

Low-dose daily interleukin-2 immunotherapy: accelerating immune restoration and expanding HIV-specific T-cell immunity without toxicity

Kendall A. Smith

There is now a great deal of interest in therapies focused on improving the function of the immune system in the treatment of individuals infected with the Human Immunodeficiency Virus (HIV). Although the antiviral drugs effectively suppress replication of the virus, they cannot cure the infection. Therefore, it now appears that both antivirals and immune system stimulants will be necessary to maximally suppress residual latent virus, thereby allowing the discontinuation of the antivirals without relapse of detectable plasma virus. Interleukin 2 (IL-2) the first cytokine to be discovered at the molecular level has been used as a therapeutic in HIV infection, because it is critical for a normal functioning immune response. IL-2 is essential for the survival and proliferative expansion of antigen-activated T cells and Natural Killer (NK) cells, and also for promoting their differentiated functions of cytokine secretion and cytotoxicity. However, as IL-2 stimulates both the innate and acquired immune responses, when used as a therapeutic it can lead to severe toxicity when given in high doses. This review focuses on low dose, daily IL-2 therapy, used to accelerate the recovery of the immune system when viral replication is suppressed maximally with antivirals. In addition, the principles of the use of IL-2 to activate HIV-specific immune reactivity are discussed. At least two signals are required to promote the proliferative expansion and function of antiviral effector lymphocytes, HIV antigens and IL-2.

© 2001 Lippincott Williams & Wilkins

AIDS 2001, 15 (suppl 1):S28–S35

Keywords: T-cell immunity, interleukin-2, immunotherapy, HIV, cytokine

Introduction

Use of highly active antiretroviral therapy (HAART) has made it possible to achieve effective suppression of HIV virus replication [1]. However, not all patients respond to therapy, and the immune system recovers slowly. There have been reports of patients who have undetectable viremia and no disease progression over long-term follow-up, even in the absence of antiretroviral therapy, so-called 'nonprogressors' [2]. Studies comparing the activity of cytolytic CD8+ T lymphocytes (CTLs) in

patients characterized as rapid progressors versus those who are nonprogressors have shown that a CTL response occurs in both types of patients after the acute infection. However, detectable CTLs quickly disappear in rapid progressors, whereas nonprogressors maintain a strong CTL immune response [3]. Accordingly, a vigorous HIV-specific CTL immune response correlates with the lack of progression to AIDS [2]. By comparison, lack of detectable HIV-specific helper T-cell reactivity is associated with more rapid progression to AIDS. In experimental animal models, it has been shown that the

From the Weill Medical College of Cornell University, New York, New York, USA.

Requests for reprints to Kendall A. Smith, M.D., Chief, Division of Immunology, Weill Medical College of Cornell University, 1300 York Avenue, New York, NY 10021, USA. Tel: (+1) 212 746 4464; fax: (+1) 212 746 4608; e-mail: kasmith@med.cornell.edu

Received 17 April 2000 **Revised** 20 October 2000 **Accepted** 21 October 2000

antigen-specific proliferative expansion of CD8+ T cells is dependent on CD4+ T helper cells and the interleukin (IL)-2 that they produce [4–7]. Accordingly, at this time, because of the inability of HAART to provide complete immune restoration of the CD4+ helper T cells, there has been a quest for innovative methods to accelerate immune system recovery. In addition, both patients and physicians alike now realize that neither antiviral drugs nor massive stimulation of the immune system has been successful in purging latent viral reservoirs [8,9]. Therefore, attention is now focused on therapies that promote the immunologic maintenance of viral latency, thereby hopefully permitting the discontinuation of antiviral medications.

IL-2 is an immune system cytokine that has been tested as a therapeutic agent for accelerating immune system recovery. It has been used successfully in clinical trials to augment the immune systems of HIV-infected individuals [10–14]. However, the US Food and Drug Administration (FDA) has not approved its use, so it is not yet available for clinical practice: despite data that show accelerated recovery of immune function, the FDA has taken the position that it must be shown to prolong life before it will be granted approval. Several different IL-2 doses have been used, ranging from low doses (i.e. ~2 million Units/day) to high doses (i.e. 15 million Units/day). As well, both intermittent dosing regimens and daily dosing regimens have been explored. This article provides the rationale and potential benefits of low-dose, nontoxic IL-2 therapy in HIV-infected patients, both for accelerating the recovery of the immune system and for the augmentation of HIV-specific immunity.

Historical overview of IL-2

IL-2 was first described as an ‘activity’ found in lymphocyte-conditioned media that functioned to promote the long-term growth of human T lymphocytes (T cells) [15]. In a series of experiments, we isolated, characterized, and purified the IL-2 molecule from lymphocyte-conditioned media, and showed that it is the molecule responsible for the T-cell growth factor activity [16–18]. We then radiolabeled the purified IL-2 and discovered the IL-2 receptor (IL-2R) [19]. Armed with purified IL-2, the IL-2 radio-receptor assay, and monoclonal antibodies reactive with both IL-2 and IL-2R, we showed that IL-2 is critical for normal cellular immune responsiveness; it stimulates both T-cell and natural killer (NK)-cell proliferation, and enhances cell-mediated cytotoxicity and cytokine production by both T cells and NK cells [20,21].

Role of IL-2 in clonal expansion of antigen-specific T cells

Development of lymphocytes from precursor cells in the bone marrow and thymus is independent of IL-2.

However, once lymphocytes populate peripheral lymphoid tissues of the spleen and lymph nodes, IL-2 becomes the principal growth factor for T cells [21]. Only antigen-activated T cells, of both the CD4+ and CD8+ subsets, produce IL-2. Therefore, IL-2 can be detected in blood only during an ongoing immune reaction, and it becomes active only on introduction of a foreign antigen to the host. Moreover, when the antigen is cleared, IL-2 once again becomes undetectable. Understanding the antigen-dependent and transient participation of IL-2 in the immune response is critical in the therapeutic use of IL-2.

The antigen specificity of the T-cell immune response is determined by antigen activation of the T-cell receptor, which activates transient expression of the IL-2Rs [22,23]. Therefore, the effects of IL-2 are restricted to cells that have recently received signals to express IL-2Rs. If the host clears the antigen, or if therapy reduces the microbe to very low levels as occurs during HAART, the T cells lose expression of their IL-2Rs and become unresponsive to IL-2. Thereafter, many of these cells die.

As in other antigen systems that have been studied, exposure to HIV leads to the clonal expansion of HIV-reactive T cells, which results from HIV antigen-specific activation of IL-2 production and expression of IL-2Rs by both HIV-specific CD4+ and CD8+ T cells [24]. It has now become established that this clonal expansion of both HIV antigen-activated CD4+ T cells and CD8+ T cells primarily occurs in the secondary lymphoid tissues; i.e., the lymph nodes and spleen [25,26]. Moreover, suppression of viral replication in the secondary lymphoid tissues with HAART leads to a rapid redistribution of cells (both CD4+ T cells, CD8+ T cells and B cells) from the lymph nodes back into the circulation [27]. Animal studies have shown that clonal expansion and immune reactivity to viral antigens are dependent on the production and effects of IL-2 [7]. This was demonstrated conclusively in viral infection experiments conducted in IL-2 ‘knockout’ mice (i.e., IL-2 gene deleted), which showed that antigen-specific T-cell clonal expansion in these mice is attenuated by greater than 90% [7]. Consequently, a persistent infection occurs instead of immune activation and viral clearance. Furthermore, the chronic infection persists even in the presence of other cytokines with T-cell growth factor activity, such as IL-4, IL-7, IL-9, and IL-15. Therefore, these data underscore the unique properties of IL-2 as the primary *in vitro* and *in vivo* T-cell growth factor for mature, peripheral antigen-activated T cells.

Previous studies have suggested that CD4+ T cells produce more IL-2 than do CD8+ T cells, and recent experiments using single-cell analysis by flow cytometry have shown that, in healthy individuals, approximately 60% of CD4+ T cells produce IL-2 on polyclonal activation, compared with only 30% of CD8+ T cells (Smith,

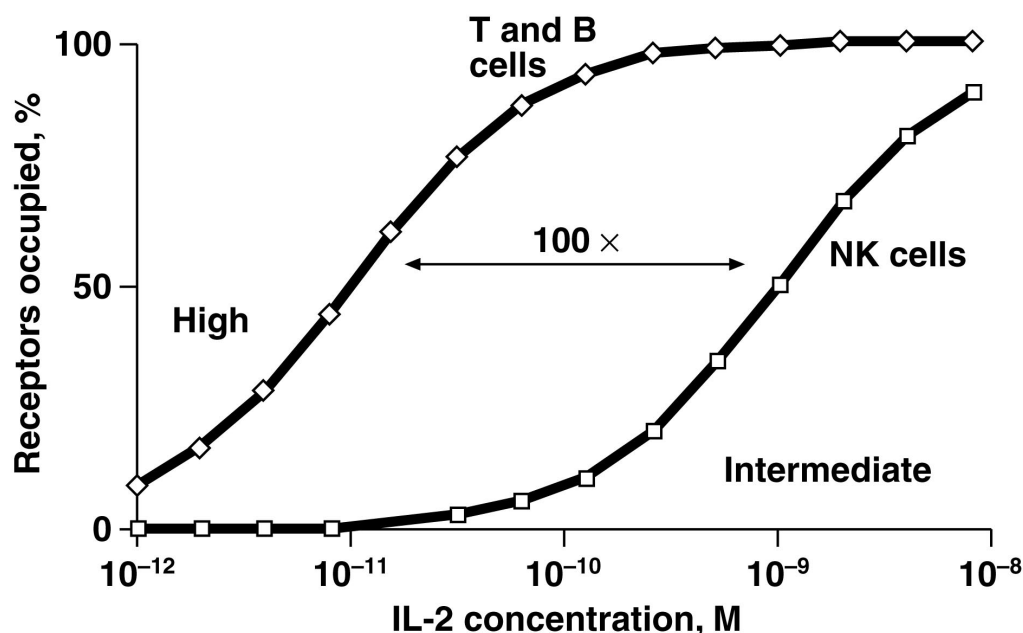


Fig. 1. Relationship between interleukin-2 (IL-2) concentration and percent of occupied IL-2 receptors on T cells and B cells (combined), and on natural killer (NK) cells.

unpublished data). In addition, there are about twice as many circulating CD4+ T cells as CD8+ T cells (900 versus 500 cells/ μ l, respectively). Consequently, the majority (80%) of IL-2 production is normally attributed to CD4+ T cells. Therefore, the ability to expand antigen-activated cytotoxic CD8+ T cells during a viral infection is highly dependent on IL-2 produced by the CD4+ T cells, which accounts for calling CD4+ T cells 'helpers'.

In addition to their effect on CD8+ T-cell clonal expansion, CD4+ T cells influence the cytotoxic capabilities of CD8+ T cells via the IL-2-promoted expression of cytolytic molecules [4]. Consequently, in the absence of CD4+ T cells and the IL-2 that they produce, CD8+ T-cell expansion and development of cytotoxicity are attenuated, leading to the persistence of the viral infection [5,6]. Experiments conducted *in vitro* and *in vivo* have also shown that the duration of CD8+ T-cell expansion is similarly dependent on exogenous IL-2 [28]. On disappearance of the antigen, the expanded antigen-specific CTLs undergo rapid apoptosis due to the withdrawal of the survival stimuli provided by IL-2. Accordingly, therapeutic IL-2 administration can preserve and extend the life span of the expanded IL-2-dependent antigen-specific T cells [28].

Role of IL-2 in innate immunity

Both T cells and NK cells are important target cells for an immune response (Fig. 1); however, NK cells differ from T cells in several ways. Natural killer cells account for only 10% of total circulating peripheral blood mononuclear

cells, whereas T cells account for 60%. In an individual with a total blood volume of 5 l, there are approximately 1 billion circulating NK cells. Unlike T cells, the effects of NK cells are antigen nonspecific, as they do not express antigen-specific receptors. Although all NK cells express IL-2Rs constitutively, approximately 90% of these cells express an intermediate-affinity IL-2R that lacks the α -chain [29]. Consequently, these NK cells have 100-fold lower affinity for IL-2 than do T cells, and require 100-fold greater concentrations of IL-2 for an equivalent response. As the IL-15R shares the IL-2R β and the IL-2R γ chains with the IL-2R, the expression of these receptor chains by NK cells is most probably a result of IL-15R expression and not IL-2R expression [30]. However, if exposed to IL-2, NK cells certainly respond. On activation by IL-2, the NK cells proliferate, become better killers and produce additional cytokines, such as interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), and granulocyte-macrophage colony-stimulating factor, all cytokines that are potent stimulators of macrophages [29].

A large part of innate host defense is dependent on the interaction between NK cells and macrophages, which are ultimately responsible for microbial destruction via phagocytosis. The secretion of IFN- γ and TNF- α by NK cells augments macrophage antiviral activity, so that secretion of these cytokines further prevents the spread of viral infection prior to the antigen-specific T-cell response. As IL-2Rs are expressed on both T cells and NK cells, IL-2 connects the antigen-specific T-cell immune response with the innate host defenses mediated by the NK cells and macrophages [24].

As discussed earlier, only 10% of NK cells express high-affinity IL-2Rs, which may be the first to be activated by the low concentrations of IL-2 produced during the initial T-cell response. Exposure of NK cells to high concentrations of IL-2 is likely to activate more of the NK cells, leading to the release of large amounts of potent pro-inflammatory cytokines, such as IFN- γ and TNF- α , that can cause systemic effects. Hence, the systemic toxicities of IL-2 are dose dependent [31].

Principles of IL-2 pharmacology

When contemplating the use of IL-2 as a therapeutic, it is vital to understand the pharmacodynamics and pharmacokinetics of IL-2, as these can serve as guides in minimizing the potential systemic toxicities of IL-2. The pharmacodynamics are based on the structure, function, and distribution of IL-2Rs, whereas the pharmacokinetics depend on the characteristics of the IL-2 molecule itself.

Pharmacodynamics of IL-2: the IL-2 receptor

The IL-2R is comprised of three separate polypeptide subunits: α , β , and γ [19,32,33]. The IL-2R α chain binds to IL-2 with a dissociation constant (K_d) of 10^{-8} mol/l, whereas the intermediate-affinity receptor, which is comprised of the β and γ chains, binds to IL-2 with a K_d of 10^{-9} mol/l [34]. The high-affinity IL-2R, IL-2R $\alpha\beta\gamma$, is comprised of all three subunits and binds to IL-2 with a K_d of 10^{-11} mol/l, allowing saturation of these receptors at very low plasma IL-2 concentrations (< 100 pmol/l) [35]. Therefore, lack of α -chain expression by the majority of NK cells results in a 100-fold lower affinity for IL-2, and thus requires 100-fold higher IL-2 concentrations for equivalent saturations (Fig. 1) [24].

The high-affinity IL-2Rs are transiently expressed by antigen-activated T cells and by only 10% of NK cells [29]. The β and γ chains of the IL-2R are expressed by both T cells and NK cells, and signal the interior of the cell, whereas the α -chain does not participate in signaling [33]. Therefore, as IL-2 doses increase, the IL-2 concentrations are eventually reached that permit binding to a substantial fraction of the β/γ IL-2Rs expressed by most NK cells [35]. The ultimate effect is activation of a cascade of secondary and tertiary cytokine release, first by NK cells and then by macrophages. Many of these secondary and tertiary cytokines are the so-called pro-inflammatory cytokines that elicit systemic symptoms such as fatigue, malaise, fever, and capillary leakage of cells and plasma. As higher and higher doses of IL-2 are administered, higher and higher IL-2 concentrations accumulate, which result in greater toxicity as more NK cells are activated. Therefore, to administer IL-2 without undue toxicity, it is necessary to understand IL-2 pharmacokinetics so that high systemic IL-2 concentrations can be avoided.

Table 1. Pharmacokinetics of interleukin-2 (Smith, unpublished data).

| |
|---|
| Absorption |
| Rapidly absorbed following subcutaneous administration |
| Plasma levels detected within 15 min |
| $C_{max} = 25$ pmol/l |
| Distribution |
| Distributed into the extracellular space (~ 14 l in a normal adult) |
| Metabolism and elimination |
| Renal excretion |
| Filtered by the glomeruli, then reabsorbed and metabolized by tubular epithelia |
| $T_{1/2}$ (subcutaneous administration) ~ 2.5 h |

C_{max} , Maximum plasma concentration; $T_{1/2}$, elimination half-life.

IL-2 pharmacokinetics

The pharmacokinetic properties of IL-2 are summarized in Table 1 [36]. IL-2, a prototypic member of the interleukin/hematopoietic cytokine family, is characterized as a globular glycoprotein of small molecular size (molecular weight, ~ 15 kDa) [17]. These physical properties allow IL-2 to freely pass between capillary endothelial cells so that, after intravenous (i.v.) administration, it distributes rapidly into the total body extracellular compartments, both intravascular and extravascular. The total extracellular space in a normal human adult is approximately 14 l. Therefore, to generate IL-2 concentrations high enough to saturate even the high-affinity IL-2Rs (100 pmol/l, 1.5 ng/ml), at least 20 μ g IL-2 must be administered, assuming that all of the molecules are native and active. Following i.v. administration of recombinant human IL-2 (16.7 μ g/m²), the initial elimination half-life ($T_{1/2}$) is 11 min, followed by a subsequent slow decay ($T_{1/2} = 45$ min) that is attributable to renal excretion (Smith, unpublished data). The IL-2 is filtered by the glomeruli, then reabsorbed and metabolized by the tubular epithelia [31].

Subcutaneous (s.c.) administration of IL-2 at identical doses results in peak plasma concentrations (C_{max}) of 25 pmol/l after 3 h (T_{max}) with a 2.8 h elimination $T_{1/2}$ (Table 1). This longer $T_{1/2}$ allows IL-2 to bind more than half of the high-affinity IL-2Rs expressed by the antigen-activated T cells and NK cells for at least 12 h. Therefore, s.c. administration is preferable to i.v. administration because it allows sufficient plasma IL-2 concentrations to bind to more than one-half of the high-affinity IL-2Rs for at least 12 h. The systemic toxicity of IL-2 is thus avoided by keeping the IL-2 concentration in the extracellular compartment below 25–30 pmol/l. This IL-2 concentration will only bind to ~ 2 –3% of the intermediate-affinity IL-2 receptors on the majority of NK cells (Smith, unpublished data) [24].

Table 2. Interleukin-2 (IL-2) doses and regimens.

| Dose | IU ^a (× 10 ⁶) | IL-2 protein (mg) | Regimen |
|--------------|--------------------------------------|-------------------|------------------------|
| Ultra-high | 150 | 10 | 3–5 days |
| High | 15 | 1 | 5 days every 8 weeks |
| Intermediate | 9 | 0.6 | 5 days every 4–8 weeks |
| Low | 2 | 0.133 | Daily |

^a Defined as 15 million international units (IU)/mg IL-2 protein, based on the IL-2 cytotoxic T-cell lines [16].

Clinical rationale for IL-2 therapy in HIV infection

Acceleration of the recovery of the immune system

In HIV disease, cytokines exert both positive and negative effects on the virus and virus-infected cells. Pro-inflammatory cytokines (TNF- α , IL-1, and IL-6) produced by the innate host cells, especially during the initial infection, facilitate viral entry to the cell, proviral integration into the cellular genome, viral replication, and production of new virions [37]. However, subsequent to the T-cell recognition and response to HIV, the IL-2 produced stimulates T-cell and NK-cell proliferation and differentiation to cytotoxic effector cells, and inhibits apoptosis of antigen-activated CD8+ T cells, so that the IL-2-promoted activation of an antiviral cellular immune response is responsible for killing the virus-infected cell [7]. Thus, as emphasized earlier, a vigorous cellular immune response correlates with control of the viral infection, so that the initial peak plasma viral concentration is reduced to lower levels after the onset of the cellular immune response [38–40].

As viral load is suppressed to undetectable levels by HAART, the number of detectable HIV-specific CD4+ and CD8+ T cells decline, probably due to decreased antigen stimulation [41]. This is thought to be due to the decline in antigen-induced production of IL-2 and lack of stimulation of expression of IL-2Rs. The rationale for the administration of IL-2 to patients undergoing treatment with HAART is to accelerate the recovery of the CD4+ T-cell concentrations [24,35,42]. However, IL-2 does not target progenitor cells of the bone marrow or thymus. Instead, IL-2 is thought to only expand the existing mature peripheral T-cell compartment. Therefore, one would anticipate that IL-2 would act primarily on those T cells in the periphery that have been recently activated by HIV antigen. Actually, in clinical trials in which either low-dose daily IL-2 [12,14] or high-dose intermittent IL-2 was administered [11,13], an accelerated recovery of the circulating CD4+ T-cell concentrations is observed, and there is an accelerated increase in both naive and memory cell subsets in the circulation. Also, there is a marked increase in the concentration of circulating NK cells when low-dose daily IL-2 therapy is administered [12,14,24,43]. By compar-

ison, intermittent high-dose IL-2 only causes a transient increase in circulating CD4+ T-cell concentrations, while NK-cell concentrations do not change [11,13].

Low-dose daily IL-2 therapy versus high-dose intermittent IL-2 therapy

There has been considerable controversy over the dose and regimen of IL-2 therapy (Table 2). This stems in part from the adaptation of the high-dose intermittent regimen originated in cancer therapy for use in HIV infection [10,44]. This high-dose intermittent approach was established empirically, and was based upon the maximum tolerable dose [31]. IL-2 causes severe dose-dependent systemic side effects characteristic of the systemic inflammatory response syndrome, which are attributable to NK cell-derived release of proinflammatory cytokines, including TNF- α , IFN- γ , and granulocyte-macrophage colony stimulating factor [45]. Unfortunately, there have been more trials employing the high-dose intermittent regimen than the low-dose daily regimen. At the high doses currently in use in intermittent regimens (i.e., 9–15 million international units/day × 5 days every 6–8 weeks), clinical Grade II and III toxicities are common [10,13]. These doses are clearly unphysiologic, in that they cause the severe pathologic signs and symptoms characteristic of the systemic inflammatory response syndrome. The intermittent regimen of administering IL-2 only every 2 months is also unphysiologic, in that the sudden withdrawal of IL-2 from IL-2-dependent cells leads to rapid apoptosis [21,28]. Therefore, the intermittent dosing regimen may very well select against the very cells that one would like to generate.

By comparison with high-dose intermittent IL-2 administration, low doses of IL-2 avoid activation of the majority of NK cells so that the secondary release of pro-inflammatory cytokines is attenuated. Consequently, this low dose of IL-2 yields physiologic IL-2 concentrations *in vivo*, so that IL-2 can be administered daily continuously [24]. We now have individuals who have self-administered IL-2 daily for more than 6 years without untoward effects (K.A. Smith, unpublished data). Moreover, by avoiding long periods without adequate IL-2 concentrations, cytokine withdrawal apoptosis is minimized. Consequently, a progressive accelerated accumulation of circulating CD4+ T cells and NK cells occurs. Moreover, because there are no systemic side effects of low-dose daily IL-2 therapy, individuals do not lose time from work or school, etc. In other words, this is the 'kinder, gentler approach' to IL-2 therapy.

Augmentation of HIV-specific immunity

It is now realized that HAART is effective therapy for HIV, capable of suppressing viral replication, but it cannot cure the infection. Thus, when HAART is discontinued after successful suppression of HIV replication, even for several years, all individuals have suffered a viral relapse within a few days [46,47]. Consequently, most

practitioners continue to treat with HAART continuously, to prevent the inevitable viral relapse. However, as the chronic long-term side effects of HAART continue to increase with prolonged therapy, the prospect of long-term HAART is becoming less and less tenable.

Often, *in vivo* assays of immunity are more representative of the capacity of a host to mount an immune response to a microbe than are *in vitro* correlates. However, until recently, it was impossible to devise *in vivo* methods to detect host reactivity to HIV. We developed a method to monitor *in vivo* antiviral reactivity in individuals who had recovered normal levels of circulating lymphocyte subsets and had achieved undetectable plasma virus RNA by the most sensitive assays [48]. After obtaining written informed consent, we perform a supervised treatment interruption (STI) and, subsequently, viral and lymphocyte dynamics are monitored carefully. In contrast to most STI protocols, we follow the course of viremia with frequent HIV determinations (i.e., twice weekly) until the HIV concentration reaches a trough, defined as at least four successive determinations that are within 25% of one another. By comparison, most STI protocols reinstitute HAART if the plasma HIV concentration exceeds an arbitrary level (e.g., ≥ 5000 RNA copies/ml). Using our approach, we have found that there is readily detectable *in vivo* antiviral reactivity, even in individuals who were infected chronically before they received HAART. Upon STI, all individuals suffer a viral relapse, with HIV becoming detectable in the plasma within a mean (\pm SEM) time interval of 19 ± 3 days. Subsequently, there is a rapid increase in plasma HIV concentration, with a doubling time of 1.6 ± 0.3 days, eventually reaching a peak concentration within 17 ± 2 days. Afterwards, the plasma virus concentration declines progressively with $T_{1/2} = 3.5 \pm 0.7$ days over the course of the next 2 weeks, eventually reaching a trough concentration within 18 ± 3 days. Coincident with the rise and fall of the plasma HIV concentration, there occurs a doubling of circulating CD8+ T cells, which remain elevated as the plasma HIV decreases. Simultaneously, there is only a transient, decrease of circulating CD4+ T cells coincident with the peak HIV concentration, while the concentration of NK cells remains unchanged.

Upon a second STI, we have found that 5/6 individuals followed thus far have had a greater than 10-fold reduction in their peak viral concentrations. We have followed these subjects for a mean of 194 days (range, 92–330 days) off HAART, and the plasma viral concentrations have consistently measured between 2000 and 20 000 HIV RNA copies/ml. Accordingly, it appears that there is an effective host response to HIV, which is detectable by monitoring viral and lymphocyte dynamics after a short-term STI. These data suggest that further experiments, exploring therapies designed to augment HIV-specific antiviral reactivity to the point where a viral relapse is prevented, are now warranted.

The role of STI in research and therapy of HIV infection

A recent publication exploring STI in individuals who were diagnosed and treated early with HAART has revealed results very similar to ours [49], which were obtained in individuals infected chronically before diagnosis and therapy [48]. The investigators involved with the early-treatment study interpreted their results as supporting the concept of the early diagnosis and treatment as important for sparing the immune system. However, our data indicate that, even in individuals infected for several years prior to the institution of HAART, there is a readily detectable *in vivo* antiviral host response [48]. This is very important, in that the vast majority of individuals are infected chronically before the diagnosis is made. In addition, data from early diagnosis and treatment studies in progress indicate that early treatment cannot prevent establishment of integrated proviral DNA. Consequently, new immunotherapy strategies are needed to target the immune system, to promote the maintenance of viral latency, and to prevent viral replication when antiviral treatment is discontinued.

At this time, it is unclear how STI should be used. There are some advocating STI to circumvent the cumulative side effects from continuous HAART. Others are attempting to use STI as a means to promote an endogenous immunization. It is important to stress that these goals are worthy, but that STI should remain an experimental procedure until its safety and efficacy have been demonstrated. Thus far, our experience indicates that 'short-term' interruption of therapy (i.e., for 8–12 weeks) is safe: we have not seen the development of drug-resistant mutants and we have observed only transient decreases in circulating CD4+ T cells. However, longer interruptions may well lead to progressive decreases in CD4+ T cells. It seems inherent that a therapeutic vaccine administered during HAART is much safer than allowing the endogenous virus to resume replication. Therefore, we plan to use the viral and lymphocyte dynamics observed after short-term STI as end-points in studies designed to examine the efficacy of immunotherapies administered during maximal suppression of endogenous viral replication with HAART. The goal of these studies will be to augment HIV-specific host defenses, so that relapse of detectable plasma viremia is prevented when HAART is discontinued.

Conclusions

Following the introduction of HAART, great advances have been made in HIV virus suppression; however, HAART does not promote the kind of immune restoration that is essential for maintaining a competent immune system and for preventing viral relapse when HAART is discontinued. Fortunately, immunotherapeutic agents such as IL-2 are available, and a great deal of basic and clinical research data indicate that IL-2 can be

used in a way that is safe and nontoxic. Consequently, the return of circulating lymphocytes to their normal concentrations can be accelerated. From the natural history of HIV infection, as well as our results and those of others of the viral and lymphocyte dynamics after STI, it is now clear that the immune system can be instrumental in containing the virus, even without aid from antiviral drugs. Therefore, the availability of nontoxic immunotherapy increases the prospects for generating effective protective immunity to HIV, as well as the prospects for the immunologic containment of latent or persistent HIV, in a fashion similar to the way the immune system contains the residual herpes viruses (e.g., Epstein-Barr virus, cytomegalovirus, herpes simplex viruses) and the way it contains latent HIV in long-term nonprogressors.

It is known that IL-2 stimulates the proliferation and differentiation of both CD4+ and CD8+ T cells, and also activation and stimulation of NK-cell proliferation and differentiation. Moreover, IL-2 prevents cytokine withdrawal apoptosis of antigen-activated T cells. Clinical evidence with IL-2 therapy thus far is yielding promising results: continuous low-dose IL-2 can be administered safely (i.e., without stimulation of viral replication) and without systemic toxicity, and the data indicate that daily IL-2 therapy accelerates the normalization of the concentrations of circulating T cells and NK cells over the course of several months.

Our data [48], and those of Rosenberg *et al.* [49] regarding the demonstration of better control of viremia after a second STI compared with the first STI, suggest that repetitive antigenic stimulation may augment antigen-specific immune reactivity. Also, the knowledge that antigenic stimulation is necessary for the production of IL-2 and for the expression of IL-2Rs suggests that two signals, antigen and IL-2, may be more effective than either alone. Therefore, combining a therapeutic vaccine with continued daily low-dose IL-2 therapy should result in marked expansion of HIV-specific CD8+ T cells, thereby promoting protective immunity. The most effective vaccines remain to be determined. However, it is now clear that the best way to monitor the effectiveness of different immunotherapeutic approaches *in vivo* is to follow viral and lymphocyte dynamics after short-term STI.

References

- Gulick RM, Mellors JW, Havlir D, *et al.* Treatment with indinavir, zidovudine, and lamivudine in adults with human immunodeficiency virus infection and prior antiretroviral therapy. *N Engl J Med* 1997, **337**:734-739.
- Rosenberg ES, Billingsley JM, Caliendo AM, *et al.* Vigorous HIV-1-specific CD4+ T cell responses associated with control of viremia. *Science* 1997, **278**:1447-1450.
- Walker B. Cellular immune response in HIV-1 infections and effects of therapy on immunologic parameters. *Int AIDS Soc* 1999, **7**:4-8.
- Kasaian MT, Leite-Morris KA, Biron CA. The role of CD4+ cells in sustaining lymphocyte proliferation during lymphocytic choriomeningitis virus infection. *J Immunol* 1991, **146**:1955-1963.
- Matloubian M, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. *J Virol* 1994, **68**:8056-8063.
- Battegay M, Moskopidhis D, Rahemtulla A, Hengartner H, Mak TW, Zinkernagel RM. Enhanced establishment of a virus carrier state in adult CD4+ T-cell-deficient mice. *J Virol* 1994, **68**:4700-4709.
- Cousens LP, Orange JS, Biron CA. Endogenous IL-2 contributes to T cell expansion and IFN-gamma production during lymphocytic choriomeningitis virus infection. *J Immunol* 1995, **155**:5690-5699.
- Davey RJ, Bhat N, Yoder C, *et al.* The noHRT trial: a prospective study of cessation of HAART in HIV-infected patients after prolonged viral suppression. *39th Interscience Conference on Antimicrobial Agents and Chemotherapy*. San Francisco, September 1999 [abstract 487].
- Zhang L, Ramratnam B, Tenner-Racz K, *et al.* Quantifying residual HIV-1 replication in patients receiving combination antiretroviral therapy. *N Engl J Med* 1999, **340**:1605-1613.
- Kovacs JA, Baseler M, Dewar RJ, *et al.* Increases in CD4 T lymphocytes with intermittent courses of interleukin-2 in patients with human immunodeficiency virus infection. A preliminary study. *N Engl J Med* 1995, **332**:567-575.
- Kovacs JA, Vogel S, Albert JM, *et al.* Controlled trial of interleukin-2 infusions in patients infected with the human immunodeficiency virus. *N Engl J Med* 1996, **335**:1350-1356.
- Jacobson EL, Piaro F, Smith KA. Rational interleukin 2 therapy for HIV positive individuals: daily low doses enhance immune function without toxicity. *Proc Natl Acad Sci USA* 1996, **93**:10405-10410.
- Davey RT Jr, Chaitt DG, Albert JM, *et al.* A randomized trial of high- versus low-dose subcutaneous interleukin-2 outpatient therapy for early human immunodeficiency virus type 1 infection. *J Infect Dis* 1999, **179**:849-858.
- Lalezari J, Beal J, Ruane P, *et al.* Low dose daily subcutaneous interleukin 2 in combination with highly active antiretroviral therapy in HIV+ patients: a randomized, controlled trial. *HIV Clin Trials* 2000, in press.
- Morgan DA, Ruscetti FW, Gallo R. Selective *in vitro* growth of T lymphocytes from normal human bone marrows. *Science* 1976, **193**:1007-1008.
- Gillis S, Ferm MM, Ou W, Smith KA. T cell growth factor: parameters of production and a quantitative microassay for activity. *J Immunol* 1978, **120**:2027-2032.
- Robb RJ, Smith KA. Heterogeneity of human T-cell growth factor(s) due to variable glycosylation. *Mol Immunol* 1981, **18**:1087-1094.
- Smith KA, Favata MF, Oroszlan S. Production and characterization of monoclonal antibodies to human interleukin 2: strategy and tactics. *J Immunol* 1983, **131**:1808-1810.
- Robb RJ, Munck A, Smith KA. T cell growth factor receptors. Quantitation, specificity, and biological relevance. *J Exp Med* 1981, **154**:1455-1474.
- Cantrell DA, Smith KA. The interleukin-2 T-cell system: a new cell growth model. *Science* 1984, **224**:1312-1316.
- Smith KA. Interleukin-2: inception, impact, and implications. *Science* 1988, **240**:1169-1176.
- Cantrell DA, Smith KA. Transient expression of interleukin 2 receptors. Consequences for T cell growth. *J Exp Med* 1983, **158**:1895-1911.
- Meuer SC, Hussey RE, Cantrell DA, *et al.* Triggering the T3-Ti antigen-receptor complex results in clonal T cell proliferation through an interleukin 2-dependent autocrine pathway. *Proc Natl Acad Sci USA* 1984, **81**:1509-1513.
- Smith KA, Jacobson EL, Emert R, *et al.* Restoration of immunity with interleukin 2 therapy. *The AIDS Reader* 1999, **9**:563-572.
- Tenner-Racz K, Stellbrinck H-J, van Lunzen J, *et al.* The unenlarged lymph nodes of HIV-infected, asymptomatic patients with high CD4+ T cell counts are sites for virus replication and CD4+ T cell proliferation. The impact of highly active antiretroviral therapy. *J Exp Med* 1998, **187**:949-959.
- Brodie S, Patterson B, Lewinsohn D, *et al.* HIV-specific cytotoxic T lymphocytes traffic to lymph nodes and localize at sites of HIV replication and cell death. *J Clin Invest* 2000, **105**:1407-1417.

27. Pakker NG, Notermans DW, de Boer RJ, *et al.* **Biphasic kinetics of peripheral blood T cells after triple combination therapy in HIV-1 infection: a composite of redistribution and proliferation [see comments].** *Nat Med* 1998, **4**:208–214.
28. Kuroda K, Yagi J, Imanishi K, *et al.* **Implantation of IL-2-containing osmotic pump prolongs the survival of superantigen-reactive T cells expanded in mice injected with bacterial superantigen.** *J Immunol* 1996, **157**:1422–1431.
29. Caligiuri MA, Zmuidzinas A, Manley TJ, Levine H, Smith KA, Ritz J. **Functional consequences of interleukin 2 receptor expression on resting human lymphocytes. Identification of a novel natural killer cell subset with high affinity receptors.** *J Exp Med* 1990, **171**:1509–1526.
30. Lodolce J, Boone D, Chai S, *et al.* **IL15 receptor maintains lymphoid homeostasis by supporting lymphocyte homing and proliferation.** *Immunity* 1998, **9**:669–676.
31. Lotze MT, Matory YL, Ettinghausen SE, *et al.* **In vivo administration of purified human interleukin 2. II. Half life, immunologic effects, and expansion of peripheral lymphoid cells in vivo with recombinant IL 2.** *J Immunol* 1985, **135**:2865–2875.
32. Smith KA. **The interleukin 2 receptor.** *Annu Rev Cell Biol* 1989, **5**:397–425.
33. Smith KA. **Cell growth signal transduction is quantal.** *Ann NY Acad Sci* 1995, **766**:263–271.
34. Wang HM, Smith KA. **The interleukin 2 receptor. Functional consequences of its bimolecular structure.** *J Exp Med* 1987, **166**:1055–1069.
35. Smith KA. **Rational interleukin 2 therapy.** *Cancer J* 1997, **3**:S137–S140.
36. Smith KA. **Interleukin 2 Immunotherapy.** In *Therapeutic Immunology*, 2nd edition. Edited by Austen F, Burakoff S, Rosen F, Strom T. Cambridge, MA: Blackwell Science; 2000.
37. Stevenson M, Stanwick T, Dempsey M, Lamonica C. **HIV-1 replication is controlled at the level of T cell activation and proviral integration.** *EMBO J* 1990, **9**:1551–1560.
38. Koup RA, Safrit JT, Cao Y, *et al.* **Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome.** *J Virol* 1994, **68**:4650–4653.
39. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. **Virus-specific CD8⁺ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection.** *J Virol* 1994, **68**:6103–6110.
40. Little SJ, McLean AR, Spina CA, Richman DD, Havlir DV. **Viral dynamics of acute HIV-1 infection.** *J Exp Med* 1999, **190**:841–850.
41. Pitcher CJ, Quittner C, Peterson DM, *et al.* **HIV-1-specific CD4⁺ T cells are detectable in most individuals with active HIV-1 infection, but decline with prolonged viral suppression.** *Nat Med* 1999, **5**:518–525.
42. Smith KA. **Lowest dose interleukin-2 immunotherapy.** *Blood* 1993, **81**:1414–1423.
43. Soiffer RJ, Murray C, Cochran K, *et al.* **Clinical and immunologic effects of prolonged infusion of low-dose recombinant interleukin-2 after autologous and T-cell-depleted allogeneic bone marrow transplantation.** *Blood* 1992, **79**:517–526.
44. Rosenberg SA, Lotze MT, Muul LM, *et al.* **Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer.** *N Engl J Med* 1985, **313**:1485–1492.
45. Mier JW, Vachino G, Klempner MS, *et al.* **Inhibition of interleukin-2-induced tumor necrosis factor release by dexamethasone: prevention of an acquired neutrophil chemotaxis defect and differential suppression of interleukin-2-associated side effects.** *Blood* 1990, **76**:1933–1940.
46. Chun T-W, Engel D, Mizell SB, *et al.* **Effect of interleukin-2 on the pool of latently infected, resting CD4⁺ T cells in HIV-1-infected patients receiving highly active anti-retroviral therapy.** *Nat Med* 1999, **5**:651–655.
47. Davey RTJ, Bhat N, Yoder C, *et al.* **HIV-1 and T cell dynamics after interruption of highly active antiretroviral therapy (HAART) in patients with a history of sustained viral suppression.** *Proc Natl Acad Sci USA* 1999, **96**:15109–15114.
48. Smith K, Jacobson E, Sohn T, Warren D, Emert R, Giordano M. **In vivo assessment of antiviral reactivity in chronic HIV infection.** *HIV Clin Trials* 2000, in press.
49. Rosenberg E, Altfeld M, Poon S, *et al.* **Immune control of HIV-1 after early treatment of acute infection.** *Nature* 2000, **407**:523–526.