

To cure chronic HIV infection, a new therapeutic strategy is needed

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With the advent of highly active anti-retroviral therapy (HAART) in 1997, most investigators felt that HIV infection would be cured with a few years of antiviral therapy. It is now clear that antiviral drugs alone cannot cure the infection, even when applied within a few weeks of initial symptoms. There are now several reports of the discontinuation of HAART after several years of effective suppression of detectable plasma virus. Relapse occurs universally within a few weeks. More promising results have been reported if HAART is initiated early after infection. However, even in this instance, most patients suffer a relapse within a few weeks. If diagnostic treatment interruptions are performed, some individuals appear to control plasma virus concentrations at low levels – <5000 HIV RNA molecules/ml. We have similar results from subjects who were infected chronically before HAART was initiated, so that it is clear that the previous dogma that HIV-specific immune reactivity is absent in individuals who are chronically infected is incorrect. Immune reactivity to HIV does exist, and is detectable *in vivo*, even when the infection becomes chronic before therapy is initiated. Consequently, we are now faced with a new therapeutic dilemma: how can a cure of this infection be achieved? This review is focused on the rationale and methods to design clinical trials directed towards achieving a cure of HIV infection.

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Abbreviations

APC	antigen-presenting cell
CMI	cell-mediated immunity
CTL	cytotoxic T lymphocyte
DTI	diagnostic treatment interruption
FDA	Food and Drugs Administration
HAART	highly active anti-retroviral therapy
LCMV	lymphocytic choriomeningitis virus
LPA	lymphocyte proliferation assay
OI	opportunistic infection

Introduction

After five years of the use of highly active anti-retroviral therapy (HAART), it is now clear that antiviral drugs can effectively suppress the replication of HIV, yet they cannot cure the infection. As soon as the drugs are withdrawn, even though plasma virus has been undetectable for several years, viral replication resumes rapidly and viremia returns within a few weeks. This alone would not be an insurmountable obstacle if HAART could simply be administered indefinitely. However, there are increasing reports of severe metabolic toxicities associated with continuous drug use that essentially preclude this option. Therefore, a new

strategy is needed to achieve a cure of HIV infection, so that HAART can be discontinued.

Toward this goal, lessons can be learned from the study of other viral infections. Since the time of Sir Edward Jenner, we have known that the immune response can act against viruses [1]. Accordingly, vaccination for smallpox works because the host defenses can be mobilized to generate protective immunity, which prevents the development of smallpox. The hallmark of the host defenses against smallpox, as for other viruses, is a strong cell-mediated immunity (CMI) response. In fact, a successful ‘take’ after immunization with vaccinia is recorded when a readily discernable delayed-type hypersensitivity (DTH) inflammatory skin reaction occurs at the site of vaccination. Moreover, the DTH response is the traditional test for CMI, as contrasted to humoral immunity, which is typified by either an immediate-type hypersensitivity skin reaction mediated by IgE and mast cells, or an intermediate-type hypersensitivity (i.e. Arthus) skin reaction mediated by IgG and macrophages.

Infection by HIV is set apart from most other infectious diseases by virtue of the fact that the primary tissue infected by HIV is the immune system itself. Consequently, the disease that results from HIV infection becomes manifest by the failure of the immune system, which is aptly termed AIDS. Moreover, the symptoms that result from AIDS are attributable to infections by multiple microorganisms that normally cannot gain entrance to the body, causing so-called opportunistic infections (OIs).

Because the disease caused by HIV results from immunodeficiency, in the past 20 years there has been a great deal of investigation focused on the mechanisms responsible for the immunodeficiency. In addition, there has been a great deal of investigation into the virus itself, and the ways in which it causes damage to the immune system. By comparison, there has been a remarkable paucity of studies focused on methods to *cure* the HIV infection. However, the fundamental mechanisms that are operative and lead to AIDS have been delineated. By virtue of their expression of CD4 (one of the receptors for the virus, as are chemokine receptors), the only cells infected by HIV are CD4⁺ T lymphocytes (‘helper’ T cells) and CD4⁺ macrophages, which are phagocytes that act as antigen-presenting cells (APCs).

HIV infection of helper T cells and APCs results in a slow, but progressive diminution of these two major types of immune system cells, ultimately severely crippling the capacity of the immune system to recognize and respond to invasion by OIs.

Since CD4⁺ helper T cells and antigen-presenting macrophages represent two of the four major cell types

responsible for CMI (the others are CD8⁺ T cells and NK cells), it is not surprising that AIDS, the disease caused by HIV, becomes manifest by OIs that are usually combated by CMI, as compared with infections caused by microorganisms that are combated primarily by humoral immunity.

The confusion with regard to a cure for HIV infection arises because HIV is a virus and therefore should be combated by CMI. At this juncture, it is important to bring to attention two of the most important aspects of HIV infection — that the infection itself is usually *asymptomatic and chronic*. It is astounding that an individual can have a persistent HIV infection for years and even decades with no symptoms attributable to the ongoing viral replication.

Primarily, virologists and infectious-disease physicians have forwarded one explanation for this paradox. It has been proposed the early during the course of an HIV infection, HIV-specific CD4⁺ helper T cells are preferentially infected and destroyed by the virus [2,3]. This hypothetical selective depletion of HIV-specific helper CD4⁺ T cells has been proposed to be responsible for allowing the persistence of viral replication and an inability of the host to completely eliminate HIV-infected cells. Subsequently, it has been hypothesized that there is a slowly progressive stochastic infection and virus-mediated destruction of other CD4⁺ helper T cells that ultimately results in a deficiency of the total number of helper T cells. Eventually this global loss of CD4⁺ T helper cells results in a general immunodeficiency and OIs.

Unfortunately, this scenario does not fit the observations made during the natural history of the infection. First, HIV is not a lytic virus capable of killing the host cells that it infects. Instead, it is a retrovirus, and one of its main strategies of persistence is to circumvent the host immune response by integrating into cellular DNA, thereby becoming latent and living along with the cell. This brings into focus the other paradox of HIV — there is a readily detectable ongoing immune response to the virus. Actually, the immune response to HIV provides the means whereby we make the diagnosis that infection has occurred. Circulating HIV-specific antibodies and CD8⁺ ‘killer’ T cells are easily detectable in all individuals who are infected chronically [4,5].

The other paradoxical observation is that there is a relative paucity of HIV-infected CD4⁺ T cells detectable even during untreated chronic infection when there is readily detectable plasma virus. Estimates from studies with peripheral blood mononuclear cells and lymph nodes indicate that very few CD4⁺ T cells are actually productively infected. For example, a plasma HIV RNA (vRNA) concentration of 30,000/ml is associated with an average of only 60 vRNA⁺ cells/million total lymph node cells (CD4⁺ cells comprise ~15%–20% of this total) — a frequency of only 0.006% [6••]. Therefore, very few cells of the total available are actually infected and producing virus.

From studies of viral dynamics performed after the initiation of HAART, it has been calculated that a productively infected CD4⁺ T cell has a half-life of only ~1 day [7,8]. Therefore, HIV infection marks these cells for destruction. It has been assumed that the virus itself is somehow responsible for the rapid death of these cells. However, *in vitro*, infected CD4⁺ T cells do not die unless CD8⁺ T cells are present. Therefore, immune-mediated damage is the most likely mechanism responsible for the destruction of productively infected CD4⁺ T cells *in vivo*, as well as *in vitro*. As in all other viral infections that have been well studied, CD8⁺ T cells kill virus-infected target cells via direct cytotoxic T lymphocyte (CTL)-mediated lysis [9,10]. As well, CD8⁺ CTLs can suppress viral replication via the secretion of antiviral cytokines, such as IFN- γ and TNF- α , as well as via the production of chemokines that compete with HIV for binding to chemokine receptors.

All of these observations indicate that HIV infection leads to the disease recognized as AIDS via an *immunopathological mechanism* — the immune system destroys itself. However, the immune system is also responsible for the *chronicity* of HIV infection, in that the total body concentration of HIV is controlled by the immune response to the virus. Moreover, most of the effect of the immune response can be attributable to the activity of CD8⁺ T cells [11••]. The enigma is that, despite a very active immune response to HIV [12], the immune system cannot totally suppress viral replication, so that the immune system cannot ‘cure’ the infection and create a state of protective immunity. Instead, the ongoing virus replication in a very few cells and continuing infection of new CD4⁺ T cells leads to a CD8⁺ T cell mediated destruction of infected CD4⁺ T cells at a rate that exceeds the capacity of the bone marrow and thymus to produce new cells. Eventually, the total number of blood CD4⁺ T cells declines and, when they fall below 200/ml, AIDS occurs (see [4]).

Some investigators have proposed that the selective destruction of HIV-specific CD4⁺ helper T cells early after infection leaves the host crippled and unable to mount an effective anti-HIV immune response [2]. This hypothesis is based on *in vitro* lymphocyte proliferation assays (LPAs) in response to HIV proteins. However, it is well known that LPAs are only semiquantitative and are relatively insensitive. LPAs require several complex biochemical pathways to be operative to register a positive response [13]. Thus, antigenic proteins must be taken up via phagocytosis by APCs, then processed into immunogenic peptides and presented to CD4⁺ T cells via MHC class II molecules. Subsequent to the recognition of MHC-peptide epitopes by the TCR, several signal transduction pathways must activate the expression of IL-2 genes and IL-2-receptor genes. Finally, the interaction between IL-2 and the IL-2-receptor must activate signal transduction pathways that promote G1 progression, DNA synthesis and mitosis.

Recently, new assays that are more direct and quantitative than LPAs and that allow the identification and enumeration of antigen-specific T cells have become available [14–16]. Using mixtures of overlapping (every four amino acids) 15-mer peptides from the entire HIV genome, it is possible to activate both CD4⁺ and CD8⁺ T cells *in vitro*, and to identify the activated cells via flow cytometry using monoclonal antibodies reactive with cell surface molecules as well as intracellular activation molecules such as cytokines. Using this approach, Koup, Picker and co-workers [11••] have recently assayed 23 chronically infected, untreated individuals and found that as many as 2.5%–25.0% of circulating CD8⁺ T cells are reactive to HIV epitopes. Even more surprising, given the absence of LPA reactivity in chronic infection, from 0.2%–2.0% of CD4⁺ T cells were found to be HIV-reactive. These data are extremely important, in that they refute the hypothesis that HIV-specific CD4⁺ T cells are totally absent in chronic infection [2]. Moreover, the high frequency of HIV-specific CD8⁺ T cells is consistent with the hypothesis that these cells are responsible for controlling the level of viremia, as they are in other viral infections [17–20].

Possible mechanisms responsible for virus persistence despite immunologic recognition

Given that HIV antigens are recognized by the immune system, particularly by both CD4⁺ helper T cells and CD8⁺ CTLs, how can one account for the persistence of HIV replication? Data are available in viral infections of experimental animals that provide for the generation of hypotheses and that lead to possible new strategies for immunotherapies. In the mouse, infection by lymphocytic choriomeningitis virus (LCMV) leads to an immunopathological inflammation of the meninges. Studies of the immune response to this virus have shown that readily detectable LCMV-specific CD8⁺ CTLs accompany an acute self-limited infection. Thus, the generation of a rapid and marked increase in LCMV-specific CTLs is responsible for clearance of the virus and the establishment of a state of long-lasting immunologic memory, which confers protective immunity against re-infection [17–20].

If mice are immunocompromised prior to infection, the virus cannot be cleared and the infection becomes persistent (chronic). Thus, both CD4⁺ T cells and CD8⁺ T cells are necessary for the generation of memory and immunity [10,21,22]. Moreover, using mice lacking the IL-2 gene, it has been found that IL-2 is responsible for the great majority (>90%) of the proliferative expansion of the LCMV-specific CD8⁺ CTLs [23,24]. Thus, infection of IL-2 ‘knockout’ mice results in a chronic LCMV infection. However, if these mice are given IL-2 replacement therapy, they are able to clear the infection [24].

It is possible to create persistently infected normal mice, either by increasing the amount of virus injected or by using virus strains that have a rapid replication rate [25]. In these mice, several different mechanisms have been uncovered

that could explain viral persistence despite an intact immune system at the time of infection [26]. Using MHC–peptide tetramer assays to identify LCMV-epitope-specific CD8⁺ CTLs, it has been shown that clonal deletion can occur. Thus, tetramer⁺CD8⁺ T cells that are readily detectable at the peak of an acute infection are no longer detectable when assayed during the conversion to a chronic infection. Antigen-mediated activation-induced cell death (AICD) is one mechanism that has been postulated to possibly account for this clonal deletion [27,28]. Of course, if there has been clonal deletion in HIV infection, especially of clones recognizing immunodominant epitopes, one would anticipate that newly produced T cells would be necessary to repair this defect in the TCR repertoire.

Another mechanism identified in persistent viral infections has been termed ‘CTL exhaustion’ [9]. Thus, tetramer⁺ cells are detectable but, when activated *in vitro*, these cells have no CTL activity and do not produce antiviral cytokines, such as IFN- γ [26]. Thus, these cells seem to be anergic. Possible mechanisms accounting for this anergic state include cytokine deprivation. For example, CTLs activated *in vitro* with antigen require IL-2 for optimal CTL activity [29] and, as well, CD8⁺ T cells depend upon IL-2 to be able to produce IFN- γ and other antiviral cytokines [30]. Accordingly, if there is a relative lack of IL-2, or inadequate CD4⁺ T cell help, a state of anergy could occur and manifest as unresponsiveness in T cells — both helper cells and CTLs.

Alternatively, cytokine-mediated suppression is another mechanism proposed to account for anergy. For example, IL-10 is a cytokine that has been found to exert feedback suppression during immune responses. To explain the exhausted phenotype, IL-10 — produced as a result of chronic stimulation — could overcome the positive signals, impinging on antigen-activated CTLs. Actually, the continuous production of IL-10 could account for the apparent unresponsiveness observed upon immunization during a persistent infection.

Viral mutations that lead to escape from CTL recognition are a third possibility that could contribute to the persistence of infection that has been described [31]. Mutations that lead to a decreased binding affinity for MHC molecules or that lead to a decreased binding affinity for the TCR have both been observed. In this instance, to be of biological importance the viral mutations that would be most effective would be those that altered immunodominant epitopes. In this regard, one of the aspects of the T cell immune response, compared with the B cell immune response, relates to the tremendous diversity of possible epitopes that can serve as antigenic sites. There are six HLA class I molecules expressed and each of these molecules has the potential to bind as many as 10⁷ distinct peptides. Thus, the HLA polymorphism creates a situation that would make it difficult for the microbe to employ mutational escape as a biologically relevant mechanism. As well, the entire

one-dimensional gene sequence of the microbe is available for the immune system to 'identify' peptide epitopes. Accordingly, the number of possible epitopes encoded in a genome as large as that in HIV is enormous.

Evidence of an *in vivo* antiviral immune response in chronic HIV infection

Given the aforementioned considerations, the dogma has arisen that — once chronic infection occurs — the immune system is irreparably damaged, so that there are very few therapeutic options beyond chronic viral suppression with antivirals [3]. Some investigators have used this dogma to justify early treatment with antivirals, to try to intervene before the putative irreparable damage to the immune system has occurred [32].

To gain information regarding the efficacy of antiviral immune reactivity in individuals infected chronically, we initiated a phase I safety trial in 1999 in which we discontinued the antivirals (i.e. HAART) in a diagnostic treatment interruption (DTI) in chronically infected patients who were in remission. Instead of re-institution of antiviral treatment as soon as plasma viremia recurred, as most investigators have done, antivirals were only re-initiated when the plasma HIV concentration reached a stable plateau. We expected that, upon relapse, the plasma HIV concentration would increase to the pre-treatment viral 'set-point', according to dogma based on viral dynamics with no role played by the host. Also in contrast to others, we administered daily low-dose IL-2 therapy after the antivirals were withdrawn. We argued that it would be safer to support the immune system with IL-2 should viremia recur.

Thus far, antivirals have been discontinued in 16 subjects for intervals ranging from 8 weeks to >1 year, while daily IL-2 therapy has been continued. The data from the first 9 subjects were recently reported and are representative of all 16 subjects [33*]. Upon cessation of HAART, all individuals have relapsed and plasma viremia recurred rapidly, within 19 ± 3 days (mean \pm standard error). Subsequently, plasma HIV concentration increased rapidly, with a doubling time of 1.6 ± 0.3 days. However, instead of leveling off, the HIV concentration reached a peak at ~4–5 weeks from the cessation of HAART, following which it declined by an average of 10-fold over the ensuing 2 weeks and reached a 'trough' by 8 weeks from the cessation of HAART.

Coincident with the decline in plasma HIV concentration, circulating CD8⁺ T cell concentrations doubled. Moreover, the rate and magnitude of decay in plasma HIV concentration correlated with the magnitude of CD8⁺ T cell increase. These findings are reminiscent of the dynamics of viral and lymphocyte changes after primary infection, with the exception that the peak and trough levels of plasma HIV during primary infection are ~100-fold higher than we observed in what is an apparent 'secondary' infection. Our data are essentially consistent with an anamnestic response, thereby providing for the interpretation that

there is a very effective anti-HIV host response operative *in vivo*, even in individuals who are chronically infected before receiving HAART.

The CD8⁺ T cell lymphocytosis is of interest, in that others who have discontinued HAART without supplementing with IL-2 have not reported an increase in total circulating CD8⁺ T cell concentrations [34,35,36*,37*]. Moreover, instead of a peak followed by a decline in plasma virus concentration, in the absence of IL-2 therapy the HIV concentration increased to a plateau. Thus, our data are consistent with the interpretation that IL-2 therapy promotes the proliferative expansion of antigen-activated CD8⁺ T cells and that these cells are responsible for regulating and controlling the viral set-point.

Several individuals remained off of HAART and continued to take daily low-dose IL-2 for periods of >1 year. During this time they maintained their CD4⁺ T cell concentrations within the normal range, with elevated CD8⁺ T cells and NK cell concentrations. Of importance, the initial viral set-point noted after eight weeks without HAART continued throughout the more prolonged intervals without antiviral therapy.

Four subjects discontinued HAART a second time after having achieved a second remission (i.e. undetectable HIV levels in plasma) following the re-institution of antiviral therapy. In three of these subjects, the peak plasma HIV concentration was >10-fold lower than after the first DTI. The CD8⁺ T cell concentrations increased upon relapse of detectable plasma virus, whereas the CD4⁺ T cell and the NK cell numbers remained stable. Accordingly, these data support the notion that repetitive exposure to HIV functions to augment acquired immune responses, mediated by CD8⁺ CTLs.

These data are extremely important in that they provide the hope that immunoenhancing therapies might be effective in augmenting immune reactivity to HIV itself and that protective immunity to HIV might be a realistic goal, even in those who are already infected chronically when diagnosed. In this regard, it is noteworthy that viremia was not prevented by the host defenses but that, once viral replication recurred, the immune system appeared to recognize and react to the virus. Consequently, at least one component of any successful immunotherapy should be HIV antigen.

A model for T cell activation: rationale for immunotherapy

Our model for T cell activation is based on a series of experiments performed soon after we had purified IL-2 to homogeneity [38,39] and had radiolabeled it [40]. We found that resting T cells do not express detectable IL-2 receptors. Moreover, resting T cells do not produce detectable quantities of IL-2. However, if activated with antigen, there appeared to be at least two distinct types of T cells — IL-2-producer cells and IL-2-responder cells [41]. Subsequent experiments revealed that most IL-2-producer cells express CD4 molecules on the cell surface, whereas most IL-2-responder cells express CD8 molecules [42]. Recently, using flow cytometry to detect

cytokine production at a single-cell level, we have found that, upon antigen activation via the TCR, ~60% of the CD4⁺ T cells produce IL-2, whereas only ~30% of CD8⁺ T cells are capable of producing IL-2 (KA Smith, unpublished data).

Since there are normally twice as many CD4⁺ T cells as there are CD8⁺ T cells, it is apparent that ~80% of the IL-2 produced during an immune reaction is produced by CD4⁺ T cells. This finding is especially important in a disease such as HIV infection, which preferentially compromises CD4⁺ T cells. If there were a relative deficiency of CD4⁺ HIV-specific T cells, then IL-2 production would be deficient. Consequently, HIV-specific CD4⁺ T cell 'help' for CD8⁺ T cells would also be deficient.

Other experiments have shown that antigen-mediated activation via the TCR without adequate IL-2 production can not only lead to the absence of activation and expansion of antigen-selected T cells, but also lead to anergy. This phenomenon has been shown by *in vitro* experiments whereby co-stimulation via the interaction of B7 on APCs and CD28 on T cells is blocked [43,44]. Of particular importance, if IL-2 is supplied exogenously the anergic reaction is circumvented even if B7-CD28 interaction is blocked.

The converse — supplying IL-2 without activation via the TCR — would also be insufficient, in that the antigen-specific cells would not express IL-2 receptors [40]. Actually, we have already observed the consequences of supplying IL-2 in the absence of antigen activation; upon discontinuation of HAART, despite continued IL-2 administration, there was not a CD8⁺ lymphocytosis until *after* the relapse of viremia. In fact, the circulating HIV concentration exceeded 10,000 HIV molecules/ml before there was a detectable CD8⁺ lymphocytosis [33*].

The other consideration is the importance of IL-2 compared with other potential T cell growth factors for the expansion of antigen-selected T cells. *In vitro*, we found that if IL-2 participation is inhibited or blocked, antigen-activated T cell clonal expansion is suppressed [29,39,45,46]. In this regard, the experiments with IL-2 knockout mice are especially revealing. Without IL-2, the expansion of antigen-activated CD8⁺ T cells is attenuated by >90% [23,24]. Thus, there appear to be no other interleukins, either *in vitro* or *in vivo*, that can substitute for IL-2.

All of these considerations lead to the conclusion that optimal immunotherapeutic intervention in chronic HIV infection should consist of at least two signals — HIV antigens and IL-2.

A new clinical trial design to test immunotherapies in chronic infection: diagnostic treatment interruption

In experimental animals, it is straightforward to design experiments to test the efficacy of vaccines and other immunotherapies. After the administration of the

immunotherapy to normal animals, it is possible to test the efficacy of the immunotherapies by injecting an infectious dose of the microbe. It is also possible to include a control group of animals that do not receive the immunotherapy, but instead receive a placebo. Exactly this experimental design has already been used in Rhesus macaques [47**]. Animals were immunized with a naked DNA vaccine containing HIV *env* and SIV antigens, followed by a boost using a poxvirus vaccine containing the HIV *env* gene and genes from SIV. Other groups of animals received the vaccine and boost together with plasmids encoding an IL-2-Ig chimera, or the IL-2-Ig molecule itself. It is particularly pertinent for our discussion that only those animals that received both the vaccines and IL-2 were able to control viral replication after an infectious challenge of an SIV-HIV chimeric virus.

Obviously, immunizing normal human volunteers followed by challenging them with HIV is impossible. Therefore, it is necessary to create a strategy to test the efficacy of immunotherapies that will provide data as to the antiviral efficacy on the host immune system. Thus far, HIV vaccines have been tested in normal volunteers. However, in this instance, the antiviral effects of the vaccines cannot be tested, so that it has been necessary to rely on immunological assays to determine whether an immune reaction to the vaccine has occurred. Unfortunately, immunological assays alone will never allow one to predict the antiviral efficacy of a particular immunotherapy or vaccine.

The situation is further compounded when contemplating testing immunotherapies in subjects who are already infected with HIV. As already mentioned, during chronic viral infections that have been well studied in experimental animals, the steady state of persistent infection includes immunosuppressive feedback mechanisms, in addition to deficiencies in cytokine concentrations and in concentrations of both helper cells and CTLs. Accordingly, the only tenable situation in which to employ immunotherapies is after first suppressing HIV replication as much as possible, to allow the immune system to right itself.

The Food and Drugs Administration (FDA) requires demonstration of clinical efficacy for approval of any new therapy. Thus, it is necessary to show that a therapy provides a cure, a prolongation of a disease-free interval or an improvement of the quality of life, if either of the first two results cannot be achieved. In HIV infection, the FDA has accepted a diminution of the plasma HIV concentration as a surrogate for a clinical end-point. This is very important for testing new immunotherapies, in that reliance on clinical end-points while antiviral drugs are continued requires improvement on results with HAART alone. This is difficult, given the rate of progression to AIDS of only ~2%/year, while receiving continuous HAART. Therefore an immunotherapy that improves the result of HAART alone by 50% would result in a rate of progression to AIDS of 1%/year. Obviously, large numbers of volunteers and

several years of therapy are required to provide for a statistically significant difference between immunotherapy with HAART versus HAART alone.

The solution to all of these difficulties is to employ immunotherapies while HAART maximally suppresses viral replication, followed by testing the efficacy of the immunotherapeutic manipulation by a DTI. Thus, the goal is to devise ways to boost HIV-specific immune reactivity that is able to *prevent relapse* and thus prevent viremia after antivirals are discontinued. If the immune system can prevent or control the rate of viral replication to the point where plasma virus remains undetectable while off of HAART, then this would constitute a *cure*, in the same sense that all herpes virus infections are cured by the immune system, which maintains residual virus in a nonreplicative state.

Actually, this clinical trial design — employing therapies for a given time interval followed by discontinuation of all therapies to ascertain the relapse rate while off of therapy — is exactly the clinical trial design used in hepatitis C virus (HCV) infection. Thus far, the standard therapy for HCV infection — IFN- γ and Ribavirin — results in a sustained viral response rate ('cure'; i.e. the % of patients who discontinue treatment) of ~50%. Although we have yet to achieve a 'sustained viral response' of even 1% in chronic HIV infection, from the data that we have generated studying the viral and lymphocyte dynamics after DTI it is now clear that it is possible to design trials with very rapid and quantitative end-points.

In New York, we have just initiated a phase II, randomized, controlled trial to test the efficacy of immunotherapies in individuals who have responded to HAART and who have circulating CD4⁺ T cell concentrations of >400 cells/ml (see www.kendallasmith.com for a complete protocol). We are testing the hypothesis that a combination of a therapeutic vaccine plus daily low-dose IL-2 will be the most efficacious in boosting HIV-specific immune responses. This hypothesis has been formulated to take into consideration that, with the onset of persistent viremia, there may have been T cell clonal deletions, so that re-exposure to viral epitopes may be necessary to activate and expand T cells with new epitope specificities. As well, if there has been mutational escape, some immunogenic epitopes may have been lost due to mutation. However, whether or not these mechanisms are operative, it is clear that before T cells become responsive to expansion and differentiation by IL-2, they must have received a recent activation signal through their TCR.

The combination of therapeutic immunization and IL-2 is based on the premise that the T cells respond to antigenic stimulation by undergoing a massive proliferative expansion that is driven by IL-2. Therefore, IL-2 is used as a supplemental therapy in this instance, ensuring that adequate IL-2 concentrations are available should the production of endogenous IL-2 be deficient. In addition, it is logical to perform these immunotherapeutic manipulations while endogenous viral antigenic stimulation is maximally suppressed with

HAART. The immune system should be maximally responsive at this stage.

A clinical-trial design of immunotherapy while on HAART, followed by a short-term DTI, is ideal in that it is possible to gain quantitative data very rapidly. Consequently, it is not necessary to perform immunotherapy trials with clinical end-points that require thousands of subjects and many years before discernible differences can be obtained. Instead, because the FDA will accept an antiviral effect as a surrogate end-point, immunotherapy trials that are designed to detect an antiviral effect after DTI should rapidly identify the most efficacious regimens and vaccines. Accordingly, the time required to identify the most promising agents can be reduced to months rather than years and the time required to identify useful vaccines should be reduced to several years instead of several decades.

Conclusions

Twenty years after the recognition of AIDS as new infectious disease, the causative agent has been identified and effective drugs that suppress the replication of the HIV have been created. Yet, there still is no cure for HIV infection. Consequently, a new therapeutic strategy is now needed. Using data derived from experimental viral infections and data about the mechanisms of how the immune system recognizes and responds successfully to viral infections, immunotherapy must be combined with antimicrobial drugs to achieve maximal and effective suppression of viral replication. Moreover, only the immune system will eventually be able to keep viral replication controlled to the point that the progression to AIDS is prevented and the antiviral drugs can be discontinued.

The immunotherapeutic approach with the most promise at this time consists of the delivery of at least two signals: HIV antigens, which can activate the adaptive T cell immune response; and low, daily doses of IL-2, which is the principal T cell growth and differentiation factor, to expand and arm cytolytic effector T cells as well as NK cells. Moreover, the clinical trial design with the most promise is the delivery of immunotherapy after HIV replication is suppressed maximally via antiviral drugs, followed by testing for an antiviral effect during a DTI. This approach promises to identify the most effective immunotherapies in the most rapid way. In addition, this therapeutic clinical trial design is applicable to the identification of the most promising vaccines and adjuvants that can be tested in prophylactic trials. Consequently, the time necessary for the development of an effective prophylactic vaccine should be shortened considerably.

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Both of these reports [36*,37*] detail the characteristics of the relapse after treatment interruption in chronically infected subjects. Earlier reports re-instituted therapy soon after the plasma virus became detectable, so that it has been difficult to determine whether there is a host antiviral response upon viral relapse. Most of the patients reported did not undergo a decline of plasma HIV. Neither of these studies reported that the CD8⁺ T cell concentration changed after the relapse.

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This report shows a clear advantage of administering a prophylactic vaccine together with IL-2 in a Rhesus macaque model system.