

MINIREVIEW

Defective Virus Drives Human Immunodeficiency Virus Infection, Persistence, and Pathogenesis

Diana Finzi, Susan F. Plaeger, and Carl W. Dieffenbach*

Basic Sciences Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland

Many aspects of the human immunodeficiency virus (HIV) life cycle are well understood, yet a key paradox remains. The primary targets for HIV are resting CD4 T lymphocytes that are not permissive to HIV replication unless activated by some external and independent event. Numerous mechanisms for this essential cellular activation step have been proposed, including coinfections, vaccination, cytokines, and endogenous microbial flora. We propose that a review of the scientific evidence reveals a simple and direct source of CD4 cell activation: HIV itself.

During HIV infection, the great majority of virus produced is noninfectious, i.e., “defective,” primarily as a result of the error-prone process of reverse transcription. Although noninfectious, this defective virus is far from innocuous. We propose that defective HIV particles provide a solution to this paradox, playing a specific, integral role in the HIV life cycle and in driving pathogenesis by (i) preferentially activating CD4 T cells, rendering them permissive for productive HIV replication, and (ii) providing a large pool of constantly changing HIV peptides that are presented on major histocompatibility complex (MHC) class II molecules to continuously stimulate resting CD4 T cells of different antigen specificities. Here we review the evidence for the central role of defective virus in fanning the flames of initial infection and aiding and abetting infectious HIV in its progression to late-stage disease.

HIV, RESTING CD4 T CELLS, AND THE IMPORTANCE OF T-CELL ACTIVATION

Most of the CD4 T cells in the body are resting cells waiting to be activated by a specific or nonspecific immunologic stimulus. Although HIV enters and undergoes reverse transcription in the cytoplasm of resting CD4 T cells, the preintegration complex (PIC) is degraded and the cell is “cured” unless the infected, resting CD4 T cell is activated within a few days (11, 14, 57, 89, 90, 93). The exact mechanisms involved in this transient, dead-end infection are not clearly understood but are thought to include actions of APOBEC (apolipoprotein B-editing catalytic subunit) or blocks in reverse transcription or in nuclear import of the HIV PIC. Interestingly, quantitative measurements of HIV have shown that the inactive, labile

form of HIV DNA represents the most common form of the virus (4, 30, 69, 71), and roughly 1% of all resting CD4 T cells in an infected individual harbor unintegrated DNA (15).

To become permissive for HIV replication, a CD4 T cell carrying a replication-competent PIC must be activated. With cellular activation, the HIV PIC is imported into the nucleus, and the HIV life cycle continues with production of progeny virus (11, 76, 89, 90). Specific activation of CD4 T cells occurs via presentation of peptide antigen in the context of MHC class II molecules on dendritic cells or other antigen-presenting cells (APCs). CD4 T-cell proliferative responses to HIV-specific proteins are present in early infection (66) and can also be induced *in vitro* by antigen-loaded dendritic cells (81). Additionally, R5 and X4 envelopes can activate resting CD4 cells through a mechanism that is dependent upon coreceptor engagement (16). Nonspecific activation can occur when CD4 T cells are stimulated by cytokines in the local environment; tumor necrosis factor, interleukin-2 (IL-2), IL-4, IL-7, IL-15, and other proinflammatory cytokines have been shown to activate resting CD4 T cells (18, 20, 78). When activated, CD4 T cells undergo several rounds of cell division and clonal expansion (9, 74) and become highly susceptible to infection by exogenous HIV. Infected cells are usually killed by cytotoxic CD8 T lymphocytes (CTL), by apoptosis, or by the cytopathic effects of infection. Occasionally, activated infected cells survive infection and become long-lived, latently infected cells. Reactivation of resting memory cells with latent proviral HIV has also been shown to result in active virus replication (15).

DEFECTIVE VIRUS PREFERENTIALLY DRIVES THE MHC CLASS II (CD4 T-CELL) RESPONSE

In most viral illnesses, the immune system clears the infection and develops immunological memory to prevent recurrence of disease. HIV defeats this process and appears to evade immunologic control by continuously evolving to escape the CTL response (2, 8, 24, 55, 60) and neutralization by antibodies (1, 3, 64, 87). The continuous evolution of HIV, deemed useful for “immunological escape,” is sustained by a high rate of mutation and a high rate of replication. The high mutation rate is driven by the error-prone process of reverse transcription (5–7, 63) and by recombination (34, 35, 94). The end result is the generation of the large range of viral diversity and a predominance of defective virus.

Perhaps by oversight, defective virus has not been a primary focus of study. From a virologic standpoint it is noninfectious

* Corresponding author. Mailing address: Basic Sciences Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, Bethesda, MD 20892. Phone: (301) 496-9112. Fax: (301) 402-3211. E-mail: cdieffenba@mail.nih.gov.

material of no consequence, and from an immunologic standpoint it is the by-product of a viral mechanism to stay one step ahead of the cellular and humoral responses. Yet vaccine studies have shown that CD4 T cells exposed to whole killed or UV-inactivated HIV (analogous to defective virus) become activated in vitro (31, 51) and proliferate through nonspecific mechanisms (68) as well as in response to HIV peptides presented on MHC class II molecules on APCs (74). Thus, defective virus that remains in the extracellular space may be the ideal antigen for the activation of CD4 T cells.

THE MHC CLASS II RESPONSE

Peptides presented to CD4 T cells are processed from exogenous antigens (such as defective virus) that are endocytosed by the APC and degraded, and the resulting peptides are displayed on the APC surface in complex with MHC class II molecules. In contrast, CD8 T cells recognize viral peptides in the context of MHC class I molecules. Unlike the peptides that are presented on MHC class II molecules, peptides presented to CD8 T cells on MHC class I molecules are processed from endogenous viral proteins made within the infected cells. Viral proteins made within an infected cell are degraded by the proteasome, transported into the endoplasmic reticulum, and loaded onto MHC class I molecules for expression on the surface of the infected cell.

Since the primary product of HIV replication is defective virus, the bulk of which remains in extracellular spaces, the law of mass action predicts that exogenous rather than endogenously produced antigen will drive the immune response. The class II response, defined by CD4 T-cell stimulation and proliferation, provides the perfect environment for HIV. The low proportion of virus that can effectively infect cells and produce viral proteins for class I presentation cannot compete with the class II response. Consistent with HIV favoring of a CD4 T-cell response is the recent finding that CD8 T-cell responses to simian immunodeficiency virus (SIV) in acute infection are "too late and too little" compared to virus replication: Reynolds et al. showed that although CD8 T-cell responses were present, their timing and magnitude lagged behind those of peak virus production (62).

By preferential induction of the CD4 T-cell immune response, HIV can target and replicate in newly activated CD4 T cells. During primary infection, HIV-specific CD4 T cells are the first CD4 T cells to be activated and then infected. This explains why HIV-specific CD4 T-cell responses are present only early in primary infection and are not found in chronically infected, untreated individuals but can be maintained if individuals are treated with antiretroviral agents during the acute stage of disease (45, 66). Over time, HIV-specific CD4 T cells are continuously used to sustain replicating virus, resulting in the eventual absence of HIV-specific CD4 T-cell responses (40, 49, 58, 85). When infected, CD4 cells are killed directly by cytopathic effects of replicating virus and by CTL. Yet there are also important mechanisms that trigger CD4 T-cell death in activated, uninfected cells. Apoptosis of uninfected cells through Fas/Fas ligand as a mechanism of CD4 T-cell depletion has been studied extensively. Also, Herbeuval and coworkers (28) have shown that noninfectious HIV can effectively and specifically trigger CD4 T-cell apoptosis with signaling through

the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) death receptor 5 and TRAIL ligand. Thus, defective virus can drive virus production via cell activation and can drive T-cell loss by induction of activation followed by apoptosis (28). Interestingly, the lack of these apoptotic mechanisms in the sooty mangabey may be associated with protection from development of disease (see below).

As CD4 T cells that are specific for the more common HIV antigens decline over the course of disease, the HIV proteins presented change as HIV mutates. Because every CD4 T cell has its own specificity and is activated by a unique peptide, each peptide generated has the potential to activate a CD4 T cell of a different specificity. It has been shown that during chronic infection the specificities of the CD4 T cells devolve to epitopes of lower avidity and frequency (85; reviewed in reference 21). With an error rate that changes every amino acid in the HIV sequence every day (54, 73, 86) and with one in four changes being frameshift mutations (5–7, 63), defective virus continuously generates new peptides. Even a single amino acid change in peptide sequence can have a major impact on peptide-MHC binding; recent data have shown that the stability of the MHC class II-peptide complex is key to the ultimate hierarchy of the elicited CD4 T-cell response to antigen (41). Furthermore, out-of-frame, cryptic peptides made in alternative reading frames of HIV sequences (recently identified for MHC class I presentation [12]) are likely to exist and increase the repertoire of peptides for MHC class II presentation. Thus, continual generation of mutations and defective viral proteins is a means of generating new peptides that stimulate CD4 T cells of many different T-cell receptor specificities.

Recent studies provide evidence for positive selection of CD4 T-helper epitopes within the virus genome. Using a molecular genetic and statistical approach, Yang and coworkers examined evolution throughout the HIV genome and found that there was widespread adaptive evolution, with a significant number of CD4 T-helper epitopes under positive selection ($P = 0.0001$) (56, 88). Though Yang and coworkers also found evidence for positive selection for CTL and antibody escape within the genome, there were fewer sites than predicted by a random distribution. Taken together, these data argue that HIV actively seeks new CD4 T-helper cell epitopes and minimizes the CTL and antibody sites within the genome.

Peptide diversity has an impact on stimulating CD4 T cells within an individual, but also within a population. There are hundreds of MHC class II alleles in the population as a whole, each with different peptide-binding specificities. Since every individual has a set of MHC molecules with different ranges of peptide-binding specificities and there are multiple variants of each MHC gene within a population as a whole, peptide diversity ensures that very different MHC molecules will find peptides that bind. It is important to note that there has been little success in correlating an MHC class II genotype with protection from disease progression (43).

ESTABLISHMENT OF INFECTION

The earliest events in acute HIV and SIV infection have recently been defined (10, 42, 50, 79, 83, 92; reviewed in reference 59). During mucosal exposure, the probability that replication-competent virus will infect a cell in an appropriate

activation state and sustain productive infection is extremely small. This is true for two reasons: normally the vast majority of susceptible CD4 cells are resting, and the bulk of the virus in the inoculum, as reflected by the viral load in plasma, is defective. The likely scenario is that a small amount of virus crosses the mucosal barrier, the replication-competent virus enters the cytoplasm of a resting cell, and defective virus remains in the extracellular space to serve as antigen. In CD4 T cells, following the two events, within the same cell, are required for productive HIV replication: (i) virus entry and (ii) cell activation. Although the order of these two events is interchangeable, both must occur within a given time frame.

Zhang et al. have shown that resting CD4 T cells are in fact the first cells infected in the first few days following primary SIV infection of macaques (91). The initial founder population of infected cells, early in primary infection, is small. In the first few hours following inoculation, infected cells have been detected by assays for viral DNA, but not for viral RNA, indicating a lag between virus entry and active replication (50). Although it has not been directly demonstrated, interruption of continued replication of HIV immediately after primary infection is possible if there is insufficient activation of the infected CD4 T cells. Transient infections may occur if virus replication is not sustained and/or if other susceptible CD4 cells (such as macrophages and dendritic cells) are not infected. This could result in very limited infection, involving small numbers of CD4 T cells. Consistent with this scenario are reports that perinatally HIV-exposed infants and high-risk adults repeatedly exposed to HIV can be transiently HIV positive by PCR (65, 67) and have T cells that respond to HIV antigens (17, 38).

INFLAMMATION AND FOCAL INFECTIONS

HIV disease is often described as a disease of immune activation, yet the causes of immune activation are still not fully explained (21, 26, 48). Activation of the immune system increases the likelihood that transmission and persistent infection will occur. Epidemiological studies of cofactors of HIV infection show that establishment of infection is a highly inefficient process and the efficiency of transmission is increased by a number of factors. For example, cofactors such as sexually transmitted diseases (25) or inflammation (29, 44, 70, 77, 80) greatly increase the efficiency of the transmission. All cofactors that have been shown to enhance infection either increase viral load in the inoculum or increase the frequency and availability of target cells in the host.

Following initial infection, SIV spreads in a highly focal pattern, first at the portal of entry and then in lymphoid tissues (50). As the number of cell-to-cell transmission events increases, there is less and less chance that the infection will become self-limiting. A model based upon this mechanism has been proposed by Haase and coworkers (50, 91). Here we extend their model by proposing that defective virus driving CD4 T-cell activation and HIV entry are inextricably linked for production of progeny virions. The two events, CD4 T-cell activation and HIV entry, can be carried out simultaneously by APCs carrying viruses from the initial site of infection. APCs effectively activate CD4 T cells and transmit virus via a process dubbed the viral synapse (36, 37).

The two events are also closely linked later in the course of disease. During chronic HIV disease there is extensive trapping of virus on the follicular dendritic cell network in lymphoid organs (53, 75). As resting CD4 T cells migrate through these lymphoid organs, they can become both infected and activated by uptake of virus and T-cell-receptor-driven stimulation by the infectious and defective material trapped in the follicular dendritic cell network (27, 32). When resting CD4 T cells encounter APCs and defective virus in the lymph nodes, they undergo a switch in surface phenotype from naïve resting to "memory." The newly stimulated cells are the ideal cells to support active virus replication. Recent stimulation of resting CD4 by APCs presenting defective virus may explain how phenotypically identified "resting cells" can produce virus (42) and why "memory" cells are more susceptible to infection and death (46, 47, 82).

Once virus enters the gut-associated lymphoid tissues (GALT), the focal infections are increased exponentially and there is a dramatic loss of CD4 T cells. The GALT contain a significantly higher proportion of activated CD4 T cells than other tissues, lymph nodes, and blood, ostensibly through chronic activation by gut microflora and ingested antigens. The constitutively higher level of activated cells found in the gut, combined with additional activation by HIV antigens, results in massive numbers of susceptible CD4 T cells that fuel virus replication in the acute phase of disease. With generalized activation, multiple mechanisms (such as direct killing by virus, CTL killing, and bystander effects) can cause CD4 T-cell depletion. An additional mechanism is the homeostatic response of activated cells to undergo apoptosis or to convert to a resting memory phenotype. It is now clear that in both HIV-infected individuals and SIV-infected macaques, approximately half of all the CD4 memory T cells are depleted from lymphoid tissues, especially the gastrointestinal tract, within the first 2 weeks of infection (10, 42, 46).

Focal infections are consistent with a model whereby a productively infected cell produces defective virus that stimulates adjacent CD4 T cells. Localized activation of CD4 T cells is conducive to foci of (mostly defective) virus driving the spread of replication-competent virus from one activated CD4 T cell to the next. Resting cells harboring replication-competent virus become permissive to infection, and adjacent uninfected activated cells become susceptible targets for productive infection. In support of this model, Cheynier and coworkers (13) have shown that individual splenic white cell pulps, less than 1 mm³ and very closely spaced, contained distinct proviral sequences as well as distinct T-cell subsets. In the SIV system, unique clones of SIV have also been identified in separate, focal regions of spleens from infected macaques (61).

INFECTION WITHOUT DISEASE

Lack of immune activation has also been postulated to protect SIV-infected sooty mangabey and African green monkeys from progressing to AIDS (39). Recently, Dunham and coworkers (22) have shown that production of cytokines (IL-2, tumor necrosis factor alpha, and gamma interferon), particularly from dendritic cells, is quantitatively reduced in sooty mangabeys compared to rhesus macaques and that expression of alpha interferon is absent in sooty mangabeys. This modu-

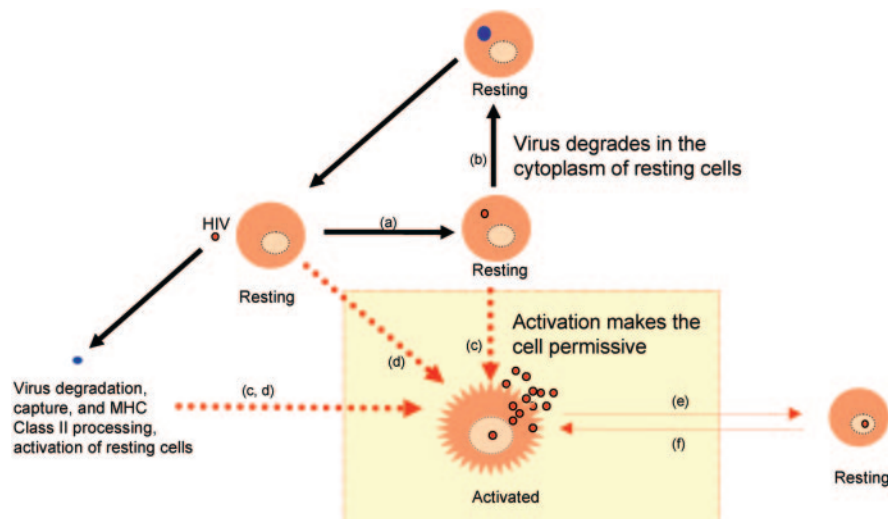


FIG. 1. Resting CD4 T cells must be activated to produce virus. (a) HIV is most likely to infect a resting CD4 T cell. (b) Resting CD4 T cells are not permissive to HIV replication, and the PIC degrades in the cytoplasm of resting CD4 T cells. (c) Activation of the resting cell is necessary for viral replication to occur. (d) An uninfected resting cell that becomes activated can be productively infected regardless of its antigenic specificity. (e) Rarely, activated infected cells survive and become long-lived memory cells with integrated, latent provirus. (f) Reactivation of these cells also makes them permissive to HIV replication.

lation in response sets the stage for nonpathogenic SIV-sooty mangabey coexistence, while the same triggers in rhesus macaques and humans result in generalized activation of the immune system and immunopathogenesis. Additionally, soon after virus infection of sooty mangabey monkeys, expression of IL-7 is increased and there is a marked decline in CCR5-expressing cells in circulation. This results in an efficient means of replenishing the CD4 T cells that are productively infected in these animals (52, 84).

Since all primate lentiviruses are nonpermissive for productive infection in resting CD4 T cells, the virus-host interaction between sooty mangabey and SIVsm has evolved to separate the activation event required to maintain virus replication from the activation processes that drive immunopathogenesis. In keeping with the model proposed here, SIVsm has been shown to generate extensive viral sequence variation throughout the course of infection (19). Yet, the two-step process of viral infection and activation that drives virus production has been decoupled from apoptosis of uninfected cells, pathogenesis, and disease progression. This decoupling may be related to the homeostatic mechanisms and cytokines involved in regulating sooty mangabey T-cell memory; several studies have shown that cytokines can exert a preventive effect on T-cell death (reviewed in reference 33).

CHRONIC DISEASE

After the acute phase of disease with explosive infection of the GALT, HIV disease returns to a pattern of focal infections within pockets of susceptible CD4 T cells. However, since many of the CD4 T cells that proliferated in response to the more common HIV epitopes are depleted, new peptides must be generated to continue activating the remaining CD4 T cells. In studying the molecular evolution of HIV, Shankarappa et al. (72) have defined the range of viral diversity within a host as

well as the divergence of the virus from the founder strain. Their studies show that HIV evolves in vivo both to generate maximum diversity and to maximize the distance from the founder strain. This has been taken as evidence that HIV is evolving to escape immune pressure, and this may be true for specific CD8 T epitopes. However, this viral evolution also creates an ever-expanding repertoire of CD4 T epitopes that effectively stimulate and then eliminate any possible response. Interestingly, Shankarappa et al. have mapped the point of maximal viral genetic diversity to a point immediately before the loss of T-cell homeostasis. Therefore, HIV may have evolved to maintain a high replicative capacity and generate a large amount of viral diversity, not to evade the immune response but rather to specifically engage and activate a sufficient number of CD4 T cells to maintain virus growth.

SUMMARY OF THE MODEL

Our synthesis draws from basic HIV biology, studies of acute HIV and SIV infection, vaccine studies, immunology, population genetics, epidemiology, and studies of transient infections. The primary components are as follows (Fig. 1).

(i) Resting CD4 T cells with a PIC represent the largest reservoir of HIV-infected cells in vivo (14, 23). Resting cells are not permissive for active HIV replication. If HIV replication is to occur, then the resting state of these PIC-containing CD4 T cells must be overcome.

(ii) HIV uses the class II antigen presentation pathway, with the antigen being the virus itself. Virus growth and evolution in an infected host generate new variants with new MHC class II specificities that maintain immune activation of CD4 T cells and keep replication at a level that sustains persistent, active infection.

(iii) The two-step process, infection and cell activation, leads to focal centers of activation that are a common feature of

primate lentiviruses. The generation of viral diversity seen in lentivirus infection drives the activation process of CD4 T cells that leads to apoptosis and is pathogenic in humans but not in the natural hosts.

This model of viral and immune pathogenesis has direct implications for HIV research in prevention, vaccine development, and therapeutics. New approaches aimed specifically at decreasing CD4 T-cell involvement rather than engaging CD4 T-cell responses are needed. Examples might include T-cell-specific immunomodulatory microbicides and/or therapies that carefully target CD8 T-cell and antibody responses while minimizing CD4 T-cell stimulation. Research in HIV pathogenesis will progress in many different directions in the coming years. We hope that this discussion has provided a model that might be worth investigating.

ACKNOWLEDGMENTS

We apologize for not being able to cite many important contributions. We thank Stuart Shapiro, Tony Conley, Kailash Gupta, Roger Miller, Ed Tramont, Gene Shearer, Peggy Johnston, David Kabat, Janet Siliciano, and Jim Mullins for their critical and insightful comments during the evolution of the concepts presented here.

REFERENCES

- Albert, J., B. Abrahamsson, K. Nagy, E. Aurelius, H. Gaines, G. Nystrom, and E. M. Fenyo. 1990. Rapid development of isolate-specific neutralizing antibodies after primary HIV-1 infection and consequent emergence of virus variants which resist neutralization by autologous sera. *AIDS* 4:107-112.
- Allen, T. M., M. Altfeld, S. C. Geer, E. T. Kalife, C. Moore, K. M. O'Sullivan, I. Desouza, M. E. Feeney, R. L. Eldridge, E. L. Maier, D. E. Kaufmann, M. P. Lahaie, L. Reyor, G. Tanzi, M. N. Johnston, C. Brander, R. Draenert, J. K. Rockstroh, H. Jessen, E. S. Rosenberg, S. A. Mallal, and B. D. Walker. 2005. Selective escape from CD8⁺ T-cell responses represents a major driving force of human immunodeficiency virus type 1 (HIV-1) sequence diversity and reveals constraints on HIV-1 evolution. *J. Virol.* 79:13239-13249.
- Arendrup, M., C. Nielsen, J. E. Hansen, C. Pedersen, L. Mathiesen, and J. O. Nielsen. 1992. Autologous HIV-1 neutralizing antibodies: emergence of neutralization-resistant escape virus and subsequent development of escape virus neutralizing antibodies. *J. Acquir. Immune Defic. Syndr.* 5:303-307.
- Bagasra, O., S. P. Hauptman, H. W. Lischner, M. Sachs, and R. J. Pomerantz. 1992. Detection of human immunodeficiency virus type 1 provirus in mononuclear cells by in situ polymerase chain reaction. *N. Engl. J. Med.* 326:1385-1391.
- Bebenek, K., J. Abbotts, J. D. Roberts, S. H. Wilson, and T. A. Kunkel. 1989. Specificity and mechanism of error-prone replication by human immunodeficiency virus-1 reverse transcriptase. *J. Biol. Chem.* 264:16948-16956.
- Bebenek, K., J. Abbotts, S. H. Wilson, and T. A. Kunkel. 1993. Error-prone polymerization by HIV-1 reverse transcriptase. Contribution of template-primer misalignment, miscoding, and termination probability to mutational hot spots. *J. Biol. Chem.* 268:10324-10334.
- Bebenek, K., J. D. Roberts, and T. A. Kunkel. 1992. The effects of dNTP pool imbalances on frameshift fidelity during DNA replication. *J. Biol. Chem.* 267:3589-3596.
- Borrow, P., H. Lewicki, X. Wei, M. S. Horwitz, N. Pfeffer, H. Meyers, J. A. Nelson, J. E. Gairin, B. H. Hahn, M. B. Oldstone, and G. M. Shaw. 1997. Antiviral pressure exerted by HIV-1-specific cytotoxic T lymphocytes (CTLs) during primary infection demonstrated by rapid selection of CTL escape virus. *Nat. Med.* 3:205-211.
- Brenchley, J. M., D. C. Douek, D. R. Ambrozak, M. Chatterji, M. R. Betts, L. S. Davis, and R. A. Koup. 2002. Expansion of activated human naive T-cells precedes effector function. *Clin. Exp. Immunol.* 130:432-440.
- Brenchley, J. M., T. W. Schacker, L. E. Ruff, D. A. Price, J. H. Taylor, G. J. Beilman, P. L. Nguyen, A. Khoruts, M. Larson, A. T. Haase, and D. C. Douek. 2004. CD4⁺ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. *J. Exp. Med.* 200:749-759.
- Bukrinsky, M. I., T. L. Stanwick, M. P. Dempsey, and M. Stevenson. 1991. Quiescent T lymphocytes as an inducible virus reservoir in HIV-1 infection. *Science* 254:423-427.
- Cardinaud, S., A. Moris, M. Fevrier, P. S. Rohrlisch, L. Weiss, P. Langlade-Demoyen, F. A. Lemonnier, O. Schwartz, and A. Habel. 2004. Identification of cryptic MHC I-restricted epitopes encoded by HIV-1 alternative reading frames. *J. Exp. Med.* 199:1053-1063.
- Cheyrier, R., S. Henrichwark, F. Hadida, E. Pelletier, E. Oksenhendler, B. Autran, and S. Wain-Hobson. 1994. HIV and T cell expansion in splenic white pulps is accompanied by infiltration of HIV-specific cytotoxic T lymphocytes. *Cell* 78:373-387.
- Chun, T. W., L. Carruth, D. Finzi, X. Shen, J. A. DiGiuseppe, H. Taylor, M. Hermankova, K. Chadwick, J. Margolick, T. C. Quinn, Y. H. Kuo, R. Brookmeyer, M. A. Zeiger, P. Barditch-Crovo, and R. F. Siliciano. 1997. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature* 387:183-188.
- Chun, T. W., D. Finzi, J. Margolick, K. Chadwick, D. Schwartz, and R. F. Siliciano. 1995. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat. Med.* 1:1284-1290.
- Cicala, C., J. Arthos, E. Martinelli, N. Censoplano, C. C. Cruz, E. Chung, S. M. Selig, R. D. Van, J. Yang, S. Jagannatha, T. W. Chun, P. Ren, R. A. Lempicki, and A. S. Fauci. 2006. R5 and X4 HIV envelopes induce distinct gene expression profiles in primary peripheral blood mononuclear cells. *Proc. Natl. Acad. Sci. USA* 103:3746-3751.
- Clerici, M., and G. M. Shearer. 1994. The Th1-Th2 hypothesis of HIV infection: new insights. *Immunol. Today* 15:575-581.
- Cohen, O. J., A. Kinter, and A. S. Fauci. 1997. Host factors in the pathogenesis of HIV disease. *Immunol. Rev.* 159:31-48.
- Demma, L. J., J. M. Logsdon, T. H. Vanderford, M. B. Feinberg, and S. I. Staprans. 2005. SIVsm quasiespecies adaptation to a new simian host. *PLoS Pathogens* 1:e3.
- Donnenberg, A. D., J. B. Margolick, and B. F. Polk. 1989. Limiting dilution analysis of in vivo-activated (IL-2 responsive) peripheral blood lymphocytes in HIV-1-infected subjects. *Clin. Immunol. Immunopathol.* 51:91-98.
- Douek, D. C., L. J. Picker, and R. A. Koup. 2003. T cell dynamics in HIV-1 infection. *Annu. Rev. Immunol.* 21:265-304.
- Dunham, R., P. Pagliardini, S. Gordon, B. Sumpter, J. Engram, A. Moanna, M. Paiardini, J. N. Mandl, B. Lawson, S. Garg, H. McClure, Y. X. Xu, C. I. Ibegbu, K. Easley, N. Katz, I. Pandrea, C. Apetrei, D. L. Sodora, S. I. Staprans, M. B. Feinberg, and G. Silvestri. 7 March 2006. The AIDS-resistance of naturally SIV-infected sooty mangabeys is independent of cellular immunity to the virus. *Blood* [Epub ahead of print.]
- Embretson, J., M. Zupancic, J. L. Ribas, A. Burke, P. Racz, K. Tenner-Racz, and A. T. Haase. 1993. Massive covert infection of helper T lymphocytes and macrophages by HIV during the incubation period of AIDS. *Nature* 362:359-362.
- Evans, D. T., D. H. O'Connor, P. Jing, J. L. Dzuris, J. Sidney, J. da Silva, T. M. Allen, H. Horton, J. E. Venham, R. A. Rudersdorf, T. Vogel, C. D. Pauza, R. E. Bontrop, R. DeMars, A. Sette, A. L. Hughes, and D. I. Watkins. 1999. Virus-specific cytotoxic T-lymphocyte responses select for amino-acid variation in simian immunodeficiency virus Env and Nef. *Nat. Med.* 5:1270-1276.
- Galvin, S. R., and M. S. Cohen. 2004. The role of sexually transmitted diseases in HIV transmission. *Nat. Rev. Microbiol.* 2:33-42.
- Hazenberg, M. D., D. Hamann, H. Schuitemaker, and F. Miedema. 2000. T cell depletion in HIV-1 infection: how CD4⁺ T cells go out of stock. *Nat. Immunol.* 1:285-289.
- Heath, S. L., J. G. Tew, J. G. Szakal, and G. F. Burton. 1995. Follicular dendritic cells and human immunodeficiency virus infectivity. *Nature* 377:740-744.
- Herbeuval, J. P., J. C. Grivel, A. Boasso, A. W. Hardy, C. Chougnnet, M. J. Dolan, H. Yagita, J. D. Lifson, and G. M. Shearer. 2005. CD4⁺ T cell death induced by infectious and noninfectious HIV-1: role of type I interferon-dependent, TRAIL/DR5-mediated apoptosis. *Blood* 106:3524-3531.
- Hillier, S. L., T. Moench, R. Shattock, R. Black, P. Reichelderfer, and F. Veronese. 2005. In vitro and in vivo: the story of nonoxynol 9. *J. Acquir. Immune. Defic. Syndr.* 39:1-8.
- Ho, D. D., T. Moudgil, and M. Alam. 1989. Quantitation of human immunodeficiency virus type 1 in the blood of infected persons. *N. Engl. J. Med.* 321:1621-1625.
- Holm, G. H., and D. Gabuzda. 2005. Distinct mechanisms of CD4⁺ and CD8⁺ T-cell activation and bystander apoptosis induced by human immunodeficiency virus type 1 virions. *J. Virol.* 79:6299-6311.
- Hufert, F. T., J. van Lunzen, G. Janosy, S. Bertram, J. Schmitz, O. Haller, P. Racz, and D. von Laer. 1997. Germinal centre CD4⁺ T cells are an important site of HIV replication in vivo. *AIDS* 11:849-857.
- Hurtrel, B., F. Petit, D. Arnoult, M. Muller-Trutwin, G. Silvestri, and J. Estaque. 2005. Apoptosis in SIV infection. *Cell Death. Differ.* 12(Suppl. 1): 979-990.
- Inoue, M., J. A. Hoxie, M. V. Reddy, A. Srinivasan, and E. P. Reddy. 1991. Mechanisms associated with the generation of biologically active human immunodeficiency virus type 1 particles from defective proviruses. *Proc. Natl. Acad. Sci. USA* 88:2278-2282.
- Jetz, A. E., H. Yu, G. J. Klarmann, Y. Ron, B. D. Preston, and J. P. Dougherty. 2000. High rate of recombination throughout the human immunodeficiency virus type 1 genome. *J. Virol.* 74:1234-1240.
- Jolly, C., K. Kashefi, M. Hollinshead, and Q. J. Sattentau. 2004. HIV-1 cell to cell transfer across an Env-induced, actin-dependent synapse. *J. Exp. Med.* 199:283-293.
- Jolly, C., and Q. J. Sattentau. 2005. Human immunodeficiency virus type 1

- virological synapse formation in T cells requires lipid raft integrity. *J. Virol.* **79**:12088–12094.
38. Kaul, R., F. A. Plummer, J. Kimani, T. Dong, P. Kiama, T. Rostron, E. Njagi, K. S. MacDonald, J. J. Bwayo, A. J. McMichael, and S. L. Rowland-Jones. 2000. HIV-1-specific mucosal CD8+ lymphocyte responses in the cervix of HIV-1-resistant prostitutes in Nairobi. *J. Immunol.* **164**:1602–1611.
 39. Kornfeld, C., M. J. Plouquin, I. Pandrea, A. Faye, R. Onanga, C. Apetrei, V. Poaty-Mavoungou, P. Rouquet, J. Estaquier, L. Mortara, J. F. Desoutter, C. Butor, R. Le Grand, P. Roques, F. Simon, F. Barre-Sinoussi, O. M. Diop, and M. C. Muller-Trutwin. 2005. Antiinflammatory profiles during primary SIV infection in African green monkeys are associated with protection against AIDS. *J. Clin. Investig.* **115**:1082–1091.
 40. Lane, H. C., and A. S. Fauci. 1985. Immunologic reconstitution in the acquired immunodeficiency syndrome. *Ann. Intern. Med.* **103**:714–718.
 41. Lazarski, C. A., F. A. Chaves, S. A. Jenks, S. Wu, K. A. Richards, J. M. Weaver, and A. J. Sant. 2005. The kinetic stability of MHC class II:peptide complexes is a key parameter that dictates immunodominance. *Immunity* **23**:29–40.
 42. Li, Q., L. Duan, J. D. Estes, Z. M. Ma, T. Rourke, Y. Wang, C. Reilly, J. Carlis, C. J. Miller, and A. T. Haase. 2005. Peak SIV replication in resting memory CD4+ T cells depletes gut lamina propria CD4+ T cells. *Nature* **434**:1148–1152.
 43. Liu, C., M. Carrington, R. A. Kaslow, X. Gao, C. R. Rinaldo, L. P. Jacobson, J. B. Margolick, J. Phair, S. J. O'Brien, and R. Detels. 2004. Lack of associations between HLA class II alleles and resistance to HIV-1 infection among white, non-Hispanic homosexual men. *J. Acquir. Immune Defic. Syndr.* **37**:1313–1317.
 44. Maher, D., X. Wu, T. Schacker, J. Horbul, and P. Southern. 2005. HIV binding, penetration, and primary infection in human cervicovaginal tissue. *Proc. Natl. Acad. Sci. USA* **102**:11504–11509.
 45. Malhotra, U., M. M. Berrey, Y. Huang, J. Markee, D. J. Brown, S. Ap, L. Musey, T. Schacker, L. Corey, and M. J. McElrath. 2000. Effect of combination antiretroviral therapy on T-cell immunity in acute human immunodeficiency virus type 1 infection. *J. Infect. Dis.* **181**:121–131.
 46. Mattapallil, J. J., D. C. Douek, B. Hill, Y. Nishimura, M. Martin, and M. Roederer. 2005. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. *Nature* **434**:1093–1097.
 47. Mattapallil, J. J., N. L. Letvin, and M. Roederer. 2004. T-cell dynamics during acute SIV infection. *AIDS* **18**:13–23.
 48. McCune, J. M. 2001. The dynamics of CD4+ T-cell depletion in HIV disease. *Nature* **410**:974–979.
 49. Miedema, F., A. J. Petit, F. G. Terpstra, J. K. Schattenkerk, F. de Wolf, B. J. Al, M. Roos, J. M. Lange, S. A. Danner, J. Goudsmit, et al. 1988. Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. *J. Clin. Investig.* **82**:1908–1914.
 50. Miller, C. J., Q. Li, K. Abel, E. Y. Kim, Z. M. Ma, S. Wietgreffe, L. La Franco-Scheuch, L. Compton, L. Duan, M. D. Shore, M. Zupancic, M. Busch, J. Carlis, S. Wolinsky, and A. T. Haase. 2005. Propagation and dissemination of infection after vaginal transmission of simian immunodeficiency virus. *J. Virol.* **79**:9217–9227.
 51. Moss, R. B., W. Giermakowska, M. R. Wallace, J. Savary, F. Jensen, and D. J. Carlo. 2000. T-helper-cell proliferative responses to whole-killed human immunodeficiency virus type 1 (HIV-1) and p24 antigens of different clades in HIV-1-infected subjects vaccinated with HIV-1 immunogen (Remune). *Clin. Diagn. Lab. Immunol.* **7**:724–727.
 52. Muthukumar, A., D. Zhou, M. Paiardini, A. P. Barry, K. S. Cole, H. M. McClure, S. I. Staprans, G. Silvestri, and D. L. Sodora. 2005. Timely triggering of homeostatic mechanisms involved in the regulation of T-cell levels in SIVsm-infected sooty mangabeys. *Blood* **106**:3839–3845.
 53. Pantaleo, G., C. Graziosi, J. F. Demarest, L. Butini, M. Montroni, C. H. Fox, J. M. Orenstein, D. P. Kotler, and A. S. Fauci. 1993. HIV infection is active and progressive in lymphoid tissue during the clinically latent stage of disease. *Nature* **362**:355–358.
 54. Perelson, A. S., P. Essunger, and D. D. Ho. 1997. Dynamics of HIV-1 and CD4+ lymphocytes in vivo. *AIDS* **11**(Suppl. A):S17–S24.
 55. Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard, C. R. Bangham, C. R. Rizza, et al. 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* **354**:453–459.
 56. Piatak, M., Jr., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K. C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson. 1993. Determination of plasma viral load in HIV-1 infection by quantitative competitive polymerase chain reaction. *AIDS* **7**(Suppl. 2):S65–S71.
 57. Pierson, T. C., Y. Zhou, T. L. Kieffer, C. T. Ruff, C. Buck, and R. F. Siliciano. 2002. Molecular characterization of preintegration latency in human immunodeficiency virus type 1 infection. *J. Virol.* **76**:8518–8531.
 58. Pontesilli, O., M. Carlesimo, A. R. Varani, R. Ferrara, G. D'Ofizzi, and F. Aiuti. 1994. In vitro lymphocyte proliferative response to HIV-1 p24 is associated with a lack of CD4+ cell decline. *AIDS Res. Hum. Retrovir.* **10**:113–114.
 59. Pope, M., and A. T. Haase. 2003. Transmission, acute HIV-1 infection and the quest for strategies to prevent infection. *Nat. Med.* **9**:847–852.
 60. Price, D. A., P. J. Goulder, P. Klenerman, A. K. Sewell, P. J. Easterbrook, M. Troop, C. R. Bangham, and R. E. Phillips. 1997. Positive selection of HIV-1 cytotoxic T lymphocyte escape variants during primary infection. *Proc. Natl. Acad. Sci. USA* **94**:1890–1895.
 61. Reinhart, T. A., M. J. Rogan, A. M. Amedee, M. Murphey-Corb, D. M. Rausch, L. E. Eiden, and A. T. Haase. 1998. Tracking members of the simian immunodeficiency virus 8B670 quasispecies population in vivo at single-cell resolution. *J. Virol.* **72**:113–120.
 62. Reynolds, M. R., E. Rakasz, P. J. Skinner, C. White, K. Abel, Z. M. Ma, L. Compton, G. Napoe, N. Wilson, C. J. Miller, A. Haase, and D. I. Watkins. 2005. CD8+ T-lymphocyte response to major immunodominant epitopes after vaginal exposure to simian immunodeficiency virus: too late and too little. *J. Virol.* **79**:9228–9235.
 63. Rezende, L. F., Y. Kew, and V. R. Prasad. 2001. The effect of increased processivity on overall fidelity of human immunodeficiency virus type 1 reverse transcriptase. *J. Biomed. Sci.* **8**:197–205.
 64. Richman, D. D., T. Wrin, S. J. Little, and C. J. Petropoulos. 2003. Rapid evolution of the neutralizing antibody response to HIV type 1 infection. *Proc. Natl. Acad. Sci. USA* **100**:4144–4149.
 65. Roques, P. A., G. Gras, F. Parnet-Mathieu, A. M. Mabondzo, C. Dollfus, R. Narwa, D. Marce, J. Tranchot-Diallo, F. Herve, G. Lasfargues, et al. 1995. Clearance of HIV infection in 12 perinatally infected children: clinical, virological and immunological data. *AIDS* **9**:F19–F26.
 66. Rosenberg, E. S., J. M. Billingsley, A. M. Caliendo, S. L. Boswell, P. E. Sax, S. A. Kalamas, and B. D. Walker. 1997. Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* **278**:1447–1450.
 67. Sahu, G. K., J. J. Chen, J. C. Huang, K. M. Ramsey, and M. W. Lloyd. 2001. Transient or occult HIV-1 infection in high-risk adults. *AIDS* **15**:1175–1177.
 68. Schmitz, J. L., T. N. Denny, A. Garcia, and J. L. Lathey. 2002. Comparison of human immunodeficiency virus antigens as stimulants for lymphocyte proliferation assays. *Clin. Diagn. Lab. Immunol.* **9**:525–529.
 69. Schnittman, S. M., J. J. Greenhouse, H. C. Lane, P. F. Pierce, and A. S. Fauci. 1991. Frequent detection of HIV-1-specific mRNAs in infected individuals suggests ongoing active viral expression in all stages of disease. *AIDS Res. Hum. Retrovir.* **7**:361–367.
 70. Serwadda, D., R. H. Gray, N. K. Sewankambo, F. Wabwire-Mangen, M. Z. Chen, T. C. Quinn, T. Lutalo, N. Kiwanuka, G. Kigozi, F. Nalugoda, M. P. Meehan, M. R. Ashley, and M. J. Wawer. 2003. Human immunodeficiency virus acquisition associated with genital ulcer disease and herpes simplex virus type 2 infection: a nested case-control study in Rakai, Uganda. *J. Infect. Dis.* **188**:1492–1497.
 71. Seshamma, T., O. Bagasra, D. Trono, D. Baltimore, and R. J. Pomerantz. 1992. Blocked early-stage latency in the peripheral blood cells of certain individuals infected with human immunodeficiency virus type 1. *Proc. Natl. Acad. Sci. USA* **89**:10663–10667.
 72. Shankarappa, R., J. B. Margolick, S. J. Gange, A. G. Rodrigo, D. Upchurch, H. Farzadegan, P. Gupta, C. R. Rinaldo, G. H. Learn, X. He, X. L. Huang, and J. I. Mullins. 1999. Consistent viral evolutionary changes associated with the progression of human immunodeficiency virus type 1 infection. *J. Virol.* **73**:10489–10502.
 73. Siliciano, R. F., T. J. Hemesath, J. C. Pratt, R. Z. Dintzis, H. M. Dintzis, O. Acuto, H. S. Shin, and E. L. Reinherz. 1986. Direct evidence for the existence of nominal antigen binding sites on T cell surface T_H1 alpha-beta heterodimers of MHC-restricted T cell clones. *Cell* **47**:161–171.
 74. Siliciano, R. F., T. Lawton, C. Knall, R. W. Karr, P. Berman, T. Gregory, and E. L. Reinherz. 1988. Analysis of host-virus interactions in AIDS with anti-gp120 T cell clones: effect of HIV sequence variation and a mechanism for CD4+ cell depletion. *Cell* **54**:561–575.
 75. Spiegel, H., H. Herbst, G. Niedobitek, H. D. Foss, and H. Stein. 1992. Follicular dendritic cells are a major reservoir for human immunodeficiency virus type 1 in lymphoid tissues facilitating infection of CD4+ T-helper cells. *Am. J. Pathol.* **140**:15–22.
 76. Stevenson, M., T. L. Stanwick, M. P. Dempsey, and C. A. Lamonica. 1990. HIV-1 replication is controlled at the level of T cell activation and proviral integration. *EMBO J.* **9**:1551–1560.
 77. Sturm-Ramirez, K., A. Gaye-Diallo, G. Eisen, S. Mboup, and P. J. Kanki. 2000. High levels of tumor necrosis factor-alpha and interleukin-1beta in bacterial vaginosis may increase susceptibility to human immunodeficiency virus. *J. Infect. Dis.* **182**:467–473.
 78. Unutmaz, D., V. N. KewalRamani, S. Marmon, and D. R. Littman. 1999. Cytokine signals are sufficient for HIV-1 infection of resting human T lymphocytes. *J. Exp. Med.* **189**:1735–1746.
 79. Vajdy, M., R. Veazey, I. Tham, C. deBakker, S. Westmoreland, M. Neutra, and A. Lackner. 2001. Early immunologic events in mucosal and systemic lymphoid tissues after intrarectal inoculation with simian immunodeficiency virus. *J. Infect. Dis.* **184**:1007–1014.
 80. Van Damme, L., G. Ramjee, M. Alary, B. Vuylsteke, V. Chandeying, H. Rees, P. Sirivongrangson, L. Mukenge-Tshibaka, V. Ettiegnre-Traore, C. Uaheowitchai, S. S. Karim, B. Masse, J. Perriens, and M. Laga. 2002. Effectiveness of COL-1492, a nonoxynol-9 vaginal gel, on HIV-1 transmis-

- sion in female sex workers: a randomised controlled trial. *Lancet* **360**:971–977.
81. **Van Gulck, E. R. A., P. Ponsaerts, L. Heyndrickx, K. Vereecken, F. Moerman, A. De Roo, R. Colebunders, G. Van den Bosch, D. R. Van Bockstaele, V. F. I. Van Tendeloo, S. Allard, B. Verrier, C. Maranon, G. Hoefel, A. Hosmalin, Z. N. Berneman, and G. Vanham.** 2006. Efficient stimulation of HIV-1-specific T cells using dendritic cells electroporated with mRNA encoding autologous HIV-1 Gag and Env proteins. *Blood* **107**:1818–1827.
 82. **van Noesel, C. J., R. A. Gruters, F. G. Terpstra, P. T. Schellekens, R. A. Van Lier, and F. Miedema.** 1990. Functional and phenotypic evidence for a selective loss of memory T cells in asymptomatic human immunodeficiency virus-infected men. *J. Clin. Investig.* **86**:293–299.
 83. **Veazey, R., and A. Lackner.** 2003. The mucosal immune system and HIV-1 infection. *AIDS Rev.* **5**:245–252.
 84. **Veazey, R., B. Ling, I. Pandrea, H. McClure, A. Lackner, and P. Marx.** 2003. Decreased CCR5 expression on CD4⁺ T cells of SIV-infected sooty mangabeys. *AIDS Res. Hum. Retrovir.* **19**:227–233.
 85. **Wahren, B., L. Morfeldt-Mansson, G. Biberfeld, L. Moberg, A. Sonnerborg, P. Ljungman, A. Werner, R. Kurth, R. Gallo, and D. Bolognesi.** 1987. Characteristics of the specific cell-mediated immune response in human immunodeficiency virus infection. *J. Virol.* **61**:2017–2023.
 86. **Wain-Hobson, S.** 1993. The fastest genome evolution ever described: HIV variation in situ. *Curr. Opin. Genet. Dev.* **3**:878–883.
 87. **Wei, X., J. M. Decker, S. Wang, H. Hui, J. C. Kappes, X. Wu, J. F. Salazar-Gonzalez, M. G. Salazar, J. M. Kilby, M. S. Saag, N. L. Komarova, M. A. Nowak, B. H. Hahn, P. D. Kwong, and G. M. Shaw.** 2003. Antibody neutralization and escape by HIV-1. *Nature* **422**:307–312.
 88. **Yang, W., J. P. Bielawski, and Z. Yang.** 2003. Widespread adaptive evolution in the human immunodeficiency virus type 1 genome. *J. Mol. Evol.* **57**:212–221.
 89. **Zack, J. A., S. J. Arrigo, S. R. Weitsman, A. S. Go, A. Haislip, and I. S. Chen.** 1990. HIV-1 entry into quiescent primary lymphocytes: molecular analysis reveals a labile, latent viral structure. *Cell* **61**:213–222.
 90. **Zack, J. A., A. M. Haislip, P. Krogstad, and I. S. Chen.** 1992. Incompletely reverse-transcribed human immunodeficiency virus type 1 genomes in quiescent cells can function as intermediates in the retroviral life cycle. *J. Virol.* **66**:1717–1725.
 91. **Zhang, Z., T. Schuler, M. Zupancic, S. Wietgreffe, K. A. Staskus, K. A. Reimann, T. A. Reinhart, M. Rogan, W. Cavert, C. J. Miller, R. S. Veazey, D. Notermans, S. Little, S. A. Danner, D. D. Richman, D. Havlir, J. Wong, H. L. Jordan, T. W. Schacker, P. Racz, K. Tenner-Racz, N. L. Letvin, S. Wolinsky, and A. T. Haase.** 1999. Sexual transmission and propagation of SIV and HIV in resting and activated CD4⁺ T cells. *Science* **286**:1353–1357.
 92. **Zhang, Z. Q., S. W. Wietgreffe, Q. Li, M. D. Shore, L. Duan, C. Reilly, J. D. Lifson, and A. T. Haase.** 2004. Roles of substrate availability and infection of resting and activated CD4⁺ T cells in transmission and acute simian immunodeficiency virus infection. *Proc. Natl. Acad. Sci. USA* **101**:5640–5645.
 93. **Zhou, Y., H. Zhang, J. D. Siliciano, and R. F. Siliciano.** 2005. Kinetics of human immunodeficiency virus type 1 decay following entry into resting CD4⁺ T cells. *J. Virol.* **79**:2199–2210.
 94. **Zhuang, J., A. E. Jetzt, G. Sun, H. Yu, G. Klarmann, Y. Ron, B. D. Preston, and J. P. Dougherty.** 2002. Human immunodeficiency virus type 1 recombination: rate, fidelity, and putative hot spots. *J. Virol.* **76**:11273–11282.