Integrase Inhibitors and Cellular Immunity Suppress Retroviral Replication in Rhesus Macaques

Daria J. Hazuda *et al.*

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attained by hybridization of total genomic DNA to an array of BACs. Thus, ROMA has additional advantages even compared with arrays with "complete" coverage of the genome, such as the 32,000-probe tiling-path BAC array (*28*). Further developments of ROMA are under way, including a 380,000-probe microarray, which promise to reveal a great deal more about large-scale polymorphism in the human genome.

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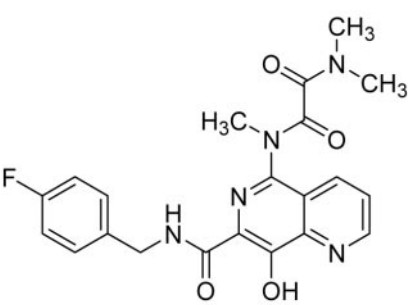
Integrase Inhibitors and Cellular Immunity Suppress Retroviral Replication in Rhesus Macaques

Daria J. Hazuda,[1]* Steven D. Young,[2]* James P. Guare,[2] Neville J. Anthony,[2] Robert P. Gomez,[2] John S. Wai,[2] Joseph P. Vacca,[2] Larry Handt,[3] Sherri L. Motzel,[3] Hilton J. Klein,[3] Geethanjali Dornadula,[1] Robert M. Danovich,[1] Marc V. Witmer,[1] Keith A. A. Wilson,[4] Lynda Tussey,[4] William A. Schleif,[4] Lori S. Gabryelski,[4] Lixia Jin,[5] Michael D. Miller,[1] Danilo R. Casimiro,[4] Emilio A. Emini,[4] John W. Shiver[4]

We describe the efficacy of L-870812, an inhibitor of HIV-1 and SIV integrase, in rhesus macaques infected with the simian-human immunodeficiency virus (SHIV) 89.6P. When initiated before CD4 cell depletion, L-870812 therapy mediated a sustained suppression of viremia, preserving CD4 levels and permitting the induction of virus-specific cellular immunity. L-870812 was also active in chronic infection; however, the magnitude and durability of the effect varied in conjunction with the pretreatment immune response and viral load. These studies demonstrate integrase inhibitor activity in vivo and suggest that cellular immunity facilitates chemotherapeutic efficacy in retroviral infections.

The substantial incidence of resistance observed in therapy-experienced patients and newly acquired HIV-1 infections (*1*–*5*) underscores the need for new antiretroviral agents, as well as the importance of maximizing the durability of available therapies. All oral agents licensed to treat HIV-1 disease target two of the three essential, virally encoded enzymes, reverse transcriptase and protease (*6*–*8*). The third HIV-1 enzyme, integrase, inserts the viral DNA into the cellular genome through a multistep process that includes two catalytic reactions: 3′ endonucleolytic processing of the viral DNA ends and strand transfer or joining of the viral and cellular DNAs (*9*, *10*). Compounds that selectively inhibit strand transfer have provided proof of concept for integrase as a chemotherapeutic target for HIV-1 infection in vitro (*11*). In this investigation we used a novel strand-transfer inhibitor, L-870812 (*12*) (Fig. 1), which exhibits potent antiviral activity in vitro against both HIV-1 and the simian lentivirus, SIV [95% inhibition concentration (IC_{95}) of 250 and 350 nM, respectively, in 50% human and rhesus serum] and favorable pharmacokinetics in rhesus macaques [oral bioavailability = 64% and half-time ($t_{1/2}$) = 5 hours] to assess the efficacy of such inhibitors in vivo. The studies were designed to evaluate integrase inhibitors as a new class of antiretroviral agents and to examine the role of viral-specific cellular immunity in chemotherapeutic intervention using SHIV 89.6P-infected rhesus macaques as an experimental model of early- and late-stage retroviral infection.

Rhesus macaques infected with SHIV 89.6P exhibit an atypical, accelerated disease marked by a profound depletion of CD4 cells concomitant with progression from acute viremia to a chronic phase at about 2 weeks after infection



L-870812

Fig. 1. The structure of L-870812, a napthyridine carboxamide that inhibits the strand-transfer activity of recombinant HIV and SIV integrase in vitro (IC_{50} = 40 nM).

[1]Department of Biological Chemistry, [2]Department of Medicinal Chemistry, [3]Department of Laboratory Animal Research, [4]Department of Vaccine Research, [5]Drug Metabolism and Pharmaceutical Research, Merck Research Laboratories, Post Office Box 4, West Point, PA 19486, USA.

*To whom correspondence should be addressed. E-mail: steve_young@merck.com, daria_hazuda@merck.com

(*13*). To evaluate integrase inhibitor chemotherapy in the context of early or late disease, this study included a cohort that initiated therapy with L-870812 before virus-mediated CD4 cell depletion (on day 10 after infection) and a second cohort in which therapy was delayed until the chronic phase was well established at day 87. Animals were treated for 77 and 45 days,

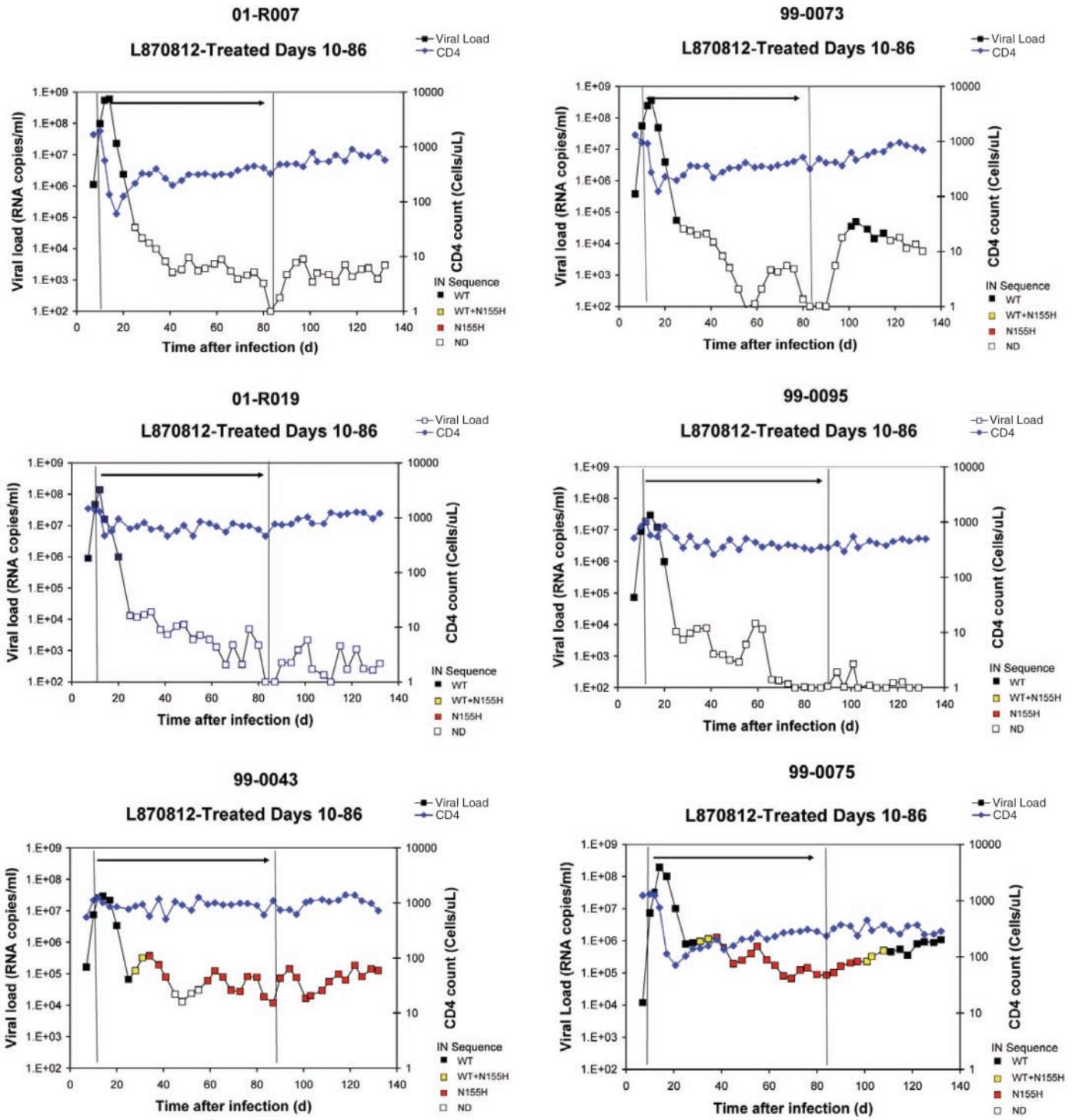


Fig. 2. Early intervention study with L-870812. Animals were infected intravenously with 50 monkey infectious doses (50 MID50) of cell-free SHIV-89.6P (*13*). In the early intervention cohort, the animals initiated therapy on day 10 and were dosed twice daily with 10 mg/kg L-870812 orally as a suspension in aqueous methyl cellulose to achieve a plasma concentration at 12 hours of about twice that required for 95% inhibition of viral replication in vitro measured in the presence of 50% rhesus monkey serum. After 4 weeks, i.e, a time experimentally determined to achieve a sustained antiviral affect, the animals were switched to a once-daily dose of 20.0 mg/kg L-870812 for convenience. Animals were maintained on L-870812 monotherapy until day 87 after infection and monitored to day 132. Plasma vRNA was measured with real-time quantitative polymerase chain reaction (*19*). CD4 cell counts (blue diamonds) and viral loads (black squares) for the individual animals are plotted in each panel. Results of the integrase sequence determinations in animals with measurable viral loads are shown as colored symbols [black, wild-type integrase; yellow, mixture of wild-type and N155H integrase; red, N155H integrase; white, not determined (ND)].

respectively, and monitored for plasma viral RNA (vRNA), CD4 cell counts, viral-specific cellular immune responses, drug exposure, and resistance.

As anticipated for this aggressive model of SIV infection, the peak of plasma viremia occurred at about day 14 after infection with a maximum titer of 10^7 to 10^9 copies of vRNA per milliliter of plasma (Figs. 2 and 3). This was followed by a decline in circulating vRNA and CD4 cell levels. In four of the six untreated animals, CD4 cells declined to less than 10 cells per microliter of plasma within 2 to 3 weeks (Fig. 3). In these animals, plasma vRNA levels during the subsequent chronic phase ranged between 1×10^4 and 5×10^6 copies/ml, with an average of 2×10^6 copies/ml. In contrast, animals treated with L-870812 starting at day 10 after infection exhibited either a minimal or a transient decrease in circulating CD4 cells, which recovered and stabilized above 200 cells/μl throughout the treatment period (at day 55, average CD4 cell count = 602, range = 261 to 1267; Fig. 2). Moreover, in four of the six

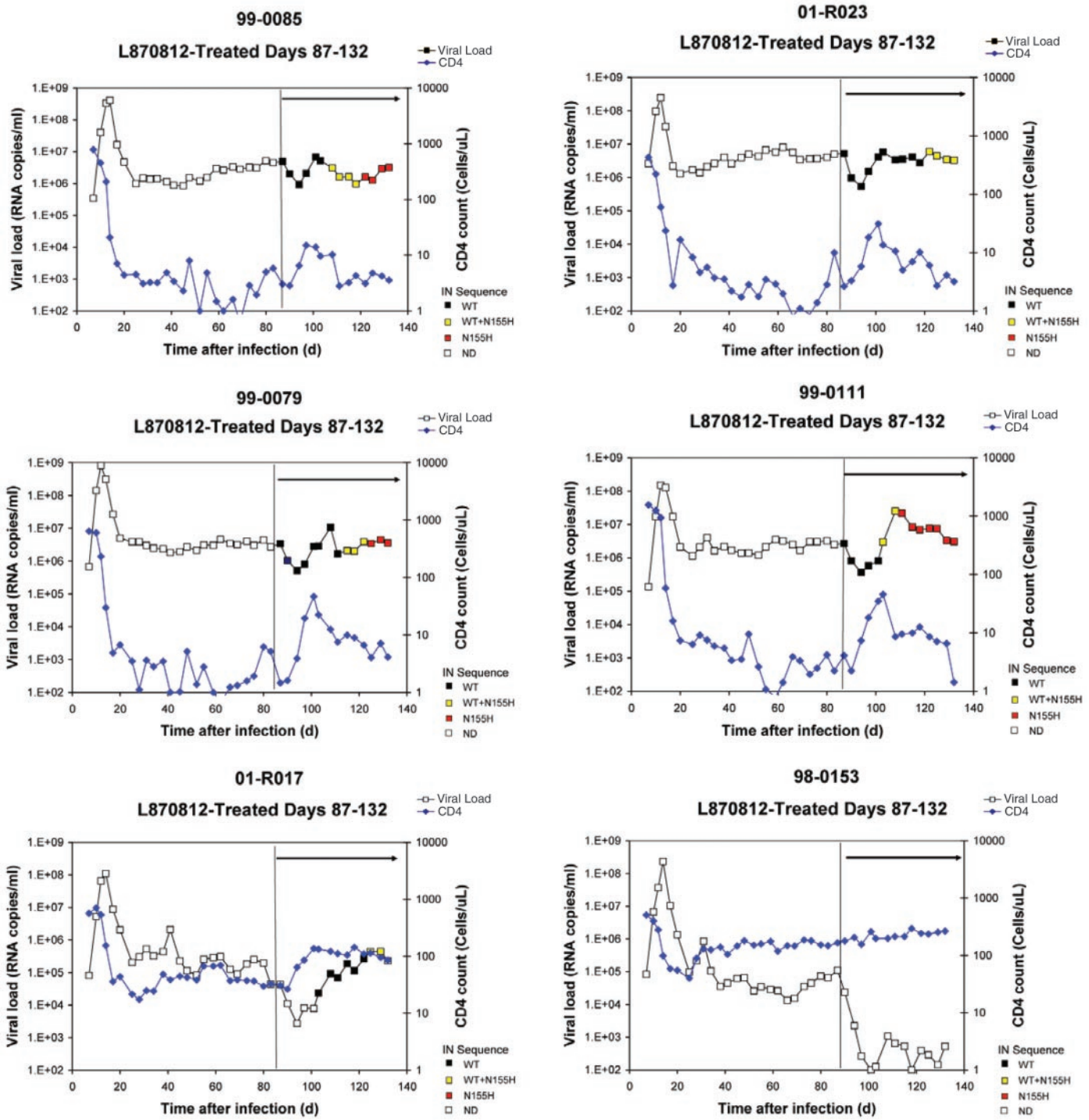


Fig. 3. Delayed treatment study with L-870812. In the late treatment cohort, L-870812 therapy was initiated on day 87 after infection as described in Fig. 2, and the animals were treated for 45 days. CD4 cell counts (blue diamonds) and viral loads (black squares) are shown for individual animals in each panel. Results of integrase sequence determinations are shown as colored symbols [black, wild-type integrase; yellow, mixture of wild-type and N155H integrase; red, N155H integrase; white, not determined (ND)].

animals, vRNA was suppressed to undetectable levels (less than 0.25×10^2 vRNA copies/ml), representing a decrease of more than four orders of magnitude relative to the untreated cohort (Fig. 2). Although the remaining two animals treated with L-870812 (99-0075 and 99-0043) did not achieve undetectable suppression, they exhibited little or no decline in CD4 cell count and maintained viral loads that were 10- and 100-fold below the average of the untreated controls during this period (3×10^4 copies/ml and 1×10^5 copies/ml, respectively).

The four animals in the early treatment cohort with undetectable viremia maintained a plasma level of L-870812 above 1.0 μM at 10 hours after dosing, as compared with 0.7 μM and 0.6 μM in animals with measurable vRNA. Because suboptimal exposure can select resistant variants, resistance to L-870812 was evaluated by sequencing the integrase coding region from treated and untreated animals with detectable viremia and by culturing virus from peripheral blood mononuclear cells (PBMCs) isolated at selected time points in the presence or absence of the inhibitor. In untreated animals, the integrase sequence remained identical to that of the challenge virus at all time points, whereas in the two treated viremic animals (99-0075 and 99-0043), a mutation at position 155 (substitution of asparagine with histidine, N155H) was detected beginning at days 28 and 32. The appearance of N155H as the predominant species on days 35 and 38 was not associated with a rebound in vRNA (Fig. 2), and there was no decline in CD4 cell count in these animals despite ongoing viral replication. In cocultures with PBMCs from treated and untreated animals isolated on days 25, 38, 80, and 84, virus was recovered from samples maintained in the absence of L-870812, but not when the inhibitor was present. In each case, the integrase sequence of the recovered viruses was wild type; this included cocultured isolates obtained from 99-0075 and 99-0043 at time points when N155H was the dominant species. When engineered into HIV-1 HXB2, the N155H mutation conferred a 25× loss of susceptibility to L-870812 and a 75% reduction in specific infectivity. These results suggest that compromised replication of the N155H mutant may contribute to the lower viral loads in viremic animals maintained on L-870812 and are consistent with the observation that discontinuation of therapy in 99-0075 resulted in a 10-fold increase in vRNA concomitant with conversion of the virus population to the wild-type integrase sequence on day 110 (Fig. 2). Although it is not known whether continued replication could select for compensatory mutations, none were observed within the time frame of the current study.

To study the effect of chemotherapy in later disease, we administered L-870812 to the untreated cohort starting on day 87 after infection (Fig. 3). At the time of initiating therapy, this group had an average viral load of 2×10^6 copies/ml and an average CD4 cell count of 35 cells/μl, with four animals having counts below 10 cells/μl. After 10 days of therapy with L-870812, a 10- to 100-fold reduction in vRNA was observed in all animals. CD4 cell counts were increased from 3 to 20 cells/μl within 14 days in the most immunosuppressed animals (99-0085, 01-R023, 99-0079, and 99-0011) and from 150 to 200 cells/μl in the least immunocompromised animal (98-0153). Although a therapeutic effect was apparent in all animals within this cohort, in contrast to the early treatment group, the subsequent durability of the response was variable and coincident with the respective pretreatment immune status and viral load of each subject. For example, vRNA was reduced by greater than 100-fold and remained below the assay limit of detection in 98-0153, which initiated therapy with the highest CD4 count and lowest viremia. In contrast, the four animals with the lowest CD4 counts and highest viral load exhibited an average 10-fold decrease in vRNA and rebound in viremia within 2 weeks. The sequence of integrase at rebound (days 105, 107, and 110) remained wild type (Fig. 3), suggesting that the apparent increase in viremia may have resulted from altered replication dynamics due to the expansion of CD4 target cells. Although the animals that experienced incomplete suppression subsequently selected for the N155H mutation within 25 days, as in the early treatment cohort, the appearance of this mutation was not accompanied by a rebound in vRNA.

The marked differences in the both the magnitude and sustained nature of the chemotherapeutic responses observed in the two treatment cohorts were also associated with significant differences in viral-specific cellular immunity (Table 1). L-870812 treatment initiated during

Table 1. Antiviral cellular immune responses in SHIV-infected monkeys.

Animal	IFN-γ ELISPOT*		IFN-γ ICS†				CM9 tetramer levels‡
			Gag		Nef		
	Gag	Nef	$CD3^+CD8^+$	$CD3^+CD4^+$	$CD3^+CD8^+$	$CD3^+CD4^+$	
L-870812-treated							
01-R019	**64**	**155**	0	173	0	233	ND
01-R007	48	**258**	362	129	170	126	ND
00-0043	**394**	**429**	**887**	**679**	45	**2176**	ND
99-0073	**1176**	**1263**	**1083**	**319**	**677**	**357**	1.93%
99-0095	**361**	**601**	**416**	**482**	0	262	ND
99-0075	**1203**	**440**	**3227**	**614**	0	**421**	2.22%
Untreated control							
01-R017	**76**	34	23	20	95	149	ND
99-0111	1	33	50	0	58	4	ND
98-0153	**753**	**621**	**1729**	**312**	64	120	1.69%
99-0079	3	**81**	0	0	160	113	ND
99-0085	30	**156**	**336**	0	183	52	0.22%
01-R023	20	18	36	0	0	160	ND

*Determinations were made using PBMC samples collected on day 41 after infection. Values are reported as the mock-corrected numbers of IFN-γ spot-forming cells (SFCs) per million PBMCs (*18*). Responses were individually measured against the complete gag and nef peptide pools. Positive responses are shown in bold type as being >50 SFCs per million PBMCs and are at least four times greater than mock levels. No responses to the viral tat protein were noted in any of the animals. Differences between the responses of both groups to nef and gag were evaluated by single-factor analyses of variance of the logarithmic values ($P < 0.0159$ and 0.0411, respectively). †PBMCs collected at day 52 after infection were analyzed by IFN-γ ICS (intracellular cytokine staining). Mock-corrected values are reported individually for the number of $CD3^+CD8^+$ or $CD3^+CD4^+$ cells per million lymphocytes. Positive responses to a pool are shown in bold type as being >300 per million lymphocytes and being three times greater than mock levels (*18*). Cohort differences in the levels of $CD4^+$-type responses to gag and nef were statistically significant ($P < 0.0017$ and 0.0309, respectively). ‡A specific major histocompatibility complex (MHC) class I tetramer reagent was used to quantitate the levels of $CD3^+CD8^+$ cells expressing T cell receptors for the immunodominant gag CM9 determinant restricted by the rhesus MHC class I allele MamuA*01. Only animals expressing MamuA*01 were analyzed. Values at day 41 after infection are expressed as % of $CD3^+CD8^+$ cells that are CM9-positive; preinfection levels have been subtracted. ND, not determined.

early infection preserved CD4 cell counts and permitted the induction of persistent SHIV-specific cellular immune responses. Stronger responses against gag and nef proteins were observed in the early treatment cohort and were evident as early as day 25 after infection (gag, $P < 0.033$; nef, $P < 4 \times 10^{-5}$). These responses involved both $CD8^+$ and $CD4^+$ T cells (Table 1) and likely contributed to the robust antiviral responses observed during L-870812 treatment and to the control of virus replication after the discontinuation of therapy (Fig. 2). Neutralizing antibodies were minimal throughout the experimental period (*14*).

Therapy with L-870812 in the delayed treatment cohort did not either induce or increase cellular immune responses. However, the animals (01-R017 and 98-1053) that expressed the best outcomes both before and during therapy exhibited preexisting SHIV-specific cellular immune responses that correlated with their respective response to treatment (Fig. 3 and Table 1). Animal 98-0153 initiated therapy with the highest CD4 cell count (150 cells/μl) and SHIV-specific cellular immune response and was the only animal in the group to achieve and maintain vRNA below 10^2 copies/ml. Conversely, 99-0111, 99-0079, 99-0085, and 01-R023 did not develop virus-specific cellular immunity and had the least durable response to L-870812. The correlation of the overall apparent efficacy of L-870812 treatment between the two cohorts and among animals within the delayed treatment group with viral-specific cellular immune responses suggests that cellular immunity may facilitate the control of retroviral replication mediated by an effective chemotherapeutic agent such as L-870812.

Integrase inhibitors represent a new class of agents to treat HIV-1 infection in therapy-naïve patients and patients harboring viruses resistant to current antiretroviral agents. The antiviral activity of L-870812 in SHIV-89.6P–infected rhesus macaques provides evidence of in vivo efficacy for integrase inhibitors in retroviral infections, demonstrating that integrase inhibitors can be engineered with the appropriate properties required for an effective therapy to treat chronic HIV-1 infection. Oral administration of L-870812 was well tolerated, and the animals in this study exhibited no clinical signs of toxicity. In animals with detectable viremia, chronic treatment with L-870812 selected a viral variant with an integrase mutation (N155H) that exhibited lower replication capacity and reduced pathogenicity, supporting observations from in vitro studies with resistant isolates selected with related inhibitors that have suggested that integrase inhibitors may present a high genetic barrier to resistance development (*11*, *15*). Consistent with studies of human acute seroconverters and antiretroviral therapy in early HIV-1 disease (*16*, *17*), L-870812 therapy was most effective in animals that initiated treatment early in infection. Early treatment with L-870812 permitted the induction of substantial antiviral-specific cellular immunity. Immune status and viral load also affected the overall response to L-870812 in animals initiating therapy later in infection. These studies therefore suggest that cellular immunity may facilitate the durability of antiretroviral chemotherapy and provide evidence to support the use of vaccines to enhance and sustain the efficacy of antiretroviral therapy as well as the reevaluation of early intervention strategies for treating HIV-1 infection with improved antiviral regimens.

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Acquired Dendritic Channelopathy in Temporal Lobe Epilepsy

Christophe Bernard,[1,3]* Anne Anderson,[1,2] Albert Becker,[4] Nicholas P. Poolos,[1,6] Heinz Beck,[5] Daniel Johnston[1]

Inherited channelopathies are at the origin of many neurological disorders. Here we report a form of channelopathy that is acquired in experimental temporal lobe epilepsy (TLE), the most common form of epilepsy in adults. The excitability of CA1 pyramidal neuron dendrites was increased in TLE because of decreased availability of A-type potassium ion channels due to transcriptional (loss of channels) and posttranslational (increased channel phosphorylation by extracellular signal-regulated kinase) mechanisms. Kinase inhibition partly reversed dendritic excitability to control levels. Such acquired channelopathy is likely to amplify neuronal activity and may contribute to the initiation and/or propagation of seizures in TLE.

Epileptic discharges correspond to highly synchronized and high-frequency activity of neurons. An augmented neuronal input-output relation is thought to be at the core of this increased excitability (*1*). Changes in the input-output relation of neurons may be caused by plastic changes on the synaptic level as reported in human and experimental epilepsy (*2*–*4*). Far less is known about changes of ion channels in the neuronal membrane, even though they control synaptic integration and intrinsic neuronal excitability. A number of rare genetic epilepsy syndromes are linked to mutations of ion channels (*5*), and acquired changes of somatic ion channels can occur after seizures (*6*, *7*). The fate of dendritic ion channels, however, has remained an enigma. As the main site for synaptic integration, neuronal dendrites could play a key role in seizure initiation and propagation (*8*–*10*). Dendrites contain a very high density of ion channels that can be targeted by antiepileptic drugs (*11*, *12*). Through their action on back-propagating action potentials (b-APs) (*13*, *14*), A-type K^+ channels are crucial modulators of information processing and synaptic plasticity in the dendrites. A reduction in A-type K^+ channel activity increases b-AP amplitude and promotes burst firing in a

[1]Department of Neuroscience, [2]Department of Pediatrics and Department of Neurology, Baylor College of Medicine, Houston, TX 77030, USA. [3]INSERM U29, 163 Route de Luminy BP13, 13273 Marseille Cédex 09, France. [4]Department of Neuropathology, [5]Department of Epileptology, Laboratory of Experimental Epileptology, University of Bonn Medical Center, Sigmund-Freud Strasse 25, 53105 Bonn, Germany. [6]Department of Neurology and Regional Epilepsy Center, University of Washington, Box 359745, 325 9th Avenue, Seattle, WA 98104, USA.

*To whom correspondence should be addressed. E-mail: cbernard@inmed.univ-mrs.fr