

Topics in HIV Medicine™

A publication of the International AIDS Society–USA

Highlights of the 9th Retrovirus Conference

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R. Paul Johnson, MD, and Amitinder Kaur, MD

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About This Issue

In this issue, R. Paul Johnson, MD, and Amitinder Kaur, MD, offer a review of new research on HIV pathogenesis and vaccine candidates presented at the 9th Conference on Retroviruses and Opportunistic Infections, held this year in Seattle, Wash, from February 24 to February 28. A list of conference abstracts cited in their text is included, and the full text of all abstracts is available on the conference Web site at www.retroconference.org.

This issue also includes 2 *Perspectives* articles from the International AIDS Society–USA continuing medical education program held in Los Angeles in March 2002. Margaret J. Koziel, MD, discussed the effects of coinfection with HIV and hepatitis C virus on the course and management of both diseases. Steven G. Deeks, MD, discussed recent findings regarding the effects of antiretroviral therapy on HIV replicative capacity and the potential implications of these findings for treatment strategies.

In a review article, John G. Gerber, MD, and Edward P. Acosta, PharmD, survey current data on the use of therapeutic drug monitoring in the treatment of HIV infection. Also in this issue, the International AIDS Society–USA Drug Resistance Mutations Group presents a redesigned update of HIV-1 drug resistance mutations. This June 2002 summary reflects new research on drug resistance mutations presented at the 9th Conference on Retroviruses and Opportunistic Infections, and represents the ongoing efforts of the group to provide a current listing of mutations to HIV clinicians and scientists. The update was also published online at www.iasusa.org.

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Highlights From the 9th Retrovirus Conference

HIV Pathogenesis and Vaccine Development

R. Paul Johnson, MD, and Amitinder Kaur, MD

Introduction

Presentations at the 9th Conference on Retroviruses and Opportunistic Infections, held in Seattle, February 24 to 28, 2002, continued to shed light on the complex interactions between HIV and the immune system. Major topics of discussion included natural hosts of simian immunodeficiency virus (SIV) infection, the dynamics of T lymphocytes, the myriad mechanisms utilized by HIV to avoid host immune responses, and the central role of dendritic cells in HIV pathogenesis. Promising new data emerged from initial results of a candidate HIV-1 vaccine able to induce more potent cellular immune responses, raising hopes for the new generation of vaccines currently entering human clinical trials.

Natural Hosts of SIV Infection

There is now little doubt that the global pandemic of HIV infection originated as a result of cross-species transmission from nonhuman primates infected with SIV; thus analysis of the biology of nonhuman primate lentiviruses may offer important insights on AIDS pathogenesis and the potential for new human lentiviral infections. In the Bernard Fields Memorial Lecture, Hahn provided an interim analysis of one of the most comprehensive ongoing surveys of the extent of SIV infection in nonhuman primates in Africa (Abstract L1). Analysis of

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specimens obtained from bushmeat (ie, monkeys killed for food) and pet monkeys revealed that antibodies to SIV were present in 13 of 16 species sampled. These results documented infection in 4 species not previously known to be infected with SIV, and molecular analysis of viral sequences amplified by polymerase chain reaction identified 5 new SIV lineages. The overall seroprevalence in all species analyzed was 20% and exceeded 90% in some sexually active nonhuman primate adults. These data dramatically reinforce the existence of a diverse reservoir of SIV strains in nonhuman primates and underscore the potential for new introductions of primate lentiviruses into the human population.

From evidence primarily obtained from captive animals, chimpanzees infected with SIV_{cpz} have long been suspected of being the primary origin of the HIV-1 epidemic. However, there have been no solid data on the prevalence of SIV infection in chimpanzees in the wild, an elusive goal that has been frustrated by the difficulties inherent in trying to obtain samples from an endangered and reclusive species. Armed with improved techniques to document SIV infection in the urine and feces from chimpanzees living in East Africa and West Africa, Hahn was able to confirm the existence of SIV-infected chimps in the wild, although the seroprevalence was surprisingly low. Only 1 in 152 animals initially tested was found to be infected with SIV_{cpz}. Interestingly, the one chimpanzee found to be SIV-infected in the initial survey was from a troop in Tanzania's Gombe National Park that has been studied for decades by Goodall and her associates. Expanded testing of chimpanzees in Gombe identified 3 additional animals infected with SIV.

These new data extend the boundary of SIV_{cpz} infection eastward into Tanzania and also document that 2 different chimpanzee subspecies (*Pan troglodytes*

troglodytes and *Pan troglodytes schweinfurthii*) can serve as reservoirs of infection. The relatively infrequent infection of chimpanzees with SIV in the wild suggests that chimpanzees may have been more recently infected by another nonhuman primate species, a possibility reinforced by recent data obtained from a survey of greater spot-nosed monkeys (Courgnaud et al, Abstract LB1). Genetic analysis of 2 complete sequences of SIV_{gsn} genomes revealed a close relationship to SIV_{cpz} in envelope, suggesting the possibility that SIV_{cpz} may have been derived from infection of chimpanzees by greater spot-nosed monkeys.

The fact that natural hosts of primate lentiviruses do not develop AIDS has prompted an intensive effort into defining the virologic and immunologic mechanisms that underlie the lack of disease. One of the best-studied natural

Information about and abstracts for the 9th Conference on Retroviruses and Opportunistic Infections can be found on the Web at

www.retroconference.org

hosts of SIV is the sooty mangabey, a species native to West Africa. Despite SIV plasma viral levels of 10⁵ to 10⁷ copies/mL, sooty mangabeys remain immunologically normal. However, cross-species transmission of the sooty mangabey virus (SIV_{sm}) into people (resulting in HIV-2) or Asian macaques (resulting in SIV_{mac}, the most widely used virus for nonhuman primate studies) induces AIDS.

Feinberg described recent efforts to better understand host-virus interactions in sooty mangabeys (Abstract S24). Debunking the theory that the lack of pathogenicity in this species may be due to a lack of cytopathic effect of the virus in vivo, he noted that administration of the reverse transcriptase

inhibitor PMPA (now approved for use in humans as tenofovir disoproxil fumarate [tenofovir]) induced a rapid decay of plasma viral levels similar to that observed in HIV-infected people and SIV-infected macaques, a result that strongly suggests the rapid death of sooty mangabey SIV-infected CD4+ T lymphocytes *in vivo*. Despite the loss of infected CD4+ T lymphocytes, the key to the sooty mangabey's ability to maintain normal immune function appears to be its ability to avoid widespread immune activation and the associated increased rates of CD4+ and CD8+ T-cell apoptosis. The precise mechanisms responsible for the lack of generalized immune activation are not clear, but may include a combination of decreased SIV-specific immune responses and lower rates of indirect cell activation and death (ie, death of cells not directly infected with SIV).

This latter point was reinforced by a presentation from Kaur and colleagues (Abstract 22), who analyzed T-cell turnover in sooty mangabeys using the nucleoside analogue bromodeoxyuridine (BrdU). Interestingly, despite the rapid turnover of SIV-infected CD4+ T cells described above, no differences in uptake or decay rates of BrdU-labeled CD4+ or CD8+ T lymphocytes were observed between SIV-infected and uninfected mangabeys. This result reinforces the concept that in this natural host of SIV infection, the virus fails to induce indirect mechanisms of accelerated T-cell activation and destruction that ultimately result in CD4+ T-cell depletion.

T-Cell Dynamics

Although loss of CD4+ T lymphocytes is a hallmark feature of HIV disease, the precise mechanisms that lead to depletion of CD4+ T cells remain incompletely understood and have often provoked contentious debate. In recent years, there has been increasing consensus that the rates of CD4+ and CD8+ T-cell turnover in HIV-infected people and SIV-infected macaques are increased by 2- to 5-fold compared with uninfected controls. However, the effects of antiretroviral therapy on T-cell turnover and the relative roles of increased CD4+ T-cell destruction and impaired T-cell produc-

tion in CD4+ T-cell depletion have remained controversial. A conference symposium on T-cell turnover and thymic function brought together several of the leading scientists in this field to attempt to resolve these issues.

Perelson (Abstract S9) highlighted the utility of mathematical modeling in the analysis of T-cell turnover data generated in HIV-1-infected subjects who underwent a 7-day infusion of deuterated (²H) glucose. Accurate modeling of CD4+ and CD8+ T-cell labeling and decay kinetics required the use of a 2-compartment model including a source of unlabeled lymphocytes. In this model, proliferation and death rates of CD4+ T cells in untreated subjects were increased by 6.3- and 2.9-fold, respectively, compared with normal controls. The calculated mean proliferation rate of CD8+ T cells was 7.7-fold higher in HIV-infected subjects, although the death rate of CD8+ T cells was not increased. Institution of highly active antiretroviral therapy (HAART) resulted in significant decreases in CD4+ and CD8+ lymphocyte proliferation and death rates after 5 to 11 weeks.

Similar data were presented by Kovacs (Abstract S10), who studied rates of labeling and decay of BrdU-labeled lymphocytes in 17 HIV-infected volunteers. Mathematical modeling of the decay of BrdU-labeled cells supported the existence of 2 populations of lymphocytes, one rapidly proliferating, the other slowly proliferating. The magnitude of plasma HIV RNA levels correlated with the size of the proliferating pool but, interestingly, not with the death rate of lymphocytes. The major effect of HAART was to decrease the size of the proliferating pool. Data supporting the existence of 2 pools of short-lived and long-lived T cells were also provided by Hellerstein and colleagues using metabolic labeling with deuterated glucose or water (Abstract 102). Hellerstein's group also observed an expansion of the short-lived, rapidly turning over lymphocyte pool in untreated HIV infection.

Miedema and colleagues (Abstract S11) addressed the multiple perturbations of T-cell homeostasis in HIV-infected patients, which include altered distribution of cells between peripheral and lymphoid compartments, increased

rates of lymphocyte turnover driven by immune activation, and increased turnover of naive T cells. HIV-1 strains able to induce syncytia and to utilize the CXCR4 coreceptor (SI/X4 strains) were uniquely able to infect and lyse naive CD4+ T cells. McCune (Abstract S12) emphasized the interplay between the thymus and the peripheral T-lymphocyte pool in attempting to maintain T-cell homeostasis in the face of HIV-mediated CD4+ T-cell destruction. Interleukin (IL)-7 is one molecule that appears to mediate feedback between the peripheral lymphoid pool and the thymus, serving to increase naive T-cell production. However, therapeutic use of this molecule is likely to be limited by its ability to increase HIV replication, and so recent studies are beginning to examine the utility of growth hormone, which has increased thymic output in rodent models. Preliminary results from 2 pilot studies (Napolitano et al, Abstract 511-M; Pires et al, Abstract 513-M) suggested beneficial effects of growth hormone on thymic tissue size and circulating naive T cells in HIV-infected subjects, but more detailed clinical studies will be required to more rigorously evaluate the utility of growth hormone in accelerating the production of naive T cells.

Taken together, these presentations highlighted the fact that untreated HIV infection results in accelerated peripheral destruction of CD4+ T cells and an associated increase in CD4+ T-cell production. CD4+ T-cell depletion is therefore likely to result from an imbalance in these rates of T-cell production and death, rather than an absolute decrease in the rate of CD4+ T-cell production. There was also a consensus regarding the ability of HAART to decrease rates of CD4+ T-cell proliferation and death, most likely by decreasing the size of the short-lived, rapidly turning over pool of T lymphocytes. These presentations also represent a shift in the debate from the magnitude of T-cell turnover in HIV infection to a renewed focus on how HIV infection perturbs T-cell homeostasis, a topic that will probably provoke ongoing controversy for years to come.

The contribution of the thymus in maintaining T-cell homeostasis was subsequently examined by Arron and colleagues (Abstract 101). Based on the decay of T-cell receptor excision circles

(TRECs) following thymectomy of juvenile rhesus macaques, the authors estimated a daily thymic production rate of approximately 10^7 T cells per day, representing about 0.01% of all T cells in a macaque. Although SIV infection reduced TREC levels in both thymectomized animals and sham-operated controls, no differences in disease progression or lymphocyte naive and memory subsets were observed between these groups. These data reinforce the notion that peripheral destruction of CD4+ T lymphocytes, rather than reduced thymic output, plays a major role in inducing CD4+ T-cell depletion in untreated SIV (and presumably HIV) infection. However, they still leave open the question of whether de novo production of naive CD4+ T cells in the setting of HAART may play a beneficial role in restoring normal immune function.

Immune Responses to HIV

One of the significant challenges in the development of an HIV vaccine has been the difficulty in generating potent and broadly neutralizing antibody responses. In his plenary talk, Wyatt (Kwong et al, Abstract L4) provided a detailed analysis of the mechanisms utilized by HIV to evade neutralizing antibody responses. A variety of structural characteristics of the HIV envelope facilitate immune evasion, including its remarkable degree of glycosylation, conformational changes of the envelope that occur during virus binding, steric barriers, shedding of the viral envelope, and the high mutation rate in the variable domains. Wyatt highlighted in particular the recessed nature of the CD4 binding site of gp120, which represents an exceptionally difficult site for antibodies to block. Binding of CD4 to gp120 induces a conformational change in the envelope protein that may be mimicked by structure-based mutants. Use of these conformationally fixed glycoproteins may prove to be effective in inducing more broadly neutralizing antibody responses.

The analysis of neutralizing antibody responses to HIV has been problematic, plagued by considerable variations in techniques and the difficulty of generating a reproducible source of autologous virus. Using a novel approach involving neutralization of recombinant HIV

strains expressing envelope proteins derived from patient samples, Richman and colleagues (Abstract LB5) described the evaluation of neutralizing antibody responses to autologous virus sequences in a cohort of 15 subjects with primary HIV infection. Although 13 of 15 subjects generated strong neutralizing antibody responses to autologous virus sequences, there was rapid evolution of envelope to escape the concurrent neutralizing antibody response. In other words, antibody responses at 25 weeks after infection were able to neutralize virus isolated at the time of infection but not virus isolated at later time points. Reproducing the finding of "original antigenic sin" previously observed in

Most individuals can
produce antibodies able
to neutralize virus
early in infection

influenza virus infection, neutralizing antibody titers able to neutralize the initial virus sequences continued to rise with time, despite the inability to neutralize concurrent HIV-1 isolates. These data provide compelling evidence that most individuals are capable of producing antibodies able to neutralize virus at time points early in infection, but that continued evolution of the virus results in a series of mutant viruses that are not efficiently neutralized by autologous antibody responses.

A leading explanation for the relatively weak CD4+ T-cell response to HIV has been that these cells are selectively infected by HIV-infected antigen-presenting cells, yet little direct evidence has been presented to support this hypothesis. Douek and colleagues (Abstract LB7; *Nature*, 2002) analyzed the frequency of HIV infection in HIV-specific CD4+ T cells identified by intracellular cytokine staining. Compared with cytomegalovirus-specific CD4+ T cells or total memory CD4+ T cells, HIV-specific CD4+ T cells were preferentially infected

by HIV. However, only a small fraction of HIV-specific CD4+ T cells were infected (0.01%-1%), an interesting finding that suggests that a substantial proportion of virus-specific CD4+ T cells are able to proliferate after they encounter antigen-presenting cells expressing HIV epitopes, yet escape infection. In individuals undergoing structured treatment interruptions (STIs), the increase in HIV infection of CD4+ T cells occurred predominantly in HIV-specific CD4+ T cells. In one such subject, up to 50% of all HIV-infected cells were HIV-specific. These data help to document one mechanism for the loss of HIV-specific CD4+ T-cell responses and also raise questions about whether STIs in chronically infected subjects will ultimately prove beneficial in boosting virus-specific CD4+ T-cell responses.

An alternative explanation for relatively weak HIV-specific CD4+ T-cell responses may lie in the intrinsic immunogenicity of the envelope protein. Grundner and colleagues described the results of immunization of mice and rabbits with recombinant core gp120 proteins from which V1 and V2 and portions of the N- and C-termini had been deleted (Abstract 105). Antibody responses to the core subunit of 2 primary isolates, but not the laboratory isolate HXBc2, were relatively weak, but could be boosted by the addition of a heterologous T helper epitope. Similar results were obtained with deglycosylated envelope proteins. Although it will be important to extend this analysis to macaques and humans, these results suggest that the envelope sequences of primary isolates may have evolved to minimize recognition by CD4+ T cells, and that inclusion of heterologous T helper sequences may enhance vaccine-induced responses against gp120.

HIV-specific CD4+ T-cell responses were also the subject of a number of poster presentations. Work from numerous laboratories clearly documented that HIV-specific CD4+ T-cell responses are present during acute and chronic infection, but are generally not detected using standard proliferation assays (Malhotra et al, Abstract 203-T; Malhotra et al, Abstract 204-T; Palmer et al, Abstract 208-T; Yassine-Diab et al, Abstract 215-T; and Iyasere et al, Abstract 216-T). This phenomenon was elegantly demon-

strated by Yassine-Diab and colleagues (Abstract 215-T), who identified HIV-specific CD4+ T cells using human leukocyte antigen (HLA) class II tetramers and showed that fluorescently labeled cells failed to divide following *in vitro* stimulation.

Research on CD8+ T-cell responses to HIV and SIV over the past several years has increasingly focused on defining mechanisms used by these viruses to evade a relatively vigorous cytolytic T-lymphocyte (CTL) response. O'Connor and colleagues (Abstract 98; *Nat Med*, 2002) described the results of a comprehensive study analyzing complete viral genomes of 21 macaques infected with the SIV_{mac239} molecular clone. By 4 weeks after infection, escape from at least 1 CTL epitope had occurred in 19 of 21 animals. Escape mutations appeared to occur selectively in high-avidity CTL epitopes. Together with the data on neutralizing antibody responses presented by Richman and colleagues (Abstract LB5), these presentations dramatically underscored the role that viral mutation plays in evading both humoral and cellular immune responses.

A role for replication senescence of HIV-specific CD8+ T cells was proposed by Brenchley and colleagues (Abstract 217-T). Using a combination of intracellular cytokine staining and fluorescent labeling to track cell division *in vitro*, these authors demonstrated that HIV-specific CD8+ T cells expressing CD57 were markedly impaired in their ability to proliferate following antigenic stimulation. Evidence for replicative senescence was provided by the fact that these cells expressed lower levels of TRECs.

Immune Reconstitution

In a plenary talk, Autran (Abstract L3) highlighted the advances that have been made in understanding the complex effects of HAART on immune function. The initial phase of CD4+ T-cell recovery after institution of HAART consists primarily of peripheral memory T cells. Thereafter, a slower, sustained increase in naive CD4+ T cells is observed, which most likely reflects *de novo* production from the thymus. A balanced recovery of both naive and memory T cells is necessary for restoration of a diverse T-cell

repertoire able to maintain T-cell memory and also to respond to new pathogens.

However, in spite of these beneficial effects of HAART, reconstitution of HIV-specific immune responses remains incomplete. HIV-specific CD4+ T-cell responses are not found in most chronically infected individuals after HAART (at least as detected by standard proliferation assays; see discussion in previous section). Moreover, HIV-specific CD8+ T-cell responses decline with the loss of antigenic stimulation. In contrast to patients with acute HIV infection, efforts to use STIs to boost host control of viral replication in chronically infected individuals have generally been unsuccessful, and have also resulted in a loss of HIV-specific proliferative responses. These results have placed a renewed emphasis on therapeutic vaccination. An ongoing European trial is examining whether vaccination with a highly attenuated poxvirus (ALVAC vCP1452), inactivated HIV (HIV Immunogen [Remune, Immune Response Corporation, Carlsbad, Calif]), or both, in combination with HAART, will be able to induce stronger immune responses that may be able to better contain HIV replication.

A key question for immune reconstitution is whether HIV-infected individuals can mount cellular immune responses to new epitopes. Altfeld and colleagues (Abstract 107) addressed this question in the context of patients undergoing STIs. The investigators examined ELISPOT responses in peripheral blood and lymph nodes to a panel of multiple epitopes selected on the basis of HLA type. The characteristic expansion of HIV-specific CD8+ T cells in peripheral blood seen with the rebound of viremia occurred largely as a result of the expansion of preexisting HIV-specific CD8+ T cells in lymph nodes rather than as an induction of new responses. Future studies will need to address this question in the context of therapeutic vaccination as well.

Intermittent infusions of IL-2 in combination with antiretroviral therapy have been well-documented as leading to sustained increases in CD4+ T-cell counts. However, detailed information on the mechanisms underlying this expansion of CD4+ T cells has been lack-

ing. Analysis by Kovacs and colleagues of lymphocyte proliferation using deuterium labeling revealed that patients receiving IL-2 had substantial proliferation of both CD4+ and CD8+ T lymphocytes and, surprisingly, a markedly prolonged survival of CD4+ T cells (Abstract 103). This latter finding may well explain why relatively infrequent IL-2 infusions may lead to prolonged increases in CD4+ T cell counts. A companion presentation by Sereti and colleagues (Abstract 104) demonstrated that the expansion of CD4+ T cells following IL-2 therapy is predominantly composed of a population of CD25+ T cells that expresses some naive markers but has a lower content of TRECs, and therefore most likely represents an expansion of peripheral T cells rather than recent thymic emigrants.

Dendritic Cells and Mucosal Immunology

The past few years have seen an explosion of research into the multifaceted properties of dendritic cells (DCs) and the critical role they play in HIV transmission, replication, and efficient induction of antigen-specific CD4+ and CD8+ T-cell responses. In a plenary talk, Pope (Abstract L2) provided a comprehensive overview of the current state of research on interactions between DCs and T cells in the context of HIV and SIV infection.

There are 2 phenotypically distinct types of DCs currently known. Myeloid DCs are CD11c+, express toll-like receptors (TLRs) 2 and 4, and respond to IL-4, granulocyte macrophage colony-stimulating factor (GM-CSF) and type I interferons. Plasmacytoid dendritic cells are CD11c-, TLR 9+, and are unique in expressing the IL-3 receptor CD123, which makes them responsive to IL-3. When stimulated, as with an initial encounter with a pathogen, plasmacytoid DCs secrete abundant type I interferons, which in turn can stimulate myeloid DC and thus provide a bridge between innate and pathogen-specific immunity. Unlike plasmacytoid DCs, which are present only in the blood and lymph nodes, myeloid DCs are also abundantly present at body surfaces, including mucosal sites. Since mucosal transmission is the predominant mode of HIV spread in the majority of infected

individuals, myeloid DCs are the first cells encountered by the virus. Since early events are important determinants of the ultimate outcome of HIV infection, the initial interactions between virus and DCs are an important area of research.

Previous work by Pope had demonstrated that in the absence of Nef, SIV replication in immature DCs is severely curtailed. She now presented data showing that the presence of Nef in immature DCs induces production of the chemokines MIP-1 α , MIP-1 β , and RANTES, and the cytokine IL-12. Further, Nef also enhances the ability of immature DCs to induce syngeneic T-cell proliferation. These effects of Nef may modulate the extent of activated T-cell recruitment and viral replication at the site of entry and thereby influence pathogenicity.

Several presentations addressed the expression of dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN), an HIV/SIV adhesion molecule, and its closely related homologue, DC-SIGNR, on DCs in vivo at different anatomical sites. DC-SIGN is a C-type lectin primarily expressed on myeloid DCs, which binds with high affinity to gp120 of HIV and SIV. Once virus is bound to DC-SIGN, it can be transmitted efficiently to susceptible T cells expressing CD4 and the coreceptors CCR5 or CXCR4.

Iwasaki (Jameson et al, Abstract 28) presented data on immunohistochemical analysis of human and rhesus macaque intestinal and genital tissue using monoclonal antibodies to DC-SIGN and DC-SIGNR. DC-SIGN was not expressed on plasmacytoid DCs. In the ileum, clusters of DC-SIGN⁺ cells that were CD11c- and CD123-negative were detected subepithelially in the dome and interfollicular regions of Peyer's patches. DC-SIGNR expression was observed on endothelial cells in the ileum. Myeloid DCs expressing high levels of DC-SIGN were present throughout the entire thickness of human rectal mucosa, and DC-SIGN⁺ cells co-expressing CD4 and CCR5 were localized just beneath the luminal epithelium. In contrast, Langerhans' cells (LCs) in the vaginal epithelium did not express DC-SIGN. Instead, DC-SIGN was moderately expressed in subepithelial DCs in the lamina propria of the vaginal mucosa.

Similar findings were observed in rhesus macaques. These results provide a physical basis for the observation of a higher risk of HIV transmission via rectal as compared to vaginal intercourse, and suggest that the proximity of DCs expressing DC-SIGN to luminal epithelium is important for mucosal transmission of primate lentiviruses.

Expression of DC-SIGN, along with CD4 and coreceptors, can also act in *cis* to promote HIV infection and replication in the DC-SIGN-expressing cells. Although DC-SIGN is almost exclusively detected in myeloid DCs, there are some reports of its presence in non-myeloid DC cells, most notably on a subset of alveolar and placental macrophages (Soilleux et al, Abstract 109), and in perivascular macrophages and micro-

The similarities between human and macaque DCs open the way to exciting future studies

glial cells in the brain (Shawver et al, Abstract 63). The expression of DC-SIGN on non-DCs may be one mechanism of expanding the tissue tropism of HIV or SIV in infected hosts. Further, molecules other than DC-SIGN may also mediate HIV transmission. This was highlighted by the finding that SIV can infect and replicate in macaque DCs in the presence of blocking antibodies to DC-SIGN (Pope, Abstract L2). The demonstration of the similarities between human and macaque DCs and the generation of macaque-specific reagents has opened the way to exciting future studies that can directly address the *in vivo* role of DCs in the early events of HIV transmission.

Although HIV contact with DCs clearly facilitates transmission and viral replication, DCs are also required for the generation of an effective anti-HIV immune response. Macaque DCs pulsed with infectious or inactivated SIV were

capable of eliciting interferon- γ secretion from SIV-specific T cells (Pope, Abstract L2). However, if HIV infection were to impair DC function, this could abrogate HIV-specific immunity and provide yet another mechanism of promoting HIV replication. Several investigators provided evidence that HIV infection can affect the number and function of myeloid and plasmacytoid DCs (Abstracts 99, 252-T, 253-T, 255-T). Both myeloid DCs and plasmacytoid DCs are decreased in individuals chronically infected with HIV-1 (Barron et al, Abstract 252-T; Jones et al, Abstract 253-T). Myeloid DCs from untreated HIV-1-infected individuals with high viral loads had an impaired ability to stimulate allogeneic T-cell proliferation in a mixed leukocyte reaction (Donaghy et al, Abstract 255-T).

Further, in one study (Loré et al, Abstract 99), mature DCs from subjects with acute HIV infection appeared to have impaired activation, as suggested by low levels of expression of CD80 and CD86 molecules, when compared to DCs isolated from individuals with acute Epstein-Barr virus infection. In all, these data suggest that impairment of DC function does occur in HIV infection and may contribute to the inability of the host to clear HIV infection.

Despite the central role that viral shedding from mucosal surfaces plays in transmission of HIV, there has been relatively little information on what cell types are predominantly responsible for release of HIV into mucosal secretions. Brodie and colleagues (Abstract 27) analyzed the distribution of HIV RNA and DNA in rectal lymphocytes, macrophages, and dendritic cells from men who were shedding HIV rectally. Although mucosal T lymphocytes were the most common cell expressing HIV RNA, HIV-infected macrophages had significantly higher levels of HIV RNA, especially in subjects with advanced disease. Rectal shedding of HIV was also detected in 6 subjects on HAART, 2 of whom had plasma HIV-1 RNA levels of less than 200 copies/mL at the time of study. These data suggest that macrophages may be a major source of viral shedding from mucosal sites, even in some patients on potent antiretroviral therapy with plasma HIV-1 RNA below detection levels.

Vaccines

In a plenary talk (Abstract L5), Emini presented data on the results of ongoing phase 1 human clinical trials with 2 candidate HIV vaccines, one a plasmid DNA, and the other a replication-defective adenoviral type 5 (Ad5) vector containing codon-optimized HIV-1 clade B *gag* DNA. The vaccines have been designed with the aim of inducing vigorous Gag-specific CD4+ and CD8+ T-lymphocyte responses. The plasmid HIV-1 *gag* DNA vaccine has been tested in 109 HIV-1-seronegative volunteers. Twenty-four were administered placebo, 42 received 1 mg DNA, and 43 received 5 mg of the DNA vaccine at 0, 4, 8, and 26 weeks. Weak interferon- γ ELISPOT responses were detected in less than 50% of individuals after 4 immunizations. Stronger and sustained interferon- γ ELISPOT responses were observed in 6 of 9 volunteers who received 3 doses of 10^8 viral particles of the Ad5-Gag vaccine. Cross-clade recognition of Gag from clades A and C HIV-1 was demonstrated in more than 75% of the responders, suggesting that this vaccine may be immunogenic across diverse populations. Subjects with preexisting high neutralizing antibody titers to Ad5 were less likely to develop Gag-specific immune responses following immunization. This barrier may be overcome by using higher doses of the Ad5-Gag vaccine, or using a DNA prime/Ad5 boost approach. Both of these modalities are currently being tested. Encouragingly, both vaccines were well tolerated and few minor adverse effects were reported.

Emini discussed a survey of HIV-1-infected subjects in the United States, Brazil, Thailand, and Malawi that showed a remarkable degree of similarity among diverse population groups in the major proteins targeted by anti-HIV-1 T-cell responses and in the magnitude of T-cell responses. A high degree of cross-clade recognition for T-cell responses against HIV-1 Gag and Nef was observed between clades A, B, and C. These results are encouraging and suggest, as mentioned previously, the possibility that vaccines encoding structural HIV proteins of one clade may be immunogenic across diverse populations.

Although promising in terms of immunogenicity, Ad5 vector-based vac-

cines may be limited in their utility in populations with a high prevalence of preexisting antibodies to the adenovirus type 5. One potential way out of this conundrum is with the use of adenoviruses derived from another primate species. Ertl (Fitzgerald et al, Abstract LB4) presented data on a novel replication-defective adenoviral vaccine carrier based on E1-deleted recombinants of the chimpanzee serotype 68 (AdC68). The AdC68 construct encoding the HIV-1 Gag protein induced a vigorous Gag-specific CD8+ T-cell response which was not inhibited in animals pre-immune to human Ad5.

Although the HIV or SIV regulatory proteins are attractive vaccine candidates and have yielded promising results in previous nonhuman primate studies, a trial using HIV-1 IIIIB or 89.6p Tat or Tat toxoid did not show any protection in rhesus macaques, despite induction of vigorous Tat-specific humoral and cellular responses in a subset of animals (Silvera et al, Abstract 289-W).

Dendritic cells are highly immunogenic and thus provide an attractive vehicle for delivery of a vaccine antigen candidate. This approach was tested in rhesus macaques (Abstracts 73, 286-W, and 312-W). In a presentation by Zhu and colleagues (Abstract 73), autologous myeloid DCs generated in vitro after positive selection and culturing with IL-4 and GM-CSF were pulsed with chemically inactivated SIV_{mne} and matured in vitro. Mature autologous DCs were infused subcutaneously 48 hours after the antigen pulse. Six infusions of DCs were given and the macaques were challenged with pathogenic SIV_{mne} 3 weeks after the last DC infusion. Seven of 10 challenged macaques had lower viral loads, and all animals maintained their CD4+ T-cell counts, in contrast to the control macaques. Surprisingly, T-cell responses as assessed by proliferative and ELISPOT assays were observed only in a subset of vaccinated animals and were generally weak. Further studies are warranted to optimize this potentially promising approach.

Liszewicz and colleagues (Abstracts 286-W and 312-W) have developed another novel vaccination approach designed to enhance mobilization of

antigen-specific DCs in vivo. They used a plasmid DNA vaccine encoding a replication- and integration-defective simian-human immunodeficiency virus (SHIV; DermaVir, Georgetown University Research Institute for Genetic and Human Therapy, Washington, DC), which is formulated in polyethylenimine-mannose (PEIm). Topical application of the DNA vaccine to shaved skin results in mobilization of LCs to the dermis. PEIm facilitates transduction of LCs in the epidermis and uptake of DNA by the LCs. DNA-expressing LCs were shown to migrate to the T-cell areas of the draining lymph node and elicit a vigorous SIV-specific CD8+ T-cell response (Abstract 286-W). When macaques with late-stage AIDS that were receiving STI-HAART were immunized topically with DermaVir, there was a dramatic decline in the rate of viral load rebound so that viral load gradually declined to undetectable levels (Abstract 312-W). Viral control was associated with induction of vigorous SIV-specific T-cell responses. These results suggest that there is a reserve of functional DCs even in late-stage AIDS, and that use of autologous DCs as a vehicle may be a promising approach. Further studies will be required to determine the efficacy of these vaccine approaches in protecting or mitigating the effects of HIV/SIV infection.

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Conference Abstracts Cited in the Text

The full text of the abstracts is available online at www.retroconference.org.

22. Dynamics of T-Lymphocyte Turnover in Sooty Mangabey, a Nonpathogenic Host of Simian Immunodeficiency Virus Infection. A. Kaur, A. F. Barabasz, M. Rosenzweig, H. McClure, M. B. Feinberg, and R. P. Johnson.

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28. Expression of DC-SIGN by Intestinal and Genital Mucosal Dendritic Cells in Humans and

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- 73.** Vaccination with Autologous Dendritic Cells Pulsed with AT-2-Inactivated Whole Virus Provides Partial Protection from Pathogenic SIV Challenge. Y.-D. Zhu, K. Koo, W. Sutton, L. Kuller, S.-L. Hu, R. Benveniste, E. Thomas, J. D. Lifson, and N. Haigwood.
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- 101.** Impact of Thymectomy on the Peripheral T-Cell Pool in Rhesus Macaques before and after Infection with SIV. S. T. Arron, A. Gettie, J. Blanchard, D. Ho, and L. Zhang.
- 102.** Kinetic Subpopulations of T Cells in Humans: Effects of HIV-1 Infection. M. Hellerstein, R. Hoh, M. Hanley, D. Cesar, D. Lee, R. Neese, and J. M. McCune.
- 103.** Prolonged and Preferential Survival of CD4+ T Lymphocytes in HIV-Infected Patients Receiving IL-2 Therapy. J. Kovacs, R. A. Lempicki, I. A. Sidorov, J. W. Adelsberger, G. Kelly, J. A. Metcalf, R. T. Davey, J. Falloon, M. A. Polis, J. Tavel, R. Stevens, L. Lambert, D. Hosack, H. J. Issaq, S. Fox, S. Leitman, M. W. Baseler, H. Masur, D. S. Dimitrov, and H. C. Lane.
- 104.** Intermittent IL-2 in HIV-Infected Patients Leads to Peripheral Expansion of a Novel Subset of Naïve CD4+ T Cells. I. Sereti, V. Natarajan, J. W. Adelsberger, J. A. Metcalf, H. Martinez-Wilson, K. Anthony, J. Kovacs, and H. C. Lane.
- 105.** HIV-1 Core Envelope Glycoproteins Deficient in T-Cell Helper Epitopes. C. Grundner, J. Kang, M. Koch, J. Sodroski, and R. Wyatt.
- 107.** Enhancement of HIV-1-Specific CTL Responses in the Peripheral Blood during STI Is Largely Due to Redistribution of Pre-Existing Virus-Specific CD8+ T Cells from the Lymph Nodes. M. Altfeld, J. V. Lunzen, N. Frahm, X. Yu, R. L. Eldridge, H. J. Stellbrink, and B. Walker.
- 109.** Constitutive and Induced Expression of DC-SIGN on Dendritic Cell and Macrophage Subpopulations in Situ and in Vitro. E. J. Soilleux, L. S. Morris, G. Leslie, J. Chehimi, Q. Luo, E. Levroney, J. Trowsdale, L. J. Montaner, R. W. Doms, D. Weissman, N. Coleman, and B. Lee.
- 203-T.** T Helper Cell Induction in Patients with Primary HIV-1 Infection and Long-Term Non-Progressive Disease. U. Malhotra, S. Holte, S. Emery, J. Lee, J. Marenza, L. Corey, and M. J. McElrath.
- 204-T.** Induction and Persistence of Envelope-Specific T Helper Cells Following Acute HIV-1 Infection. U. Malhotra, T. Zhu, E. Delpit, C. Huntsberry, A. Sette, R. Shankarappa, and M. J. McElrath.
- 208-T.** HIV-1 Replication *in Vivo* Is Associated with Suppression of HIV-1-Specific CD4+ T-Cell Proliferation but not with Loss of HIV-1-Specific IFN-gamma Producing CD4+ T Cells. B. Palmer, E. Boritz, and C. C. Wilson.
- 215-T.** Anergy State of HIV-Specific CD4+ T Cells Detected by Class II Tetramers but Suppressed by Several Stimuli. B. Yassine-Diab, G. Breton, S. Younes, M. Connors, M. Lainesse, N. F. Bernard, K. MacDonald, M. Legault, J. P. Routy, and R. P. Sekaly.
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- 217-T.** Replicative Senescence of HIV-Specific CD8+ T Cells Defined by CD57. J. Brenchley, D. Ambrozak, N. Karandikar, M. Betts, J. Kuruppu, D. Douek, and R. Koup.
- 252-T.** Defects in Number and Surface Expression of Co-Stimulatory Molecules on CD11c+ and CD11c- Blood Dendritic Cells in HIV-1-Infected Individuals. M. A. Barron, N. Blyveis, B. Palmer, S. MaWhinney, and C. C. Wilson.
- 253-T.** Dendritic Cell Numbers and Function in an HIV-1-Infected Ugandan Population. G. Jones, P. Amjadi, C. Watera, J. Morlese, P. Kaleebu, J. Whitworth, F. Gotch, and J. Gilmour.
- 255-T.** Impaired Function of Isolated Blood Myeloid and Plasmacytoid Dendritic Cells from HIV-1-Infected Patients. H. Donaghy, B. Gazzard, F. Gotch, and S. Patterson.
- 286-W.** A Novel Topical DNA Vaccine (DermaVir) for the Induction of T-Cell Immunity. J. Lisziewicz, J. Xu, J. Trocio, L. Whitman, P. Markham, S. Arya, J.-P. Behr, and F. Lori.
- 289-W.** Vaccination of Rhesus Macaques with HIV-1 Tat Protein. P. Silvera, J. Greenhouse, J. Yalley-Ogunro, M. W. Richardson, J. Mirchandani, K. Khalili, J.-F. Zagury, M. G. Lewis, and J. Rappaport.
- 312-W.** Control of Viral Load Rebound during Treatment Interruptions in Macaques with AIDS Induced by a Novel Topical DNA Immunization (DermaVir). J. Lisziewicz, J. Xu, J. Trocio, L. Whitman, M. G. Lewis, and F. Lori.
- 511-M.** Increased Thymic Mass and Circulating Naïve CD4+ T-Cell Counts in HIV-1-Infected Adults Treated with Growth Hormone. L. Napolitano, J. Lo, M. Gotway, K. Mulligan, J. D. Barbour, D. Schmidt, R. Halvorsen, C. Stoddart, M. Schambelan, J. M. McCune.
- 513-M.** Growth Hormone Enhances Thymocyte Development and Induces Differentiation into Functional Peripheral T Cells in HAART Treated HIV-1+ Patients. A. Pires, J. Pido-Lopez, G. Moyle, B. Gazzard, F. Gotch, and N. Imami.
- L1.** SIV Reservoirs and Human Zoonotic Risk. B. H. Hahn.
- L2.** Dendritic Cell Responses and Host Interactions. M. Pope.
- L3.** Immune Reconstitution and Immunologic Responses to Antiretroviral Therapies: Implications for Therapeutic Strategies. B. Autran.
- L4.** Analysis of HIV-1 Envelope Glycoproteins and Neutralizing Antibodies. P. Kwong, S. Majeed, S.-H. Xiang, R. Gupta, L. Wang, M. Doyle, D. Casper, W. Hendrickson, J. Sodroski, and R. Wyatt.
- L5.** A Potential HIV-1 Vaccine Using a Replication-Defective Adenoviral Vaccine Vector. E. Emini.
- LB1.** Molecular Characterization of a Novel Simian Immunodeficiency Virus with a Vpu Gene from Greater Spot-Nosed Monkeys (*Cercopithecus nictitans*) from Cameroon. V. Courgnaud, M. Salemi, X. Pourrut, E. Mpoudi-Ngole, B. Abela, P. Auzel, F. Bibollet-Ruche, B. Hahn, A. M. Vandamme, E. Delaporte, and M. Peeters.
- LB4.** A Novel Adenoviral Vaccine Based on a Chimpanzee Derived Isolate as a Vaccine Carrier for HIV-1 Gag. J. Fitzgerald, G.-P. Gao, J. Wilson, and H. C. J. Ertl.
- LB5.** Rapid Evolution of the Neutralizing Antibody Response following Primary HIV Infection. D. D. Richman, T. Wrin, S. Little, and C. Petropoulos.
- LB7.** HIV Preferentially Infects HIV-Specific CD4 T Cells. D. Douek, J. Brenchley, M. Betts, D. Ambrozak, B. Hill, Y. Okamoto, J. Casazza, J. Kuruppu, K. Kunstman, M. Dybul, A. Oxenius, D. Price, M. Connors, and R. Koup. *Published as:* Douek D, Brenchley J, Betts M, et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature.* 2002;417:95-98.
- S9.** T-Cell Dynamics in HIV Disease. A. S. Perelson.
- S10.** Identification of Dynamically Distinct Subpopulations of T Lymphocytes that are Differentially Affected by HIV. J. A. Kovacs.
- S11.** Role of Immune Hyperactivation and Failing Thymic Homeostasis in the Pathogenesis of AIDS. F. Miedema, M. D. Hazenberg, S. A. Otto, H. Schuitemaker, and R. J. de Boer.
- S12.** Mechanisms of T-Cell Depletion in HIV Disease. M. McCune.
- S24.** Nonpathogenic Infections in Animal Models. M. Feinberg.

Drug Resistance Mutations in HIV-1

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New information continues to accumulate on drug resistance mutations in HIV-1 and their relevance to clinical practice. The Drug Resistance Mutations Group of the International AIDS Society-USA (IAS-USA), originally a subgroup of the IAS-USA Resistance Testing Guidelines Panel,¹ monitors the influx of information and maintains a current list of mutations that impact drug susceptibility. This list is published as the IAS-USA Drug Resistance Mutations Figures and is updated regularly. The most recent update and adaptation was published in this journal and posted on the IAS-USA Web site (www.iasusa.org) in December 2001.² The new graphic presented here includes relevant data presented at the 9th Conference on Retroviruses and Opportunistic Infections, held in Seattle, Wash, in February 2002.³

New Graphic Display

These new figures feature a simpler graphic display of the information for easier reference. The nucleotide reverse transcriptase inhibitor (nRTI) category, including only tenofovir disoproxil fumarate (tenofovir DF), has been combined with the nucleoside reverse transcriptase inhibitor (nRTI) category. For all mutations, the codon number, rather than a mark, appears on the gene. Finally, the nRTI-associated mutations (NAMs) are indicated as pink lines in the background. Where a codon number marks the NAM, data indicate that the

mutation confers resistance to the specific drug.

Content Changes

In this revision, "primary" or "secondary" mutations in the protease gene have been redefined as "major" or "minor" mutations to avoid confusion regarding the order in which the mutations may occur. In general, major mutations are either (1) selected first by the drug; or (2) are shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or replication. By themselves, major mutations have an effect on phenotype. In general, these mutations tend to be the major contact residues for drug binding. On the protease inhibitor figure, the codon numbers for major mutations are marked in boldface type (see key).

Minor mutations, in general, appear later than major mutations, and by themselves have not been shown to have a significant effect on phenotype. In some cases, their effect may be to improve replicative fitness of virus carrying major mutations. On the protease gene figure, the codon numbers for minor mutations are marked in lightface type (see key).

Other changes to the figures include noting the effect of the E44D mutation on response to zidovudine, clarifying the role of the V108I and P225H mutations to efavirenz resistance, and adding an explanation of the M184V mutation and

its contribution to phenotypic resistance to abacavir.

Figure Pocket Card Available

The IAS-USA Drug Resistance Mutations Figures are now available on a pocket-sized folding card. To order copies, please call the IAS-USA at (415) 561-6720 or e-mail your request to resistance@iasusa.org.

Future Updates

The IAS-USA Drug Resistance Mutations Group will consider the next update after the 6th International Workshop on HIV Drug Resistance and Treatment Strategies, to be held in June 2002 in Seville, Spain. In the meantime, we welcome evidence-based comments to this paper. Please send your comments, along with the relevant supporting reference citations, to resistance@iasusa.org; submissions will be considered for the next update.

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MUTATIONS IN THE REVERSE TRANSCRIPTASE GENE ASSOCIATED WITH RESISTANCE TO REVERSE TRANSCRIPTASE INHIBITORS

Nucleoside and Nucleotide Reverse Transcriptase Inhibitors

Multi-nRTI Resistance: 151 Complex	A	V	F	F	Q					
	62	75	77	116	151					
	V	I	L	Y	M					
Multi-nRTI Resistance: 69 Insertion Complex ¹	M	A	D	▼	K			L	T	K
	41	62	67	69	70			210	215	219
	L	V	N	insert	R			W	Y	Q
								F	E	
Multi-nRTI Resistance ² (NAMs)	M		D		K			L	T	K
	41		67		70			210	215	219
	L		N		R			W	Y	Q
								F	E	
Zidovudine ³⁻⁵	M		D		K			L	T	K
	41		67		70			210	215	219
	L		N		R			W	Y	Q
								F	E	
Didanosine ⁶			K		L					M
		65			74					184
		R			V					V
Zalcitabine			K	T	L					M
		65	69		74					184
		R	D		V					V
Stavudine ^{3,7}	M		D		K	V		L	T	K
	41		67		70	75		210	215	219
	L		N		R	T M S A		W	Y	Q
								F	E	
Abacavir ⁸	M		K	D	K	L		Y		M
	41		65	67	70	74		115		184
	L		R	N	R	V		F		V
										W
										Y
										Q
										E
Lamivudine ⁹	E							V		M
	44							118		184
	D							I		V
										I
Tenofovir DF ^{3,10}			K							
		65								
		R								

Nonnucleoside Reverse Transcriptase Inhibitors

Multi-NNRTI Resistance ¹¹					K					Y
					103					188
					N					L
Multi-NNRTI Resistance (accumulation of mutations) ¹²			L	V				Y	G	M
			100	106				181	190	230
			I	A				C	S	L
								I	A	
Nevirapine			L	K	V	V		Y	Y	G
			100	103	106	108		181	188	190
			I	N	A	I		C	C	A
								I	L	
									H	
Delavirdine ¹³					K			Y		P
					103			181		236
					N			C		L
Efavirenz ^{13,14}			L	K	V			Y	Y	G
			100	103	108			181	188	190
			I	N	I			C	L	P
								I	S	H
									A	

Footnotes

¹The 69 insertion complex, consisting of a mutation at codon 69 (typically T69S) and followed by an insertion of 2 or more amino acids (S-S, S-A, S-G, or others), is associated with resistance to several nRTIs. The 69 insertion is often accompanied by mutations at other sites. Some other amino acid changes from the wild-type T in codon 69 without the insertion may also be associated with broad nRTI resistance.

²Multi-nRTI-associated mutations (NAMs): mutations associated with cross-resistance to nRTIs (except lamivudine).

³The reverse transcriptase mutation M184V may enhance susceptibility. This effect may be overcome by an accumulation of NAMs. The clinical significance of this effect is not known.

⁴One study reported that the E44D or V118I mutation confers lamivudine resistance in a zidovudine-resistant background (Hertogs et al, *Antimicrob Agents Chemother*, 2000). Analysis from AIDS Clinical Trials Group 241 associated the E44D mutation with a significantly worse response to treatment with zidovudine and didanosine, with or without nevirapine (Precious et al, *AIDS*, 2000).

⁵The D/C/S substitutions in reverse transcriptase codon 215 do not confer zidovudine resistance and suggest that virus evolved from the zidovudine-resistant mutant T215Y to a variant that is more fit in the absence of drug. In vitro studies indicate that T215Y may emerge quickly from T215D/C/S in the presence of drug; in vivo relevance is possible but not yet proven.

⁶One of the following (K65R, L74V) by itself or a combination of a few of the following (NAMs, E44D, T69D/N, V118I) can lead to didanosine resistance.

⁷V75T/M/S/A are seldom observed in patients in whom stavudine has failed.

⁸When present with NAMs, the M184V mutation is selected by abacavir and contributes to phenotypic resistance to

abacavir. However, when present alone, the M184V mutation does not appear to be associated with a reduced virologic response to abacavir.

⁹One article reports that the E44D or V118I mutation confers low-level resistance to lamivudine when accompanied by several of the NAMs (M41L, D67N, L210W, T215Y/F, K219Q/E) in the absence of a concurrent M184V mutation (Hertogs et al, *Antimicrob Agents Chemother*, 2000). One abstract (D'Arminio-Monforte et al, 8th CROI, 2001), reported no association over the short term between E44D or V118I and virologic response to a lamivudine-containing combination regimen.

¹⁰In vitro data suggest that 4 or more NAMs (M41L, D67N, K70R, L210W, T215Y/F, K219Q/E) will lead to a significant degree of resistance; the actual clinical cut-off for tenofovir DF IC₅₀ or a detailed relationship between specific multiple NAMs and tenofovir DF IC₅₀ has not yet been published. Clinical trial results indicate reduced plasma HIV-1 RNA responses to tenofovir DF in groups of patients in whose plasma virus 3 or more NAMs, including either M41L or L210W, were identified (Miller et al, 9th CROI 2002). The group of patients with plasma virus in which any accumulation of D67N, K70R, T215Y/F, or K219Q/E were identified (in the absence of detection of M41L or L210W) did not have a diminished average HIV-1 RNA response to tenofovir DF in that data set.

¹¹The K103N or Y188L mutation by itself can substantially reduce the clinical utility of all currently approved NNRTIs.

¹²Accumulation of these mutations (2 or more) substantially reduces the clinical utility of all of the currently approved NNRTIs.

¹³There are some in vitro data suggesting that the Y318F mutation, alone or in the presence of K103N and Y181C, decreased susceptibility to delavirdine in primary HIV infection. This mutation was observed only rarely in clinical isolates. An effect of the Y318F mutation on efavirenz susceptibility in vitro was detected if the K103N mutation was also

present. However, confirmatory data and/or analyses in clinical HIV infection are needed to confirm clinical relevance.

¹⁴V108I and P225H each contribute to efavirenz resistance when present in combination with other NNRTI mutations. Although V108I or P225H alone does not confer measurable resistance in laboratory strains of HIV-1, their presence in a clinical isolate may indicate prior selection for efavirenz-resistant variants.

¹⁵The Drug Resistance Mutations Group has reclassified resistance mutations in the protease gene as either “major” or “minor” rather than “primary” or “secondary” (if known).

Major: In general, major mutations are either (1) selected first by the drug; or (2) are shown at the biochemical or virologic level to lead to an alteration in drug binding or an inhibition of viral activity or viral replication. By themselves, major mutations have an effect on phenotype. In general, these mutations tend to be the major contact residues for drug binding.

Minor: In general, minor mutations appear later than major mutations, and by themselves have not been shown to have a significant effect on phenotype. In some cases, their effect may be to improve replicative fitness of virus carrying major mutations.

¹⁶Accumulation of these mutations (4 or 5 or more) is likely to cause multi-protease inhibitor resistance.

¹⁷For indinavir, the mutations listed as major may not be the first mutations selected, but they are present in most patient isolates in combination with other mutations.

¹⁸Major and minor mutations have not been designated for lopinavir/ritonavir-associated resistance since there are currently no clear data defining degrees of influence with this drug combination. The accumulation of 6 or more of these mutations is associated with a diminished response to lopinavir/ritonavir. The product information states that 7 or

8 mutations confer resistance to the drug. However, more recent data suggest as few as 4 mutations can be associated with high-level resistance (Prado et al, *AIDS*, 2002). Further clinical experience and research are needed to better define the mutations that affect the effectiveness of lopinavir/ritonavir.

¹⁹Protease mutation L63P is common in viruses that have never been exposed to protease inhibitors (Kozal et al, *Nat Med*, 1996) and may be more prevalent in viruses from patients in whom a protease inhibitor-containing regimen has failed. However, by itself, protease mutation L63P does not cause any appreciable increase in the IC₅₀ for any protease inhibitor. L63P is listed for lopinavir/ritonavir (and not any other protease inhibitor) because the prescribing information approved by the US Food and Drug Administration lists it as one of the numerous mutations that together predict a lack of viral load response to lopinavir/ritonavir-containing regimens.

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Perspective

New Insights in HIV and Hepatitis C Virus Coinfection

At the International AIDS Society–USA course in Los Angeles in March 2002, Margaret J. Koziel, MD, discussed recent studies of HIV and hepatitis C virus coinfection and its effect on the course and management of both diseases.

Characteristics of HCV Infection

Initial infection with hepatitis C virus (HCV) is characterized by rapid viral replication. Alanine aminotransferase (ALT) levels increase dramatically over the course of the initial 1 to 3 months but may be highly variable thereafter. Following the initial period of viremia, humoral and cellular immune responses develop, but in most cases are insufficient to clear the infection (Liang et al, *Ann Intern Med*, 2000). HCV has 6 major subtypes; in the United States, approximately 70% of HCV isolates from infected patients are genotype 1. Multiple strains of HCV are present in an individual patient; hence, the virus exists as a quasi species.

HCV infection rates based on antibody-positive status in individuals at risk for HIV infection include rates of 70% to 80% in injection drug users and 8% to 10% in men who have sex with men. A recent study in an AIDS Clinical Trials Group population, which was representative of all patients with HIV in the United States, showed an overall HCV seroprevalence rate of 37% (Sherman et al, *Clin Infect Dis*, 2002). In contrast, studies in volunteer blood donors in the United States indicate a seroprevalence rate of 0.4%.

Approximately 80% to 85% of individuals with acute HCV infection develop chronic infection (defined as continuing viral replication). The mechanisms that permit apparent clearance in the 15% to

20% in whom chronic infection is not established remain unclear, although infection with a narrow range of viral quasi species and more vigorous humoral and cellular immune responses may be major factors (Farci and Purcell, *Semin Liver Dis*, 2000).

Over the course of about 20 years, approximately 20% of individuals with chronic infection develop cirrhosis and some patients may progress to death from liver failure or hepatocellular cancer. However, progression appears to be highly variable. In studies of progression to cirrhosis after known exposure, rates have varied from 2.4% over 17 years (in a study of women in Ireland who were infected via contaminated anti-D[RH1] immune globulin) to 32.3% over 7.5 years (in patients in Italy infected via transfusion). Factors associated with poor prognosis for chronic HCV infection include male sex, age at HCV acquisition of more than 40 years, alcohol consumption, iron overload, and immunosuppression (Thomas et al, *JAMA*, 2000).

The incidence of HCV infection dropped dramatically after the mid-1980s; the reasons for this decrease remain unclear, although it likely reflects

changes in practice in injection drug use. However, the prevalence of chronic infection is increasing and is expected to triple from current rates by about 2015 (Armstrong et al, *Hepatology*, 2000). This projected increase is of great concern, since the health care system is already stressed at current levels of liver transplantation and expenditures for chronic liver disease.

Rational use of HCV diagnostic tests in patient management includes serologic testing and qualitative HCV RNA testing for diagnosis, and liver biopsy for prognosis. ALT level can be quite variable and is not a reliable indicator of severity of disease. Studies in asymptomatic individuals positive for HCV RNA indicate that ALT levels are normal in 31%, increased by less than 2 times the upper limit of normal (ULN) in 42%, and increased by greater than 2 and 3 times the ULN in 15% and 12%, respectively. Similarly, neither the genotypic nor the viral load is an accurate indicator of the degree of histologic injury (Goedert et al, *J Infect Dis*, 2001). The degree of fibrosis on initial liver biopsy provides important prognostic information; as shown in Figure 1 (Yano et al, *Hepatology*, 1996), the rate of progression

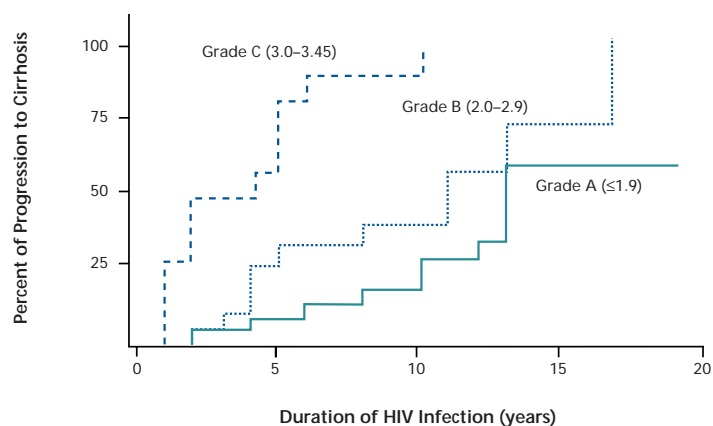


Figure 1. Rate of progression to cirrhosis in chronic hepatitis C virus (HCV) infection according to degree of fibrosis on initial liver biopsy (grades A to C in the METAVIR scoring system for fibrosis indicate progressively higher fibrosis score and worsening fibrosis on a 0-4 scale). Adapted with permission from Yano et al, *Hepatology*, 1996.

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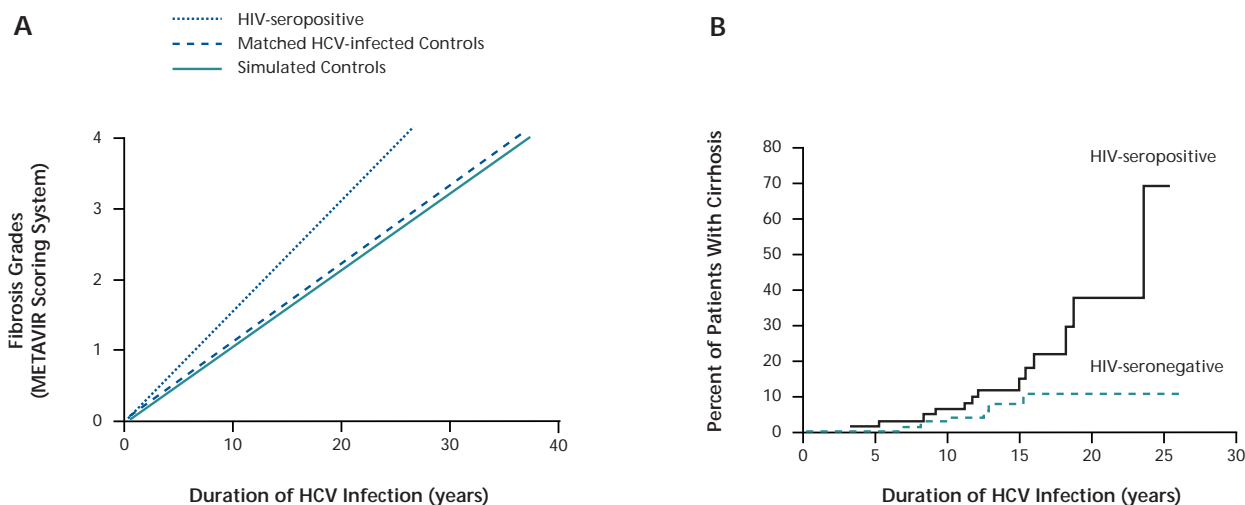


Figure 2A. Rates of progression of liver fibrosis in chronic hepatitis C virus (HCV) infection in patients with HIV infection (n=122), matched HCV-infected controls without HIV infection (n=122), and simulated controls (for which fibrosis progression was predicted using a mathematical model that corrects for age at infection, sex, and alcohol consumption [grams/day]) (n=122). Adapted with permission from Benhamou et al, *Hepatology*, 1999. **B.** Rates of progression to cirrhosis in HCV-infected patients with and without HIV disease. Adapted with permission from Di Martino et al, *Hepatology*, 2001.

to cirrhosis is markedly increased in those with more severe fibrosis.

Genotyping and assessment of HCV viral load are useful for determining duration of anti-HCV treatment; however, response to treatment can be assessed by quantitative HCV RNA assay. Management of patients with HCV infection must include counseling regarding avoidance of alcohol consumption and any drug that might damage the liver (including limiting the use of acetaminophen). It is also important that patients receive vaccination against hepatitis A virus, especially those at increased risk of acquiring infection (eg, men who have sex with men and injection drug users), since the relative risk of hepatic failure is increased 8-fold in patients with HCV infection who acquire hepatitis A infection.

Effect of HIV Infection on HCV Disease

A variety of data indicate that coinfection with HIV and HCV results in increased HCV viral load as immune deficiency progresses, increased risk of HCV perinatal and sexual transmission, and possible alteration of HCV-specific immune responses and accelerated pro-

gression of HCV disease. However, the most important clinical issue is whether HIV coinfection results in more rapid progression of liver disease. Figure 2A shows the more rapid advance in fibrosis grade occurring in coinfecting patients versus HCV-infected matched controls in a study using paired liver biopsies (Benhamou et al, *Hepatology*, 1999). Figure 2B shows the more rapid progression to cirrhosis among coinfecting patients in another study using paired liver biopsies, with risk of cirrhosis at 20 years being about 40% in coinfecting patients compared with about 10% in those with HCV disease alone (Di Martino et al, *Hepatology*, 2001).

A recent meta-analysis of 6 studies used liver biopsy data, documented cirrhosis, or data on hospitalized patients dying from liver failure or hepatocellular carcinoma to assess outcome (Graham et al, *Clin Infect Dis*, 2001). The results indicate that risk of progression to cirrhosis in HCV disease is increased 3.6-fold in HIV-infected patients. A recent study by Bica and colleagues (*Clin Infect Dis*, 2001) shows a marked increase in mortality from end-stage liver disease (ESLD) among cohorts of HIV-infected patients, with the proportion of deaths attributable to ESLD-related deaths increasing from 11% in 1991 and 14% in

1996 to 50% in 1998. One third of patients in the 1998 cohort had a recent history of discontinuing potent antiretroviral therapy due to hepatic toxicity. More than half who died with ESLD had either plasma HIV RNA levels below assay detection limits or CD4+ cell counts greater than 200/ μ L 6 months prior to death. However, many important questions remain, such as whether these rates will change in the era of potent antiretroviral therapy.

Effect of HCV Infection on HIV Disease

Coinfection with HCV appears to be associated with diminished immune reconstitution with antiretroviral therapy in HIV-infected patients. In a cohort study of 3111 HIV-infected patients, HCV infection was associated with a significantly reduced likelihood of achieving a CD4+ cell increase of greater than or equal to 50/ μ L (Greub et al, *Lancet*, 2000). This significant association was maintained when analysis was restricted to the 1596 patients with plasma HIV RNA levels persistently less than 400 copies/mL. Although diminished capacity for immune reconstitution has been observed in other studies as well, not all

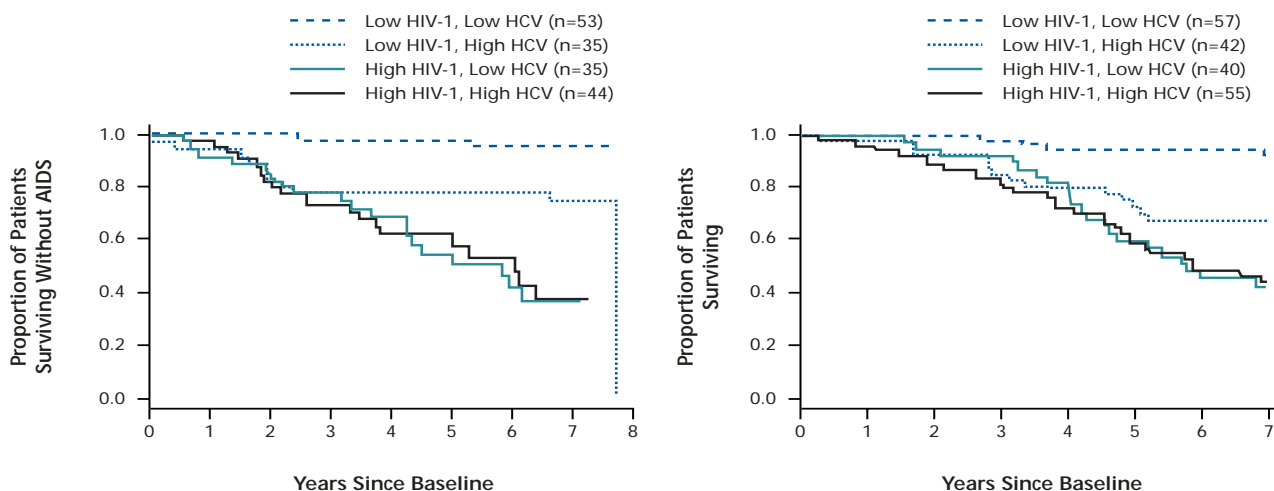


Figure 3. Proportion of patients coinfecting with HIV and hepatitis C virus (HCV) surviving without AIDS (left) and proportion surviving (right) according to low or high HCV and HIV viral load values at the time of study enrollment (baseline). Adapted with permission from Daar et al, *J Infect Dis*, 2001.

studies support this finding. The impact of HCV infection on HIV disease progression to AIDS remains unclear. As shown in Figure 3, however, there are data indicating a more rapid progression to AIDS and to death among coinfecting patients with low HIV viral load who have high HCV viral load than among those with low HCV viral load (Daar et al, *J Infect Dis*, 2001).

HCV infection may also complicate management of HIV disease by increasing the risk of liver toxicity of protease inhibitors and toxicity of antituberculosis medications. One study has indicated increased risk of nephrolithiasis associated with indinavir in coinfecting patients (Brodie et al, *AIDS*, 1998). In addition, there have been case reports of liver failure during immune reconstitution; it has been suggested that such failure may occur in some patients as a result of the increased recognition and destruction of HCV-infected hepatocytes during immune reconstitution under potent antiretroviral therapy (Price et al, *J Clin Virol*, 2001; John et al, *AIDS*, 1998).

Effect of HCV Infection on Antiretroviral Therapy

A number of studies indicate that HCV-infected patients are at increased risk of hepatotoxicity associated with potent antiretroviral regimens. In one recent

study in HIV-infected patients receiving potent therapy, grade 3 or 4 hepatotoxicity was more common in patients with HCV or hepatitis B virus infection than in patients without such coinfection. It was noted, however, that 88% of the coinfecting patients tolerated non-ritonavir regimens without grade 3 or 4 toxicity (Sulkowski et al, *JAMA*, 2000). Risk factors for hepatotoxicity included ritonavir use and CD4+ cell count increase to greater than 500/ μ L.

In a subsequent study, hepatotoxicity, defined as any increase in liver enzymes, occurred in 14.9% of HCV-infected patients versus 5.6% of patients

without HCV coinfection (Aceti et al, *J Acquir Immune Defic Syndr*, 2002). Severe toxicity, defined as a greater than 5-fold increase in enzymes, occurred in 4.8% versus 1.1% of patients. Risk of increased ALT was associated with all protease inhibitors, but only ritonavir use was associated with severe hepatotoxicity. In another study (Monforte et al, *J Acquir Immune Defic Syndr*, 2001), the hazard ratio for aspartate aminotransferase elevation to greater than 200 U/L was 4.01 for HCV-infected patients compared with those without HCV infection. This study found no association of liver enzyme increases with any particular

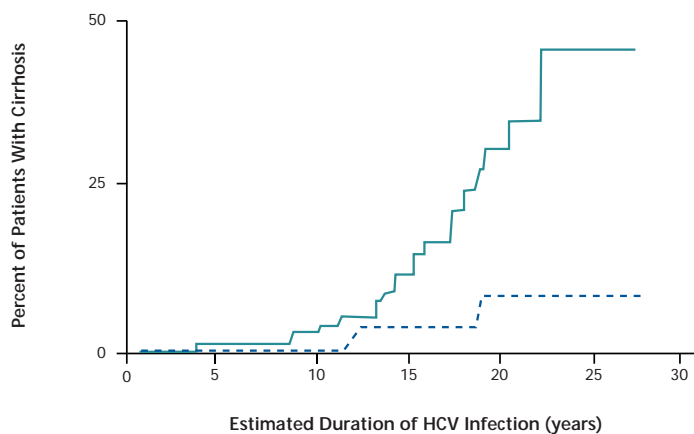


Figure 4. Proportion of patients coinfecting with HIV and hepatitis C virus (HCV) with cirrhosis according to treatment (blue) or no treatment (green) with protease inhibitor-containing regimens. Adapted with permission from Benhamou et al, *Hepatology*, 2001.

antiretroviral regimen. It is important to note that trials of new agents often specifically exclude patients with evidence of chronic liver disease due to HCV infection, so that understanding the true degree of risk from a particular regimen may be difficult until there is extensive phase 4 experience.

Effect of Antiretroviral Therapy on HCV Disease

One study has shown a large reduction in progression to cirrhosis in HIV/HCV-coinfected patients in association with the use of protease inhibitor-containing regimens versus no protease inhibitor treatment (Figure 4; Benhamou et al, *Hepatology*, 2001). However, other recent data do not support this finding. It is possible that studies comparing the effects of protease inhibitor therapy versus non-protease inhibitor treatment are open to selection bias, since patients with more severe HCV-associated liver disease may be precluded from receiving protease inhibitor treatment owing to increased risk of hepatotoxicity. This is obviously an important area for future research.

Treatment of HCV Infection in Patients With HIV Disease

The current recommended drug treatment for HCV infection is pegylated interferon alfa plus ribavirin. Pegylated interferon alfa-2b/ribavirin treatment was associated with sustained virologic response (absence of detectable virus for 6 months after treatment) in 54% of 511 patients, compared with a 47% response rate with the formerly used regimen of thrice weekly standard interferon alfa-2b/ribavirin (Manns et al, *Lancet*, 2001). Among patients with genotype 1 HCV infection, who are the majority of patients in the United States, sustained response rates with the combination interferon alfa/ribavirin regimens have been reported at 42% with pegylated interferon alfa-2b at 1.5 µg/kg (significantly greater than standard interferon alfa/ribavirin treatment), 34% with pegylated interferon alfa-2b at 0.5 µg/kg, and 33% with standard interferon alfa at 3 million units. Response rates with these regimens in patients infected with HCV genotypes 2 or 3 were reported at 82%, 80%, and 79%, respectively.

A number of small studies have examined the effects of interferon

alfa/ribavirin treatment in patients with HIV disease (Table 1), with most having been reported only in abstract form. The 3 fully reported studies showed sustained response rates of 35%, 14%, and 40% in small groups of patients (Landau et al, *AIDS*, 2000; Zylberberg et al, *Gut*, 2000; Sauleda et al, *Hepatology*, 2001). Only 1 study has reported the effect of pegylated interferon alfa/ribavirin in coinfecting patients, with a 33% sustained response rate (Pérez-Olmeda et al, 9th CROI, 2002). However, this European study included a much greater proportion of patients with genotype 3 HCV infection than is characteristic of HCV-infected populations in the United States, which would tend to increase the proportion of sustained responders. Although the virologic response rates at least appear to be lower in coinfecting patients than in those without HIV infection based on the extant literature, there are data suggesting that histologic outcome of treatment does not differ between coinfecting patients and those without HIV infection. As shown in Figure 5, a recent study showed no significant differences in changes in inflammation grade and changes in fibrosis grade with treatment for HCV liver dis-

Table 1. Reported Studies of Interferon Alfa/Ribavirin Treatment for HCV Disease in Patients with HIV Infection

Study	N	Overall Response		Sustained Response		Comments
		Number	Percent	Number	Percent	
Landau et al, <i>AIDS</i> , 2000	20	10/20	50%	7/20	35%	Median CD4+ cell count of 350/µL. No change in plasma HIV RNA levels.
Zylberberg et al, <i>Gut</i> , 2000	21	6/21	29%	3/21	14%	2/10 subjects with plasma HIV RNA levels below detection limit at baseline had HIV RNA increase to detectable levels.
Bochet et al (abstract)	56	-	-	10/56	18%	26% dropout rate.
Sauleda et al, <i>Hepatology</i> , 2001	20	-	-	-	40%	2/10 subjects with plasma HIV RNA levels below detection limit at baseline had HIV RNA increase to detectable levels.
Kostman et al (abstract)	53	-	26%	-	-	54% dropout rate.
Bini et al (abstract)	32	-	-	-	22%	25% dropout rate.
Pérez-Olmeda et al (abstract)*	65	-	50%	-	33%	14% dropout rate.

HCV indicates hepatitis C virus.

*Treatment was pegylated interferon alfa/ribavirin.

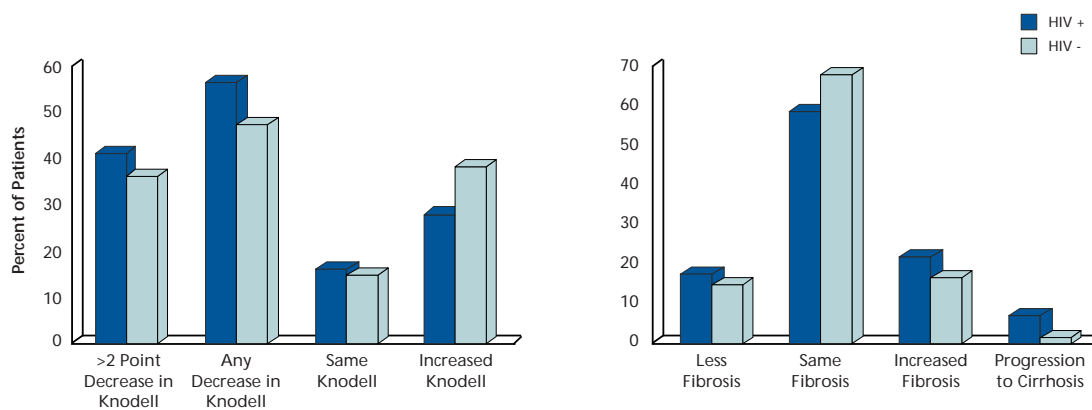


Figure 5. Histologic outcome after treatment of hepatitis C virus (HCV) disease in patients with (dark blue) or without (light green) HIV disease. Change in inflammation on Knodell scale (decrease = less inflammation) is shown on left; change in fibrosis is shown on right. Adapted from Di Martino, *AIDS*, 2002.

ease according to HIV infection status (Di Martino et al, *AIDS*, 2002).

Adverse effects of interferon alfa treatment include a characteristic flu-like syndrome, anemia (myelosuppression) gastrointestinal symptoms, alopecia, somnolence, depression (including suicidal ideation), thrombocytopenia, and neutropenia, including a reduction in CD4+ cell count but not percentage of CD4+ cells. Ribavirin, which is a nucleoside analogue, causes a hemolytic anemia in 8% to 10% of nonimmunocompromised patients. In addition, there are theoretical concerns that ribavirin may interact with nucleoside reverse transcriptase inhibitors (nRTIs) to decrease efficacy of the nRTIs by decreasing intracellular phosphorylation and increase the risk of mitochondrial toxicity and metabolic adverse effects. Ribavirin treatment has been associated with lactic acidosis in patients receiving potent antiretroviral therapy, and zidovudine and ribavirin may be antagonistic in terms of their antiretroviral effects.

Summary

Available data suggest that patients coinfecting with HCV and HIV have an accelerated HCV disease course. HCV infection may impact HIV disease course by inhibiting immune reconstitution and impairing ability to use potent antiretroviral therapy and other HIV-related medications. There is a potential role for drug treatment for HCV infection when histologic disease is present in patients with HIV disease, although

additional data on the effectiveness of treatment in this population are needed. If interferon alfa/ribavirin therapy is used in HIV-infected patients, it is important to monitor for hepatotoxicity, HIV RNA level, CD4+ cell percentage, anemia, and immune reconstitution-related hepatic destruction during HCV therapy.

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Perspective**Incomplete Viral Suppression Under Potent Antiretroviral Therapy: Determinants of Treatment Outcome**

At the International AIDS Society–USA course in Los Angeles in March 2002, Steven G. Deeks, MD, discussed recent findings regarding the effects of antiretroviral therapy on HIV replicative capacity and the potential implications of these findings for treatment strategies.

Virologic failure of potent antiretroviral therapy remains common in clinical practice, and is often associated with the emergence of a drug-resistant HIV. One recent population-based study demonstrated that approximately 78% of viremic patients in clinical care harbor drug-resistant HIV (Richman et al, ICAAC, 2001). Many factors are known to contribute to the inability of potent antiretroviral therapy to completely suppress viral replication, including preexisting resistance, limited drug potency, nonadherence, altered drug metabolism, tissue compartmentalization, and, for unclear reasons, advanced HIV disease.

Although much is known about the causes of virologic failure, much remains unknown about its long-term clinical consequences. Patients in whom virologic failure occurs often continue to respond to their combination treatment, in that plasma HIV-1 RNA level does not return to pretreatment levels and CD4+ cell counts remain elevated for a prolonged duration. CD4+ T-cell count changes were recently studied in a cohort of 291 patients experiencing continuous virologic failure of protease inhibitor therapy, with most patients being on a second “failing” regimen (Deeks et al, *AIDS*, 2002). The majority of patients maintained some gain in CD4+ cell count above pretreatment levels after more than 3 years of

continuous virologic failure (Figure 1). The change in plasma HIV-1 RNA levels below the pretreatment baseline level was the single most important predictor of CD4+ T-cell outcomes in this cohort. Specifically, sustained CD4+ T-cell gains were observed as long as patients maintained a change in HIV-1 RNA level of at least 0.7 log₁₀ copies/mL below baseline.

Still, it is clear that virologic failure ultimately results in immunologic decline and clinical progression. A recent report from the Swiss HIV Cohort showed that the relative risk for progression to a new AIDS event or death was markedly increased according to increments in average plasma HIV-1 RNA level over time from the start of potent antiretroviral therapy. This result indicates that residual viral replication during therapy is predictive of disease progression (Egger et al, 9th CROI, 2002). In other words, although some patients do well for years with low to moderate levels of viremia, it is clearly preferable to achieve and maintain a plasma HIV-1 RNA level as low as possible.

Why Does Viral Load Often Remain Below Pretreatment Levels After Emergence of High-Level Resistance?

Plasma HIV-1 RNA levels often remain below pretreatment baseline levels after emergence of high-level drug resistance. Factors that may contribute to this phenomenon include viral replicative capacity or fitness, characteristics of the HIV-specific cellular immune response, and persistent activity of some drugs against resistant variants. The role of viral replicative fitness in viral replication under antiretroviral drug pressure has recently been investigated.

In an attempt to identify factors in the prolonged maintenance of relatively low and stable plasma HIV-1 RNA level despite the likelihood of significant antiretroviral resistance, investigations were performed (Deeks et al, *N Engl J Med*, 2001) in a group of patients with virologic failure who underwent a structured treatment interruption. All pa-

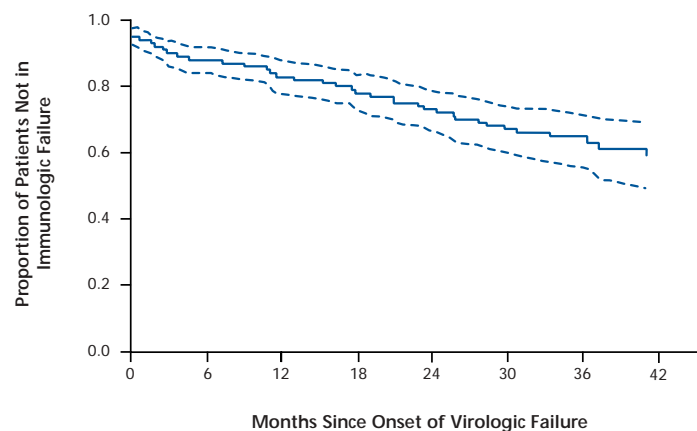


Figure 1. Proportion of 291 patients maintained on failing protease inhibitor-containing regimen in whom CD4+ cell count remained above pretreatment baseline level (ie, not in immunologic failure) for more than 3 years. Adapted with permission from Deeks et al, *AIDS*, 2002.

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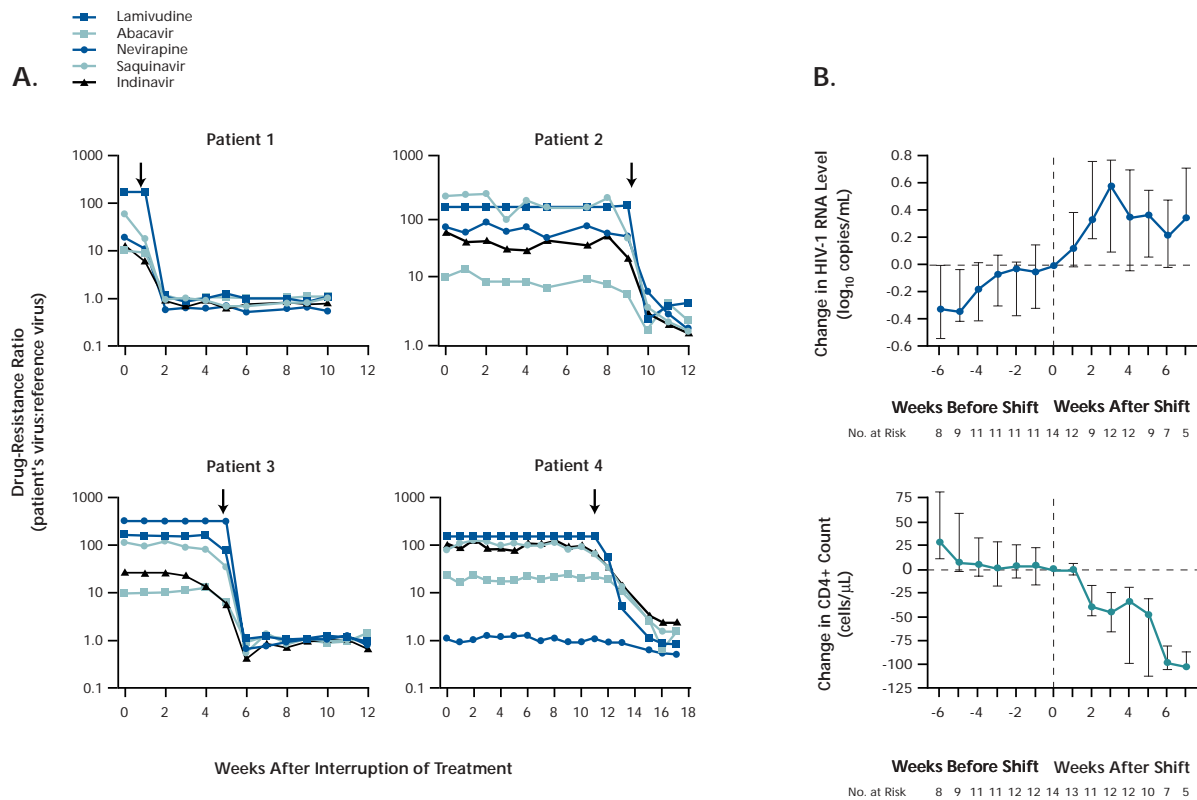


Figure 2A. Changes in drug resistance ratios after treatment interruption in 4 patients with continuous virologic failure. Arrows indicate onset of rapid switch to susceptible phenotype for all drugs simultaneously and also show time 0 in Figure 2B. B. Change in plasma HIV-1 RNA level (top) and CD4+ cell count (bottom) from time of switch from resistant to susceptible phenotype according to time before and after switch. Median values and interquartile ranges are shown. Number of patients contributing data at each time point is shown in the “at risk” line. Adapted with permission from Deeks et al, *N Engl J Med*, 2001.

tients were on long-term protease inhibitor regimens, had detectable plasma viremia, and exhibited clear evidence of protease inhibitor resistance. All patients stopped antiretroviral drugs and were followed up weekly.

With regard to drug resistance characteristics, most patients exhibited a similar pattern, as shown by data from 4 representative patients (Figure 2A). Levels of phenotypic resistance to protease inhibitors remained relatively stable over a period of weeks following treatment interruption. At a time point that differed among patients, a rapid dramatic shift took place in viral phenotype from resistant to wild-type virus, with a complete shift occurring over a median of 2 weeks. This rapid shift in drug resistance generally occurred for all drugs simultaneously. Analysis of plasma HIV-1 RNA level and CD4+ cell count changes before and after the shift in viral phenotype showed that the shift was associated with a marked increase in the

rate of HIV-1 RNA increase and the rate of CD4+ cell count decrease (Figure 2B).

The investigators hypothesized that the rapid increase in viremia in these patients during treatment interruption was due to the greater replicative fitness of the wild-type virus that rapidly emerged in this setting. To assess this hypothesis, they used a recently developed assay to measure viral replicative capacity. It should be noted that “replicative fitness” refers to the ability of a species or strain to compete in a defined environment with other species or strains (eg, the resistant virus is more fit than wild-type virus in an environment defined as a patient on potent antiretroviral therapy). Assays that measure viral fitness thus require direct competition between strains in vitro (mixing experiments) or in vivo; these traditional assays are laborious and expensive and can only be performed on a small number of samples. In contrast, the replicative capacity assay provides a

noncompetitive measure of the inherent ability of a given strain to replicate. It can be used with a large number of specimens and requires only a small amount of plasma.

In this recombinant virus assay, which was devised by modifying a phenotypic assay, vectors containing patient-derived HIV reverse transcriptase or protease undergo a single round of replication. The vector contains a luciferase gene that permits quantitation of replication that is then compared to the level of replication of a wild-type HIV reference strain. The result is provided as the ratio of study-strain replication to reference-strain replication. One potential drawback is that the assay measures only reverse transcriptase and protease activity, and thus does not account for other potential compensatory mechanisms in the virus that could affect replicative ability.

Use of the replicative capacity assay in samples from patients in the treat-

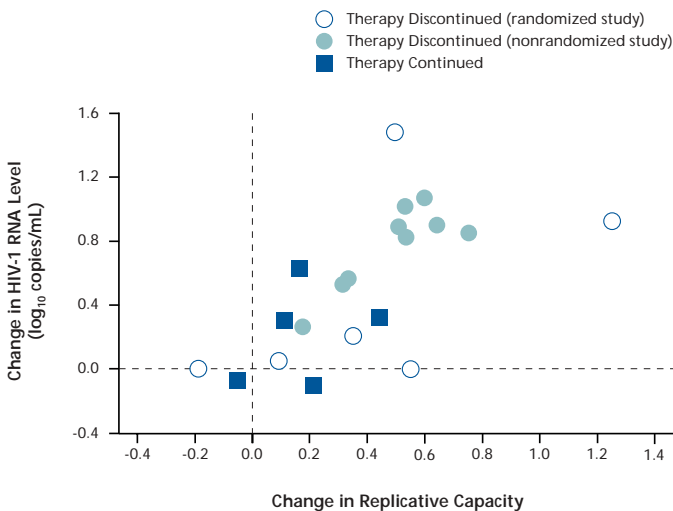


Figure 3. Association of change in plasma HIV-1 RNA level with change in replicative capacity after switch to wild-type phenotype in patients in whom therapy was discontinued (circles) and with continued therapy in patients with continuous virologic failure (squares). Adapted with permission from Deeks et al, *N Engl J Med*, 2001.

ment interruption study showed that change in replicative capacity from baseline to 12 weeks after treatment interruption was strongly correlated with change in plasma HIV-1 RNA level over this period (Figure 3). The relative fitness of the resistant and wild-type strains was assessed by quantitating relative proportions of the resistant and wild-type phenotypes in weekly samples from each patient, allowing calculation of the slopes of decrease in drug-resistant virus and increase in wild-type virus. These studies showed that the fitness differences between the wild-type and resistant virus averaged more than 50% per generation. The findings using this gold-standard measure of fitness correlated very strongly with findings on the replicative capacity assay, suggesting that the latter may provide a good indication of what is occurring in vivo. Overall, these findings indicate that the benefit observed in patients with persistent viremia on protease inhibitor regimens is at least in part a reflection of maintenance of a viral population with reduced replicative capacity.

Is There a Role for Viral Fitness Measurements in Clinical Management?

If measurement of viral fitness is to have a role in clinical management of HIV dis-

ease, it needs to provide information that is useful in predicting outcome in patients in whom virologic failure has occurred. To assess the predictive capability of fitness measurements, Barbour and colleagues (9th CROI, 2002) investigated viral evolution parameters in a separate cohort of 20 patients who remained on protease inhibitor regimens despite incomplete viral suppression. Plasma HIV-1 RNA level remained at less than 10,000 copies/mL over 24 months on the failing regimen in more than half of the patients studied. Ongoing viral replication in the presence of drug was associated with increasing protease inhibitor and nRTI resistance in most patients. Increased resistance was observed in some patients even as the plasma HIV-1 RNA level remained stable and low. Replicative capacity was low and remained low in most patients. Achievement of the steady-state level of viremia was characterized by a substantial reduction in viral evolution, and the drug-resistant variants remained the primary virus population at the end of follow-up.

These data suggest that it may be possible to monitor viral fitness over time and to predict long-term treatment outcome based on replicative capacity and level of drug resistance. However, there are other factors involved in maintenance of treatment benefit despite virologic failure, with additional data

suggesting that although reduced replicative capacity may be a necessary condition for durable benefit, it is not a sufficient condition. For example, it has previously been observed that immunologic and virologic benefit might not be achievable in patients with advanced disease despite presence of virus with extremely low replicative capacity. As with untreated patients, numerous factors are likely important determinants of long-term outcome in partially treated patients, and more than one such factor might be needed to ensure durable treatment benefit in the presence of drug-resistant HIV.

Altered Pathogenicity of Drug-Resistant HIV

It is clear that plasma HIV-1 RNA levels can remain partially suppressed in some patients despite the emergence of drug-resistant variants, and that this partial suppression is related to several factors. It is also clear that CD4+ T-cell counts can remain elevated in such patients, and that this sustained CD4+ T-cell benefit is partially explained by the treatment-mediated decrease in plasma HIV-1 RNA level. What remains unclear is whether sustained CD4+ T-cell gains occur for other reasons that are independent of the level of partial viral suppression. In other words, is there any evidence that for any given level of viremia, immunologic outcomes are improved for patients with drug-resistant HIV versus wild-type HIV?

The relative virulence or pathogenicity of the protease inhibitor-resistant virus was addressed in a study that examined the immunologic mechanisms underlying sustained increases in CD4+ cell count in patients with protease inhibitor-resistant viremia (Deeks et al, *J Infect Dis*, 2002). The investigators observed CD4+ cell turnover in vivo in untreated patients with wild-type virus, in patients with virologic failure with drug-resistant virus maintained on stable antiretroviral therapy, and in patients with plasma HIV-1 RNA below detection limits on potent therapy. The median fractional replacement rate of the total peripheral CD4+ T-cell population in patients with drug-resistant virus was significantly lower than that

observed in the untreated group ($P < .001$). However, the difference between the virologic failure group and the group with undetectable HIV-1 RNA levels was not significant ($P = .32$). Based on these fractional replacement rates, the estimated median CD4+ T-cell half-lives for the 3 groups were 68 days (virologic failure group), 22 days (untreated group), and 82 days (virologic "success" group). Similar trends were observed in the CD8+ T-cell population. These data indicate that the drug-resistant virus may be less virulent (ie, cause less CD4+ T-cell turnover) in vivo than the wild-type virus, even after controlling for the level of plasma viremia. Further research is needed to elucidate the complex dynamic between the host immune system and characteristics of the virus. In summary, some patients experience durable virologic and immunologic benefit despite incomplete viral suppression and the emergence of drug-resistant HIV. The mechanisms underlying this benefit are likely multifactorial and interrelated.

Host Genetics as Predictor of Treatment Outcome

Host genetic factors likely have a major influence on both HIV disease course and response to treatment. One intriguing strand of research in this regard involves the role of human leukocyte antigen (HLA) haplotypes in determining treatment outcome. Class I HLA molecules occur on all cells and present peptides to receptors on CD8+ T cells, whereas class II HLA molecules occur on antigen-presenting cells and present peptides to receptors on CD4+ T cells. HLA haplotypes vary widely among individuals, and it is likely that in every individual infected with HIV, only some components of the virus are presented to the immune system. Given the strong influence of genetic polymorphisms within HLA and the chemokine receptors on disease progression in untreated HIV infection, it is reasonable to assume that such polymorphisms are likely to predict the response to potent antiretroviral therapy. However, data supporting this hypothesis have been inconsistent (O'Brien et al, *AIDS*, 2000; Malhotra et al, *J Clin Invest*, 2001).

In a recent study to assess effects of viral and host genetics on drug resistance (Moore et al, 9th CROI, 2002), viral genotype and presence of specific host haplotypes were determined in a large cohort of patients with continued detectable viremia while receiving potent antiretroviral therapy. The V82A protease mutation associated with indinavir resistance was, as would be expected, significantly more common in patients receiving indinavir than in patients not receiving indinavir (odds ratio, 4.3). However, the presence of the HLA-A2 haplotype, which recognizes a portion of HIV protease overlapping the V82 amino acid residue, had an independent effect in predicting the presence of the V82A mutation (odds ratio, 5.4).

Such findings indicate that there are strong independent effects of drug and host immune pressure on viral evolution. Similar studies may help explain why, for example, the majority of patients who develop nelfinavir resistance do so via the D30N resistance mutation rather than the L90N pathway. Continued research in this area may ultimately allow prediction of viral evolutionary pathways in individual patients, enabling selection of drug treatment to maximize response and to avoid pathways that lead to cross-resistance. It is likely that the near future will bring a number of retrospective analyses of defined patient cohorts that shed additional light on the influence of host genetics on treatment response.

Conclusions

Maintenance of viral suppression to levels below 50 HIV-1 RNA copies/mL is not feasible in many patients with current antiretroviral therapy. Thus, a thorough understanding of the determinants of viral evolution and disease progression under potent antiretroviral therapy is needed to define better treatment strategies. In addition to using resistance testing, therapeutic drug monitoring, and adherence monitoring to optimize treatment outcome, clinical management may ultimately also include measures of viral replicative fitness, viral pathogenicity, and assessment of host genetics.

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Review

The Potential Role of Therapeutic Drug Monitoring in the Treatment of HIV Infection

John G. Gerber, MD, and Edward P. Acosta, PharmD

Abstract. Therapeutic drug monitoring (TDM) is increasingly being used in clinical practice in order to improve the therapeutic outcome in HIV infection. The use of TDM requires certain pharmacologic, analytical, and clinical criteria in order to interpret the plasma concentrations appropriately. In this context, we have reviewed whether there are enough data to recommend the widespread use of TDM in the treatment of HIV infection. Nucleoside reverse transcriptase inhibitors are prodrugs that require intracellular metabolism for activity, so as a group they would not qualify for TDM in plasma. Although TDM potentially could be helpful in improving the efficacy and reducing the toxicity of protease inhibitors and nonnucleoside reverse transcriptase inhibitors, without clearly defined therapeutic ranges for many of these drugs and with few prospective TDM trials showing efficacy, plasma TDM has to be considered as an experimental tool in most clinical settings.

Therapeutic drug monitoring (TDM) is defined as a strategy by which the dosing regimen for a patient is guided by repeated measurements of plasma drug concentrations. If the concentration is not within a predefined target range, the dose is adjusted to bring this level within this target range (Figure 1). The 2 main reasons to undertake TDM in clinical situations are to avoid drug toxicity and to improve therapeutic efficacy. The history of TDM in clinical medicine is relatively brief, even though the concept that both efficacy and toxicity of a drug are dose- and concentration-dependent has been well established. Use of TDM in clinical medicine has been clearly linked to the development of assays that are accurate, sensitive, specific, and have a rapid turnaround time.

Although TDM is used in the treatment of several diseases, there is very little rigorous scientific evidence that its use has improved clinical outcome in patients.¹ The use of TDM in the prevention of drug toxicity has a stronger basis than the use of TDM for improved efficacy. This should not be surprising since therapeutic outcome is multifactorial and includes the importance of individual drug-taking behavior. For the treatment of HIV infection, TDM has another layer of complexity: incomplete viral suppression during therapy may result in HIV mutations so that drug susceptibility may become a moving target. This is quite unique compared with other diseases where TDM has been applied, in which the target concentration range remains the same throughout therapy.

The use of TDM in the pharmacotherapy of HIV infection is gaining momentum despite the fact that there are no clear-cut therapeutic ranges established for any of the antiretroviral drugs. HIV treatment requires the concomitant use of multiple drugs for durable viral suppression; however, TDM usually involves the monitoring of only a single drug concentration. The utility of TDM to improve therapeutic outcomes in HIV-infected patients has not been definitively demonstrated in large clinical trials, and therefore its use should be considered investigational. In this article we will review the criteria required for the use of TDM in clinical medicine and evaluate how antiretroviral drugs fare in this regard. In addition, we will review data from the few prospective clinical trials that have been published or presented that address the utility of TDM in the management of HIV infection. Unfortunately, there are no prospective studies that have attempted to measure the cost-effectiveness of TDM in the treatment of HIV infection.

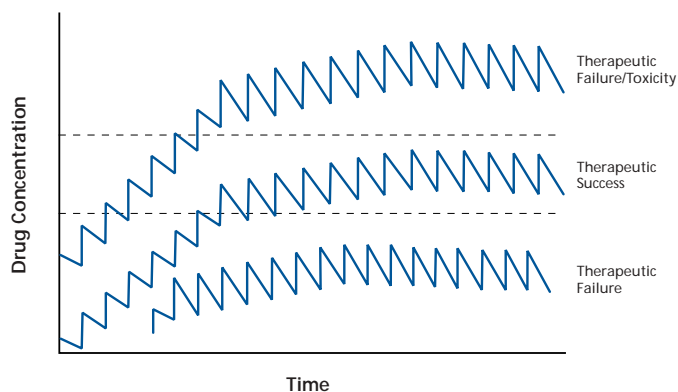


Figure 1. The mechanism by which therapeutic concentrations for a drug can be established. The ordinate represents drug concentration and the abscissa represents a time factor on therapy. The bottom saw-toothed line shows concentrations where therapeutic failure results from suboptimal drug concentration. The middle line represents the optimal drug concentration for therapeutic success. The top line shows the concentrations that result in therapeutic failure secondary to toxicity from high drug concentrations.

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Table 1. Characteristics of Antiretroviral Drugs Applicable for Therapeutic Drug Monitoring

Criteria	Evaluation
Analytical	
Drug assay is accurate, specific, precise, requires small sample volume, yields rapid results, and is inexpensive	√
Pharmacokinetic	
Pharmacokinetic data are available	√
Significant interpatient pharmacokinetic variability exists	√
Pharmacologic	
Pharmacologic effect is related to drug concentration	√
Drug has a narrow therapeutic index	±
Drug has constant pharmacologic effect over extended period of time	±*
Clinical	
Clinical studies have documented the therapeutic and toxic ranges of the drug	±

√ indicates sufficient data are available for antiretroviral drugs to meet the specified criterion; ± indicates some data are available but the criterion has not been met. *Depends on the extent of viral inhibition. Adapted from Spector et al, *Clin Pharmacol Ther*, 1988.

Criteria for TDM Use in Clinical Medicine

The required criteria for the use of TDM in clinical medicine were described by Spector and colleagues in 1988.² These criteria are reviewed below in the context of antiretroviral drugs and characteristics that make drugs candidates for TDM (Table 1).

Analytical Criteria

A drug assay is available with high specificity, small sample volume requirements, reasonable cost, and rapid turnaround time.

Very sensitive and specific assays are available for all the protease inhibitors, nonnucleoside reverse transcriptase inhibitors (NNRTIs), and nucleoside reverse transcriptase inhibitors (nRTIs) using high-performance liquid chromatography with ultraviolet (HPLC-UV) detection or liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). However, nRTIs are more complex than protease inhibitors and NNRTIs since they circulate in the plasma as prodrugs and require intracellular phosphorylation to the active triphosphate metabolite. As a result, plasma concentrations of nRTIs may not always reflect activity in the intracellular compartment. A good example is didanosine, which has a very short plasma half-life but a long intracellular half-life and duration of action.³ Since it has been

very difficult to measure accurately the intracellular concentrations of many nRTI triphosphates, nRTIs may not be good candidates for TDM and thus will not be included in the discussion of the other criteria for TDM. The analytical criteria required for application of TDM is met by the protease inhibitors and NNRTIs; the cost and turnaround time criteria will likely be met as more laboratories undertake the analysis of these drugs.

Pharmacokinetic Criteria

There is significant interindividual variability in pharmacokinetics, resulting in large variability in achieved plasma concentrations. Adequate pharmacokinetic data concerning the drug are available.

Although a fixed dose is administered to all adults taking protease inhibitors and NNRTIs, large variability in achieved plasma concentrations has been well documented for all the drugs in clinical use.⁴⁻⁷ A number of pharmacokinetic studies have been conducted on currently available antiretroviral drugs as reviewed in a recent position paper on TDM.⁸ All protease inhibitors have large intersubject variability in achieved plasma concentration following fixed-dose administration (Figure 2). This variability becomes a concern when the achieved trough concentration is below the concentration necessary for inhibition of viral replication, thereby creating the potential for the evolution of drug-resistant isolates. Administration of ritonavir with other protease inhibitors tends to reduce the pharmacokinetic variability of the protease inhibitors, but the intersubject variability still remains high.⁹ Large interindividual variability is also present with achieved plasma concentrations of NNRTIs, yet it is unclear whether failure of NNRTI-based therapy is due to low plasma concentrations. Achieved plasma concentrations for nevirapine are orders of magnitude higher than what is required for viral inhibition in vitro.¹⁰ However, quasi species with high-level drug resistance circulate as minority strains, and therefore adequate exposure to concomitant nRTIs is likely crucial in maintaining successful therapy with most of the NNRTIs.

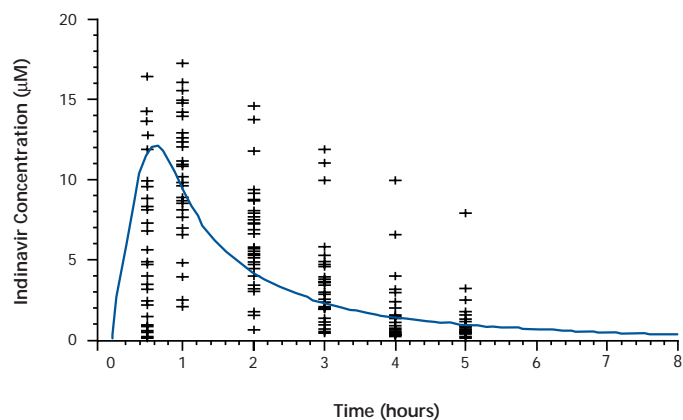


Figure 2. The extreme variability of the pharmacokinetics of a protease inhibitor (indinavir) following standard dosing (800 mg q8h). Similar data showing large intersubject variability in pharmacokinetics are available for other protease inhibitors. Adapted with permission from Acosta et al, *Pharmacotherapy*, 1999.

There are many reasons behind pharmacokinetic variability of these lipophilic drugs. There is undoubtedly variability in drug solubilization and absorption from the intestinal tract. Multidrug-resistant proteins such as P-glycoprotein likely impede the bioavailability of protease inhibitors. In addition, the large variability of CYP3A expression in the small intestine and the liver can result in significant variability in the drug's bioavailability and systemic clearance. Based upon the large intersubject pharmacokinetic variability of protease inhibitors and NNRTIs and the sufficient data available on their pharmacokinetic profiles, both drug classes qualify for TDM.

Pharmacologic Criteria

Pharmacologic effect is proportional to the plasma drug concentration. A narrow range exists between the efficacious and toxic concentrations. A constant pharmacologic effect over an extended period of time exists.

Higher plasma concentrations of protease inhibitors have resulted in a greater HIV-1 RNA response during initial dose-ranging studies as well as in clinical trials. This is not surprising since the basic tenet of pharmacology is that a concentration-response relationship exists with all clinically active drugs. Under certain circumstances, however, this relationship may not always hold true. One is the presence of active metabolites that can contribute to the overall therapeutic activity of the drug. Of the available protease inhibitors and NNRTIs, only nelfinavir is associated with a measurable active metabolite in the plasma.¹¹ The hydroxylated metabolite is designated as M-8 and is generated by nelfinavir's oxidation by CYP2C19.¹² M-8 and nelfinavir have equipotent in vitro activity against HIV. At this point, it is unclear to what extent the M-8 metabolite contributes to the overall antiretroviral activity after nelfinavir administration. It is likely that M-8 plasma binding is significantly less than nelfinavir plasma binding, so that the fraction of the drug that equilibrates intracellularly may be higher for M-8 than for nelfinavir. More research is required since in clinical use nelfinavir appears to be more effective than what would be predicted from its plasma concentration alone.

Protein binding is another factor influencing the concentration-response relationship for protease inhibitors because changes in overall binding of these drugs could affect the way total drug concentrations are interpreted. HIV replication occurs within cells, and protease inhibitors have to reach their intracellular target for activity. At equilibrium, the unbound concentration of a drug in plasma should be equivalent to the unbound concentration in the cell as long as there are no energy-dependent pumps that can transport drugs against a concentration gradient. The importance of the unbound concentration of a drug for activity was clearly demonstrated by the lack of clinical activity of the protease inhibitor SC-52151.¹³ Because of its high degree of protein binding, the drug showed no activity despite achieving seemingly adequate plasma concentration based on in vitro anti-HIV activity.

Protease inhibitors are organic bases that are mostly bound in plasma to α_1 -acid glycoproteins (AAGs), which are acute-phase reactants whose concentrations increase under conditions of infection and acute inflammation.¹⁴ The extent of protein binding of protease inhibitors is dependent upon the con-

centration of AAGs.¹⁵ Higher concentrations of AAGs result in increased protein binding of protease inhibitors. Changes in protein binding for drugs like indinavir, which is only 60% plasma bound, will not greatly affect plasma-unbound concentrations and antiretroviral activity. In contrast, changes in protein binding for highly protein-bound drugs, such as nelfinavir and lopinavir, will significantly alter their activity at an equivalent plasma concentration. Thus when total plasma concentration of a highly protein-bound drug is interpreted, the same concentration with variable protein binding may not translate into equivalent antiretroviral activity. Nonetheless, there is some evidence that the pharmacologic effect of protease inhibitors is proportional to the plasma concentration in drug-naive subjects.¹⁶

The treatment of HIV infection requires multiple drugs for durable efficacy. For NNRTIs, the concentration-response relationship is less firmly established, but data with efavirenz increasingly point in that direction.⁷ It is presently unclear if concomitant use of all the nRTIs will result in an equivalent antiretroviral efficacy at a specific protease inhibitor or NNRTI concentration. It is this high level of pharmacologic complexity in the treatment of HIV infection that makes the relationship between plasma concentration of protease inhibitors and antiretroviral response difficult to predict.

The therapeutic ranges for protease inhibitors and NNRTIs are difficult to evaluate because most drugs cannot be pushed to maximally tolerated doses. There are both absorption and tolerability limitations for these agents. It is likely that most drugs used in the treatment of HIV infection have a narrow range between the tolerated dose and the systemic concentration required for durable suppression. Gatti and colleagues clearly demonstrated that adverse effects of ritonavir are correlated to both peak and trough concentrations.¹⁷ The tolerability of ritonavir is dose dependent and most patients do not tolerate the usual dose necessary for durable antiretroviral efficacy, 600 mg twice daily. Consequently, ritonavir is used mainly as a metabolic inhibitor of other protease inhibitors at a lower and better-tolerated dose.

For nelfinavir, for which most of the toxicity is gastrointestinal, there is no relationship between plasma concentration and the development of diarrhea.¹⁸ Nephrotoxicity caused by indinavir has been related to a peak plasma concentration (C_{max}) above 10 $\mu\text{g/mL}$.¹⁹ Since indinavir has a very short plasma half-life, a large amount of drug has to be administered to maintain adequate trough concentrations (C_{trough}). As a result, the peak/trough ratio for indinavir is the highest among the protease inhibitors. The high C_{max} of indinavir can be manipulated by using lower indinavir doses with low-dose ritonavir to prolong the drug's plasma half-life.²⁰

For NNRTIs, central nervous system (CNS) toxicity associated with efavirenz has been shown to be related to plasma concentration, and the concentration necessary for maximal activity is not far from the concentration that results in CNS toxicity.⁷

The presence or evolution of resistant viral strains will determine whether antiretroviral drugs exhibit a constant pharmacologic effect over an extended period of time. The HIV reverse transcriptase gene does not contain a "proofreading" mechanism, and as long as the virus is replicating, it can generate mutations that confer reduced susceptibility to antiretroviral drugs. If viral replication is contained, a constant pharmaco-

logic effect over an extended period of time does indeed occur for both the protease inhibitors and the NNRTIs. If mutated and phenotypically less susceptible viral strains evolve during therapy, the pharmacologic effect is not constant over even a short period of time. Higher concentrations of drugs may need to be achieved to control viral replication to the same extent as during initial therapy.

Based on these pharmacologic criteria, the applicability of TDM to antiretroviral drugs is variable. Since treatment of HIV infection requires the concomitant use of multiple drugs, monitoring only a single drug (eg, a protease inhibitor or NNRTI) may not be appropriate. Both the therapeutic efficacy and the toxicity of protease inhibitors and NNRTIs may demonstrate synergy, antagonism, or additive effects when combined with the various nRTIs or each other. The presence of baseline minority drug-resistant mutations and the evolution of drug resistance over time can make the concentration necessary for antiretroviral efficacy a moving target.

Clinical Criteria

Clinical studies exist that define the therapeutic and toxic ranges of the drug.

A therapeutic range has not been formally defined for all drugs in clinical use for HIV, but concentration-response data are available for most of the protease inhibitors and NNRTIs. One problem is the uncertainty as to which pharmacokinetic parameter best defines the therapeutic and toxic exposures of the drugs. The C_{trough} is usually monitored to determine adequate drug exposure because it is the easiest to collect for both the patient and the investigator; however, this parameter requires an accurate recall of when the last dose of the drug was administered. Calculating an accurate area-under-the-curve value would require obtaining specimens over an extended period of time, which is unrealistic in a busy clinical setting. Logically, C_{trough} should define the lowest drug concentration during a dosage interval and thus define the minimum effective concentration of the drug, but this has not been prospectively validated for any of the antiretroviral drugs.

In terms of toxicity, the trough and the peak drug concentrations each may play an important role. For example, C_{max} may best approximate the risk of indinavir-induced nephrotoxicity,¹⁹ but other toxicities such as skin and nail abnormalities may be related to total drug exposure. Marzolini and colleagues attempted to define the therapeutic range of efavirenz by retrospectively correlating CNS toxicity with plasma concentrations in a small group of subjects, some of whom were experiencing virologic failure.⁷ Although this may be one way to define the therapeutic ranges for drugs, prospective studies that validate these drug concentrations with observations of efficacy and toxicity would certainly strengthen the argument for TDM.

In drug-naïve subjects, the C_{trough} necessary for continued viral suppression has been defined best for indinavir. This concentration is approximately 100 ng/mL, which is close to the protein-binding-corrected 95% inhibitory concentration for indinavir in vitro.¹⁶ However, the determination of the C_{trough} necessary for durable virologic suppression is made in the presence of concomitant nRTIs, and whether all of the nRTIs interact with the protease inhibitors at the same potency in vivo has not been

studied. Abacavir, for example, which is a much more potent antiretroviral drug than stavudine, may quantitatively contribute to successful therapy more than stavudine when used concomitantly with protease inhibitors.^{21,22}

Prospective Clinical Trials

There are only a few prospective studies that have examined the utility of TDM in the treatment of HIV. ATHENA was a prospective trial of TDM in which analyses of a subgroup of patients on indinavir or nelfinavir were performed.²³ Ninety-two treatment-naïve patients were randomized to receive nelfinavir 1250 mg twice daily using TDM or no TDM. A concentration ratio (CR) was used to assess drug exposure and make dosing modifications in the TDM group. A measured drug level (drawn at any time following an unobserved dose) was compared with a population average concentration-time curve. A ratio of 1 meant the patient's concentration was the same as the population average on that occasion. If the first nelfinavir CR was less than 0.9, taking the drug with food was discussed with the patient. If the subsequent CR remained less than 0.9, the dose was increased to 1500 mg twice daily, and low-dose ritonavir was added if the third CR was low. The average turnaround time for the assay results was 4 weeks.

By intent-to-treat (ITT) analysis, the proportions of patients achieving plasma HIV-1 RNA levels below 500 copies/mL at 1 year were 81% and 59% in the TDM and no-TDM groups, respectively ($P=.03$). The authors suggested that these results reflected differences in drug efficacy because the main reason for drug discontinuation in the no-TDM group was virologic failure, which was more frequent for the no-TDM group.

The ATHENA study also examined the effect of TDM in subjects receiving indinavir 800 mg 3 times a day, indinavir 800 mg with 100 mg ritonavir twice daily, or indinavir plus ritonavir at 400 mg each twice daily. The acceptable CR for indinavir was defined as 0.75 to 2.0. This analysis showed more favorable outcomes in the group randomized to TDM. By ITT analysis, 75% and 48% of subjects had plasma HIV-1 RNA levels below 500 copies/mL in the TDM and no-TDM arms, respectively, at 1 year. This difference was secondary to toxicity, since there were very few virologic failures and far fewer subjects in the TDM arm dropped out of the study for toxicity reasons than those in the no-TDM arm. These data suggest that TDM may be potentially useful in the management of antiretroviral therapy for both efficacy and toxicity reasons. Although these substudies of the ATHENA trial had positive results, concerns remain regarding the statistical power of the study to detect differences between groups and whether clinicians truly followed dose change recommendations.

PharmAdapt was a prospective study comparing the use of TDM versus no TDM in treatment-experienced patients in whom therapy failed.^{24,25} A total of 257 subjects enrolled in this study, but the presented data were limited to 180 subjects receiving protease inhibitor-containing regimens. Ninety-six subjects were in the control group and 84 subjects were in the TDM group. All subjects had genotypic resistance testing prior to randomization, and drug therapy was determined on the basis of these results. The TDM group had a dose modification at week 8 based on week 4 trough concentrations. The targeted protease

inhibitor exposure was a trough concentration above the protein-binding-corrected median inhibitory concentration (IC_{50}) of wild-type HIV described in the literature. At week 8, 6% of the control group had a physician-determined protease inhibitor dose modification. Physician- and protocol-driven dose modifications occurred in 17% of subjects in the TDM group. At week 12, the change in plasma HIV-1 RNA level was equivalent in both groups ($-2.61 \log_{10}$ in the control group; $-2.32 \log_{10}$ in the TDM group). Also at week 12, plasma HIV-1 RNA levels below 200 copies/mL were observed in 52% of the control group and 45% of the TDM group. A 32-week analysis reported virologic effects in the 2 groups similar to those at week 12.

This TDM study was the first to report negative results; however, concerns regarding the study design deserve comment. It is unclear how the protein-binding-corrected IC_{50} was chosen as the appropriate target concentration for protease inhibitors. In addition, it is likely that wild-type IC_{50} concentrations were too low for an adequate antiviral response in the majority of drug-experienced subjects. Furthermore, dosage adjustment at week 8 may have been too late to prevent evolution of the virus in a group of subjects in whom protease inhibitor-based regimens were failing. Finally, a power analysis was not performed. If 17% of subjects required dose modification in the TDM group versus 6% in the control group, a sample size greater than 180 may have been necessary to demonstrate virologic differences across treatment approaches.

A prospective, randomized clinical trial evaluating concentration-controlled versus fixed-dose therapy with zidovudine/lamivudine/indinavir in 40 treatment-naive patients has been reported by Fletcher and colleagues.²⁶ Subjects in the fixed-dose arm received standard zidovudine 300 mg twice daily, lamivudine 150 mg twice daily, and indinavir 800 mg every 8 hours. Subjects randomized to the concentration-controlled arm received doses to maintain plasma concentrations of at least 0.17, 0.4, and 0.13 mg/L for zidovudine, lamivudine, and indinavir, respectively. Treatment duration was 52 weeks, and adherence was measured using the ratio of medication taken (derived from pill counts) to that prescribed. Intensive pharmacokinetic analyses were made at week 2, and secondary pharmacokinetic measurements were made at week 28. Dose changes in the concentration-controlled arm were implemented at week 4 based on the pharmacokinetic data collected at week 2, and a Bayesian-estimation feedback algorithm was employed using subsequent single concentration-time points for further dosage refinement. Sample-size calculation was based on variability in drug exposure, toxicity, and differences in plasma HIV-1 RNA level at the 5% significance level with 80% power. Seven subjects terminated the study early and were not included in the pharmacokinetic or virologic analyses. Zidovudine, lamivudine, and indinavir doses were altered in 44%, 31%, and 81% of the concentration-controlled recipients, respectively.

At 52 weeks, in an ITT analysis, 15 of 16 subjects (94%) in the concentration-controlled arm and 9 of 17 subjects (53%) in the fixed-dose arm achieved plasma HIV-1 RNA levels below 50 copies/mL ($P=.017$). The concentration-controlled group reached undetectable plasma HIV-1 RNA levels more rapidly than the fixed-dose group: 108 days versus 225 days, respectively ($P=.01$). No significant differences were found between groups in terms of occurrence of common adverse events,

including anemia, neutropenia, and nephrolithiasis. Average adherence rates exceeded 90% for all 3 drugs in both arms and were not statistically different between the treatment groups.

This is the longest prospective TDM trial reported to date, and the first one to apply TDM to all drugs in a regimen of highly active antiretroviral therapy (HAART). The study investigators concluded that a single-dose approach to treat all patients with HIV infection may not be optimal. Although this latter study is the most rigorously conducted of the three, the use of indinavir alone as the protease inhibitor in a HAART regimen is now fairly unusual. Many physicians now routinely prescribe indinavir with low-dose ritonavir, which results in higher plasma indinavir concentrations than indinavir alone.

Conclusions

Where are we in terms of using TDM for managing antiretroviral drug use in clinical HIV medicine? Widespread use of TDM is not appropriate at this time. Obtaining random plasma concentrations of drugs with very short plasma half-lives makes interpretation of these data difficult. Using TDM to assess adherence to therapy is also not an appropriate use of plasma concentrations for drugs with short half-lives.

It is critical to determine why multidrug therapy for HIV infection fails. If in most cases failure is secondary to poor adherence, then TDM is not the correct way to improve outcome. Data from Fischl and colleagues, which compared the results of using directly observed therapy (DOT) with self-administered therapy in antiretroviral drug-naive subjects, indicate that a major hurdle to therapeutic success in these patients is adherence to drug administration.²⁷ In that study, results at week 88 showed that all subjects in the DOT group had plasma HIV-1 RNA levels below 400 copies/mL; in the self-administered therapy group, approximately 80% had levels below 400 copies/mL. The percent achieving plasma HIV-1 RNA levels below 50 copies/mL in the 2 groups was 93% and 60%, respectively.

If the intent of TDM is to identify a small percentage of patients who are rapid metabolizers of drugs, performing TDM early in therapy after reaching a presumed steady-state concentration may be appropriate. TDM may also be an appropriate way to reduce drug toxicity if data from retrospective clinical studies indicate a concentration of the drug above which many patients develop toxicity. However, a prospective study would be important to confirm that this toxicity can be circumvented using TDM. Cost-effectiveness analysis should also be a component of all clinical trials utilizing TDM.

TDM also may be appropriate in cases where another drug needs to be added to a successful therapeutic regimen in which a drug-drug interaction is possible. Obtaining a plasma concentration of the current drug prior to the addition of the new drug would define the current drug's therapeutic concentration for that person, which would then be the target concentration after the addition of the new drug. For example, if phenytoin, an inducer of drug-metabolizing enzymes, needs to be added to a successful protease inhibitor-based regimen, the protease inhibitor concentrations will likely be much lower following the addition of phenytoin, and checking a drug level may be useful to ensure it is not below the average level observed in patients.

In the treatment of drug-experienced subjects who have drug-resistant virus, determining a protein-corrected IC_{50} may be useful. Applying TDM to then achieve a concentration several-fold above that IC_{50} would seem reasonable. Evaluation of the inhibitory quotient (IQ), in which the phenotypic sensitivity of the virus to the drug and the plasma concentration of the drug determine the IQ, is being studied in clinical trials. Well-designed and adequately powered prospective studies need to be performed before IQ can be generally recommended for clinical practice.

The important message about TDM is that it may ultimately prove useful, but at this time there are not enough data to recommend its use outside of very specific circumstances. TDM should currently be viewed as an investigational tool to explore means of improving therapeutic outcome and reducing toxicities in the treatment of HIV infection.

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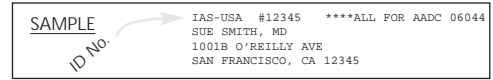


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