed with 400 uninfected lymphocytes. After isolation of total cellular DNA, fractionated high (H) and low (L) molecular weight DNA (*12*) were analyzed by PCR with primers specific for HIV-1 *pol* (*25*). (**B**) HIV-1 PCR analysis of high and low molecular weight DNA fractions from 2000 MT-4 cells (human $CD4^+$ T cell line) infected with an integration minus HIV-1 mutant (*6, 14*). (**C**) Four 8E5 lymphocytes were mixed with 2000 uninfected T lymphocytes and distributed in five replicate fractions (1 to 5). Total cellular DNA was isolated, and HIV-1 and tubulin DNA were amplified by 30 and 20 cycles of PCR, respectively. PCR product sizes are in base pairs.

Table 1 summarizes the results of our analysis on the arrangement of HIV-1 DNA within individual infected T lymphocytes from eleven asymptomatic and seven AIDS/ARC (AIDS-related complex) patients. The number of peripheral blood lymphocytes (PBL) harboring HIV-1 genome (Table 1) varied among individuals within asymptomatic and AIDS groups; however, the increased virus DNA load in AIDS patients is in agreement with other studies (*2, 15*). The HIV-1 *pol* primers detect both complete and incomplete products of reverse transcription. However, use of primers [long terminal repeat (LTR)–*gag*] spanning the primer binding site and 5′ LTR-*gag* junction—synthesized after both template switching events (*16*)—identified late products in reverse transcription and gave identical results to those with the *pol* primers (Fig. 2).

In all patients analyzed, the presence of integrated and unintegrated HIV-1 DNA forms within the same fraction was rarely observed. When both forms were detected in the same fraction, this was due to the presence of more than one infected cell in that fraction, and preparation of a second dilution series containing fewer cells in each fraction resolved this. For example, in AIDS patient B12, analysis of replicate cell fractions in aliquots of 250 cells per fraction revealed integrated and unintegrated HIV-1 DNA in 40% of the replicate samples (Table 1). Repeat analyses of the same lymphocyte preparation with 100 or 50 cells per fraction resulted in the presence of both DNA forms in 18.75% and 0% of the replicates, respectively (Table 1). Limiting dilution and discrimination of integrated and extrachromosomal HIV-1 DNA in all patients in this study demonstrated that superinfection did not appear widespread in lymphocytes of individuals infected with HIV-1. This is not surprising because infected quiescent cells are not productive for virions and thus do not provide the conditions for superinfection. On the other hand, superinfection of a permissive (activated) T lymphocyte may be restricted because of receptor interference (*10, 11, 17*) or may be a rare and rapid event, difficult to detect in vivo. In any event, because of the low numbers of infected cells within patients (≤1%), superinfection is statistically unlikely.

Monoclonal antibody to the major histocompatibility complex class II human lymphocyte antigen (HLA)–Dr, which is expressed on macrophages and activated T lymphocytes, but not quiescent T lymphocytes (*18*), was used to deplete patient lymphocyte populations of activated T cells. After depletion of activated T cells, the proportion of cells harboring exclusively unintegrated viral DNA increased from 35 to 80% for patient B28 and from 20 to 100% for patient B30 (Table 1). Depletion of interleukin-2 receptor–positive lymphocytes of asymptomatic patient B24 demonstrated a similar striking predominance of extrachromosomal HIV-1 DNA forms in quiescent lymphocytes (*19*).

The biological activity of extrachromosomal HIV-1 DNA forms in infected individuals was assessed after T cell activation in vitro. Lymphocytes from patient B4 (HIV-1–positive, asymptomatic) were isolated; half of these were activated with phytohemagglutinin (PHA) and half of these were untreated. After 24 hours, 20 replicate cell fractions were prepared from the quiescent and activated cultures, and each replicate fraction was then analyzed for the presence of integrated and extrachromosomal viral DNA (*12*). High concentrations (7 μg/ml) of soluble CD4 (sCD4), which has been shown to completely inhibit HIV-1 binding and infection (*20*), were incorporated in the culture medium to prevent new rounds of infection by virus released from permissively infected (activated) lymphocytes. Of ten infected replicate cell aliquots (400 cells per fraction) from patient B4, six had exclusively extrachromosomal HIV-1 DNA forms (Fig. 2 and Table 1), as detected with the use of HIV-1 *pol* primers or HIV-1 LTR-*gag* primers that detect late products in reverse transcription. Two cell aliquots displayed exclusively integrated provirus, and two aliquots had both DNA forms (Fig. 2 and Table 1). After T cell activation in vitro and fractionation, all replicate cell samples containing HIV-1 genome displayed exclusively integrated provirus (Fig. 2 and Table 1). Thus, a large proportion of HIV-1 genome in asymptomatic individuals exists as full-length, extrachromosomal DNA, which retains the ability to integrate upon activation of the host cell.

To provide additional verification for the presence of full-length, extrachromosomal

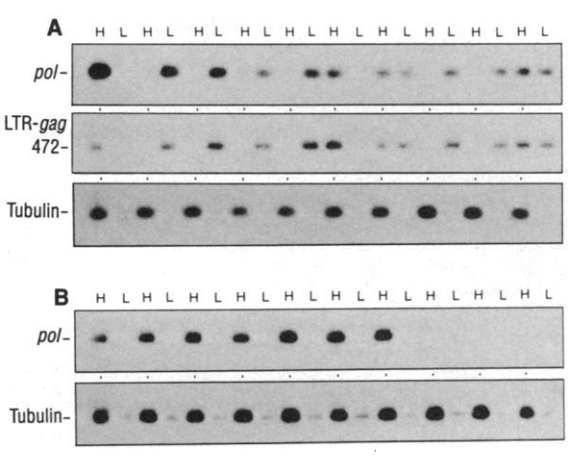

Fig. 2. Arrangement of HIV-1 DNA before and after T cell activation in vitro. Replicate macrophage-depleted cell samples (400 cells per sample) were prepared from lymphocyte cultures of patient B4 (asymptomatic) before (**A**) and after (**B**) activation of the culture in vitro with PHA. Presence of HIV-1 DNA in high and low molecular weight fractions was determined by PCR with primers specific for HIV-1 *pol* or primers directed to a region spanning the 5′ LTR-*gag* terminus (*25*). The separation of genomic DNA in high and low molecular weight fractions was confirmed using α-tubulin–specific primers.

HIV-1 DNA with quiescent PBLs, we isolated total cellular DNA from HLA-Dr–depleted lymphocytes of AIDS patients B28 and B29 and analyzed this DNA with a ligation-mediated PCR protocol (*21*). The method was adopted so that free blunt 3′ LTR termini could be identified within enriched quiescent T lymphocytes of HIV-1–infected individuals. The blunt 3′ LTR terminus, which comprises full-length (−) strand (complementary to genomic viral RNA) and (+) strand (same polarity as genomic viral RNA) viral DNA, exists only after completion of reverse transcription (*22*). The presence of free HIV-1 DNA termini in cells infected with an integration-defective HIV-1 mutant (HIV-1 ΔIN) was evident (Fig. 3C, upper panel), whereas no amplification products, even after two rounds of amplification (Fig. 3C, lower panel), were detectable in DNA isolated from 8E5 cells, which contain one integrated provirus per cell and completely lack extrachromosomal HIV-1 DNA (*13*). Integrated provirus and circular forms of unintegrated HIV-1 DNA (containing one or two LTRs) (*23*) are not detected by this approach because these viral DNA forms do not provide free 3′ LTR termini for attachment of the common linker that is required for subsequent PCR amplification (Fig. 3). Application of this ligation-mediated PCR method to DNA from HLA-Dr–depleted lymphocytes from patients B28 and B29 confirmed the presence of complete HIV-1 (−) strand cDNA terminating at nucleotide 9718 (Fig. 3A, step 1), as evidenced by the amplification of a 155–base pair (bp), HIV-1 LTR terminus–specific product (Fig. 3D). Use of HIV-1–specific primers directed to the 5′ LTR-*gag* junction demonstrated the abundance of HIV-1 DNA in each sample analyzed (Fig. 3B, upper panel). Sequence analysis of ligation-mediated PCR products (two rounds of amplification) by means of the nested LTR R primer (Fig. 3A, step 4) confirmed the amplification of complete 3′ LTR ends containing a GCAGT terminus (*19*). In a second series of PCR reactions, double-stranded DNA extracted from HLA-Dr–depleted lymphocytes from patients B28 and B29 was ligated directly to the common linker as in Fig. 3A, step 3 (essentially bypassing DNA denaturation and primer extension steps in Fig. 3A, steps 1 and 2). PCR amplification with the nested R primer and longer oligomer of the common linker resulted in amplification of a 155-bp product for DNA from both patients' lymphocytes and from $CD4^+$ cells infected with the HIV-1 integrase mutant, but not for 8E5 cell DNA (Fig. 3C). This evidence points to the presence of full-length (+) strand in extrachromosomal HIV-1 DNA in quiescent T lymphocytes of infected individuals.

Our results demonstrate the existence of a quiescent T cell reservoir where HIV-1 integration is restricted and HIV-1 DNA is harbored in an extrachromosomal state. The demonstration that a large fraction of HIV-1 DNA can exist in an extrachromosomal state in infected individuals is in agreement with other reports (*24*). However, the results presented in this study attribute the extrachromosomal DNA pool in HIV-1–infected individuals to infection of quiescent T cells, restricted integration, and establishment of a latent and inducible HIV-1 reservoir. Because T cell activation is a prerequisite for viral DNA integration, the contribution of factors affecting T cell activation (mitogenic agents, opportunistic infection) to reactivation of extrachromosomal HIV-1 DNA forms thus becomes apparent.

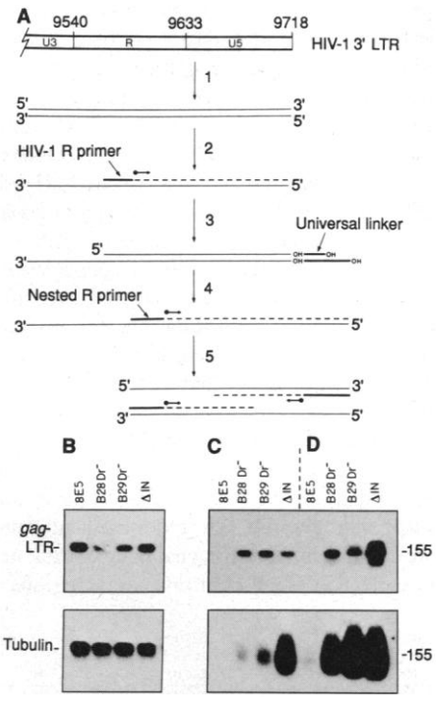

Fig. 3. Ligation-mediated PCR analysis of 3′ LTR termini in enriched quiescent lymphocyte populations. (**A**) The ligation PCR protocol was essentially as described (*21*). A representation of the HIV-1 3′ LTR and locations of the U3-R, and R-U5 junctions (*26*) are shown at the top of the figure. Total cellular DNA from HLA-Dr–depleted quiescent lymphocytes of HIV-1–infected individuals is denatured by alkali (1) and annealed to an HIV-1 R-specific LTR primer (2) complementary to the (−) strand of the HIV-1 LTR. DNA polymerase extension from the R primer (2) results in formation of double-stranded blunt-end 3′ LTR terminus, the end of which is defined by the U5 terminus. The newly created blunt-end 3′ U5 LTR terminus provides a substrate for ligation of a universal linker (3). The composition of the universal linker is as described (*21*). Annealing and extension from the R-specific LTR primer results in a blunt-end LTR terminus only in linear extrachromosomal HIV-1 DNA forms. A second polymerase extension step (4) from a nested (−) strand R-specific primer results in formation of a new (+) strand that is complementary to the LTR (−) strand and that incorporates the sequence of the longer oligomer component of the universal linker. The resultant double-stranded products provide suitable substrates for PCR (5) by means of the nested R-specific LTR primer and the longer oligomer component of the universal linker. The expected PCR product size of 155 bp includes 130 bp of HIV-1 LTR [extending from nucleotide 9591 (*26*) at the HIV-1 R-specific primer binding site to the 3′ GCAGT terminus of U5 at nucleotide 9720] and 25 bp of incorporated common linker sequence. (**B** through **D**) PBLs from two HIV-1–infected individuals with ARC (B28 and B29) were depleted of macrophages and activated T cells by magnetic affinity sorting with HLA-Dr antibody–conjugated magnetic particles (*27*). Total cellular DNA from the HLA-Dr^- lymphocyte population was subject to direct PCR (B) with primers to the HIV-1 5′ LTR-*gag* junction (upper panel) and to the human α-tubulin gene (lower panel) or to a ligation-mediated PCR approach (C and D), as outlined in (A), using a single round (upper panel) or double round (lower panel) of PCR. In (D), samples were processed exactly as outlined in (A), in that the substrate for ligation of the universal linker was provided after DNA denaturation and polymerase extension from an annealed HIV-1 R-specific primer. In a second series of reactions (C), lymphocyte DNA was ligated directly to the universal linker, essentially bypassing the DNA denaturation and primer extension steps [steps 1 and 2 in (A)]. 8E5 is a human $CD4^+$ T cell line that contains one integrated noninfectious HIV-1 provirus per cell and completely lacks extrachromosomal HIV-1 DNA (*13*). ΔIN represents DNA from $CD4^+$ lymphocytes infected with an integration minus mutant of HIV-1 (*6, 14*).

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12. All patients from whom PBLs were derived for this study were registered in the Clinical AIDS Unit at the University of Nebraska Medical Center, and only patients not undergoing azidothymidine (AZT) treatment were recruited. CD4 and CD8 cell counts were determined at the time blood was drawn for analysis. Isolation of PBL and depletion of monocytes and macrophages was performed essentially as described (*6*). Analysis and quantitation of PCR-amplified products was as described (*6*). The autoradiographic signal of PCR-amplified products from doubling dilutions of DNA from patient lymphocytes was compared with that of PCR-amplified products from doubling dilutions of DNA extracted from 8E5 cells that contain one noninfectious HIV-1 provirus per cell (*13*). HIV-1 DNA copy number in each patient was quantitated after normalization of the autogradiographic signal of HIV-1–specific products to those generated from PCR amplification of primers specific for α-tubulin on parallel dilutions of 8E5 and patient lymphocyte DNA. After quantitation of proviral DNA copy number, lymphocytes were distributed in multiple (10 to 40) aliquots, each of which contained sufficiently few lymphocytes so that there was not more than one infected cell in each aliquot. Typically for asymptomatic individuals, each aliquot contained 400 to 1000 cells, whereas for AIDS patients each aliquot contained 50 to 400 cells. Total cellular DNA was extracted from each aliquot, mixed with purified carrier salmon sperm DNA (1 μg) and resolved on 0.8% low gelling temperature agarose–tris borate gels at 2.5 V/cm for 10 to 14 hours. After electrophoresis, DNA was visualized by ethidium bromide staining. Gel regions containing DNA of 4 to 15 kb (low molecular weight fraction) and over 15 kb (high molecular weight fraction) were excised. Each gel fragment was melted at 68°C, and DNA was extracted and purified as described elsewhere (*6*). Purified DNA from each high and low molecular weight fraction was pelleted, dried, and resuspended in 10 μl H_2O for analysis by PCR.
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25. PCR was performed essentially as described (*6*), with the following modifications. PCR was performed in a reaction volume of 25 μl, and each cycle of amplification comprised a 30-s denaturation step (95°C), a 30-s annealing step (56°C), and a 1-min extension step (72°C). In addition, there was one 5-min step at 72°C at the end of each series of cycles to ensure complete extension of amplified DNA. The sequences of all primers used in this study are available from M. Stevenson on request.
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Stereospecific Effects of Inhalational General Anesthetic Optical Isomers on Nerve Ion Channels

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Although it is generally agreed that general anesthetics ultimately act on neuronal ion channels, there is considerable controversy over whether this occurs by direct binding to protein or secondarily by nonspecific perturbation of lipids. Very pure optical isomers of the inhalational general anesthetic isoflurane exhibited clear stereoselectivity in their effects on particularly sensitive ion channels in identified molluscan central nervous system neurons. At the human median effect dose (ED_{50}) for general anesthesia, the (+)-isomer was about twofold more effective than the (−)-isomer both in eliciting the anesthetic-activated potassium current $I_{K(An)}$ and in inhibiting a current mediated by neuronal nicotinic acetylcholine receptors. For inhibiting the much less sensitive transient potassium current I_A, the (−)-isomer was marginally more potent than the (+)-isomer. Both isomers were equally effective at disrupting lipid bilayers.

THE POSSIBILITY THAT OPTICAL ISOMERS of inhalational general anesthetics might display differential biological activities has generally been discounted because of the traditional view that general anesthetics act by nonspecific perturbation of lipid membranes (*1–3*). However, accumulating evidence (*1, 3*) suggests that these "nonspecific" agents may act by binding directly to particularly sensitive protein targets in the central nervous system (CNS). We have observed stereoselective effects of the optical isomers of the widely used inhalational general anesthetic isoflurane on neuronal ion channels, which are generally accepted as the most likely targets for these agents.

Very recently, extremely pure optical isomers of isoflurane (Fig. 1A) were prepared, and the enantiomers of halothane, enflurane, and isoflurane were resolved with the use of capillary columns (*4*). We found differential effects of the two isomers of isoflurane in activating the anesthetic-activated potassium current $I_{K(An)}$ in a previously described (*5*) anesthetic-sensitive neuron in the right parietal ganglion of *Lymnaea stagnalis* (Fig. 1B). This neuron was impaled with two electrodes and then completely removed from the ganglion (*6*), which facilitated rapid exchange of solutions and ensured that the effects of anesthetic were on the neuron itself rather than on the surrounding nerve network. The data in Fig. 1B show that when the cell was voltage-clamped, the $I_{K(An)}$ current induced by bath application of (+)-isoflurane was about twofold greater than that induced by (−)-isoflurane (7). This effect was invariably observed and appeared to be independent of anesthetic concentration in the range of isoflurane partial pressures from 0.006 to 0.031 atm, which corresponds to 0.5 to 2.4 times the human minimum alveolar concentration (MAC) of 0.013 atm (*8*). The ratio I^+/I^- of currents induced by the (+)-isomer and the (−)-isomer, respectively, averaged 2.01 ± 0.04 (mean ± SEM, n = 34). The current induced by the racemic mixture was intermediate between those induced by the individual isomers. The current-voltage (I-V) curves for the current activated by each of the enantiomers at a partial pressure of 0.025 atm are shown in Fig. 1C. These curves demonstrate that the differential effects are essentially independent of membrane potential.

The (+)-isomer of isoflurane is also more effective than the (−)-isomer at inhibiting currents (Fig. 2A) induced by the bath application of 200 nM acetylcholine (ACh). For these experiments, we used isolated identified neurons (*9*) from the right parietal ganglion that were very sensitive to ACh but relatively insensitive to isoflurane alone. The neurons were nonetheless clamped at a potential in the range from −70 to −80 mV (near the K^+ reversal potential), which minimized the effect of anesthetic-activation of $I_{K(An)}$, and a low concentration of ACh was used to reduce desensitization. This ACh-induced current had characteristics of a cur-

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