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Interleukin-2: Use in Immune Restoration and During Structured Treatment Interruption

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LIKE HIV ITSELF, UNDERSTANDING OF HOW cytokines work is a relatively young field of scientific study. But with the explosion of immunology-based research over the last 20 years, much has been learned about how HIV impairs the immune system and the potential role cytokines play in preventing—and perhaps reversing—the damage incurred by this virus. And while cytokine therapy has yet to be included into the armamentarium of standard therapies to treat HIV disease, research is quickly homing in on a potential role for these therapies, particularly recombinant interleukin-2 (IL-2).

IL-2 101

CONSIDERED AS A WHOLE, CYTOKINES ARE A group of regulatory proteins produced by cells, which in turn act to control the proliferation and activity of cells. IL-2, originally called T-cell growth factor, is produced by antigen-activated T-lymphocytes. It acts in an autocrine or paracrine fashion on T-cells to promote their division and activates other cells in the immune system such as natural killer (NK) cells and B-lymphocytes. Other biological activities of IL-2 include: the generation of lymphokine-activated killer (LAK) cells; the activation of macrophages; the stimulation of T-cells to produce other cytokines; and the proliferation of oligodendrocytes.

Soon after the discovery of a T-cell growth factor activity in lymphocyte culture media by Drs. Doris Morgan and Frank Ruscetti in Dr. Robert Gallo's laboratory at the National Cancer Institute (NCI) in 1976, Dr. Smith and his colleagues—then at Dartmouth Medical School—conducted a series of experiments over a five-year period that led to

the discovery of the IL-2 molecule. Much to Dr. Smith's credit, these data—published in more than ten peer-reviewed scientific journals—paved the way for Dr. Tadatsugu Taniguchi and his colleagues in Japan to clone the IL-2 gene in the early 1980s, making it possible to produce large quantities of recombinant IL-2.

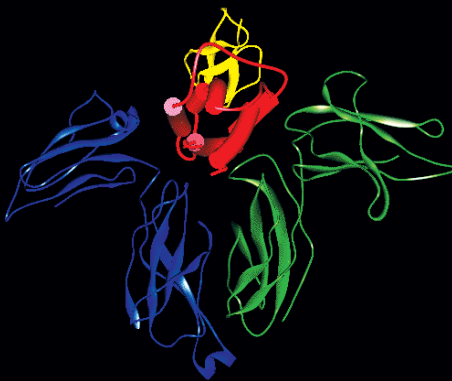
IL-2 is composed of 133 amino acid residues that are organized into four main anti-parallel amphipathic alpha helices. The molecule is stable in extremes of pH and heat, with a strong hydrophobic core and a hydrophilic exterior. IL-2 is variably glycosylated, but the carbohydrates are not involved in mediating its biological activity.

Only antigen-activated T-lymphocytes, which include CD4+ and CD8+ cells, produce IL-2. Upon being exposed to a foreign antigen, T-cell receptors (TCRs) signal the expression of IL-2 receptors. Conversely, IL-2 receptors are not expressed in the absence of antigen.

There are actually three different classes of IL-2 receptors—a high-affinity class, consisting of α , β , and γ chains; an intermediate-affinity class, consisting of β and γ chains; and a low-affinity class, consisting only of an α chain. And while all IL-2 receptors serve as IL-2 binding sites, those receptors containing the β chain and γ chain are also involved in creating the signal associated with cellular IL-2 production. Understanding the relationship between IL-2 receptor signaling and antigen stimulation is important, particularly with respect to Dr. Smith's more recent research (discussed below).

NK cells also express IL-2 receptors, albeit with a much lower affinity than those expressed by T-cells; only 10% of the overall NK cell population express high-affinity IL-2 receptors. Once stimulated by IL-2, NK cells produce their own set of cytokines—mainly interferon-gamma ($\text{IFN}\gamma$), tumor necrosis factor-alpha ($\text{TNF}\alpha$), and granulocyte-macrophage colony stimulating factor (GM-CSF)—that stimulate macrophages. And because NK cell/macrophage activity is an important front-line approach to preventing the spread of infection before antigen-specific T-cell immune responses emerge, the potential role of IL-2 in promoting their activity cannot be ignored.

The IL-2 and IL-2 receptor complex



Interleukin-2 is represented schematically in red. The receptor-alpha chain is blue, the beta chain is yellow, and the gamma chain is green.

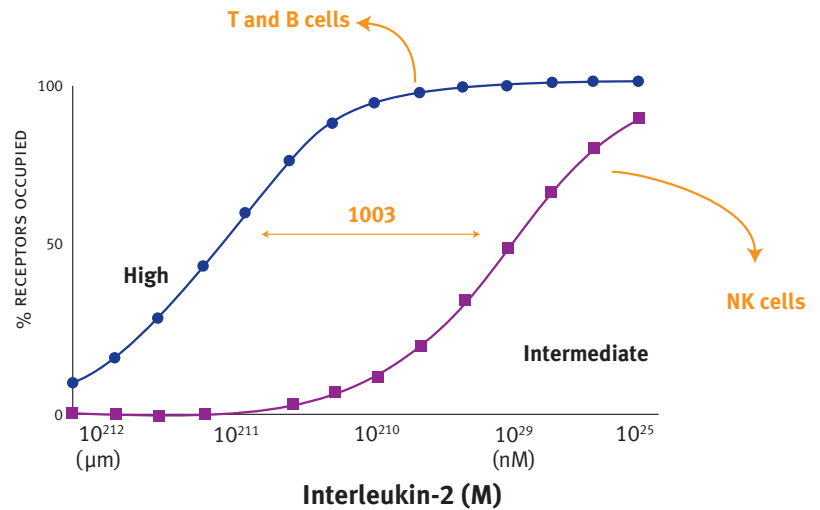
Source: Curtis Sather (Claremont McKenna College). Based on molecular structure data contained within the Brookhaven Protein Data Bank (PDB).

Therapeutic Implications

RECOMBINANT IL-2 WAS FIRST USED IN A 1985 clinical trial involving patients with refractory metastatic cancer. As reported by Dr. Steven Rosenberg and colleagues at the NCI, high-dose IL-2 therapy (50 million IU q8h for five days) was associated with a 44% response rate, when used in combination with autologous LAK cells (Rosenberg, 1985). These data were subsequently debated

Figure 1. Interleukin-2 receptor (IL-2R) occupancy is shown as a function of IL-2 concentration by plotting the percentage of receptors bound by IL-2 against levels of plasma IL-2. High-affinity IL-2Rs, expressed by antigen-activated T-cells and B cells, bind IL-2 at very low concentrations, between 1 and 100 pM. By comparison, intermediate-affinity IL-2Rs have a 10-fold lower affinity for IL-2. As a result, 100-fold higher IL-2 concentrations (100 pM to 10 nM) are necessary to saturate these intermediate-affinity IL-2Rs, which are expressed by most natural killer (NK) cells.

Source: Smith, 1999. (*The AIDS Reader*: SCP Communications).



by the author, and it was determined that autologous LAK cell administration was not necessary and that the response rate was really 20%, not 44%, with complete responses seen in 10% of the patients studied (Rosenberg, 1997).

Studies dating back to as early as 1980, many of which were conducted by Dr. Smith and his colleagues, found that IL-2 plays a critical role in generating various immune responses. Numerous *in vitro* studies have demonstrated that high doses of recombinant IL-2 are necessary to saturate all IL-2 receptors, particularly T-cells and NK cells with intermediate- and low-binding affinities. Yet high doses of IL-2 can lead to a cascade of cytokine events, including the secretion of the pro-inflammatory cytokines (e.g., IFN γ and TNF α), resulting in extreme toxicities such as fatigue, malaise, fever, and capillary leakage. In turn, it is vital to understand how best to maximize efficacy while at the same time minimizing the dosages used in both clinical research and practice.

With respect to formulations, both Amgen's and Chiron's recombinant IL-2 have been studied in clinical trials. And while Amgen's IL-2 formulation is more potent than the Chiron formulation, only Chiron's recombinant version of IL-2 aldesleukin (Proleukin) has been approved by the Food and Drug Administration (Smith, 1999). [Editor's Note: For the sake of consistency, all doses listed herein, regardless of which IL-2 brand was studied, reflect those associated with aldesleukin therapy.]

Dr. Smith suggests that subcutaneous injections of IL-2 provide a bigger bang for the buck; Subcutaneous (sc) injections are associated with slower decay rates than identical iv doses, in that IL-2 must first be absorbed into the bloodstream. In turn, low doses of IL-2 injected sc allow for concentrations sufficient to bind to high-

affinity IL-2 receptors, but are too low to bind to most intermediate-affinity IL-2 receptors expressed by most NK cells.

To fully saturate both high- and intermediate-affinity receptors, greater than 150 million IU/day—which translates into 10 mg of IL-2 protein—would be needed. This “ultra-high” dose regimen, administered in three divided doses every eight hours for three to five days, is associated with severe grade 3 or 4 toxicities, and should be administered only in the setting of supportive hospital care. This dose has not been studied in HIV-infected patients, nor are there plans to initiate such a study.

Clinical studies and treatment protocols for HIV infection at the National Institutes of Health typically use doses of 12 to 18 million IU/daily (approximately 1 mg IL-2 protein), administered by continuous intravenous infusion or in two divided subcutaneous doses, both for five days every eight weeks. This relatively high dosing schedule is associated with high- and intermediate-affinity saturation of approximately 99% and 82%, respectively. According to the results of several studies, these doses resulted in a progressive increase in CD4+ cell counts, along with other immunologic parameters (Davey, 1999, 1999a, 1997; Kovacs, 1996). However, a significant number of grade 3 or 4 toxicities were reported, including capillary leakage, hypotension, marked lymphopenia, and viral load increases.

An intermediate dose of IL-2—approximately 9 million IU/daily (0.6 mg IL-2 protein)—saturates approximately 98% of all high-affinity IL-2 receptors and 32% of all intermediate-affinity receptors. This dose,

divided into twice-daily sc injections self-administered for five days every two months, has been studied in HIV-infected patients with CD4+ counts between 200 and 500 cells/mm³ also receiving highly active antiretroviral therapy (HAART) (Levy, 1999). Compared to patients receiving HAART alone, intermediate doses of IL-2 in combination with potent antiretroviral therapy were associated with tenfold increase in CD4+ cells. While this dose is much better tolerated than either of the two higher doses of IL-2 discussed above, fatigue and fever are common toxicities, occurring in 25% and 42% of patients, respectively.

Various HIV clinical studies employing low doses of IL-2 therapy have been reported. In one study conducted by Dr. Elizabeth Jacobson and Dr. Smith and their colleagues at Cornell Medical College, 2 million IU sc of IL-2—administered daily and continuously for six months—was found to be the maximum dose that can be administered on a continuous basis without any adverse effects (Jacobson, 1996). This dose can be self-administered by patients and, thus, does not interfere with daily activities such as work.

Why continuous IL-2 therapy? According to Dr. Smith, intermittent therapy is associated with apoptosis after the completion of each high-dose cycle. In other words, cell populations exposed to IL-2 remain in continuous demand for the protein in order to survive and thrive. And while low doses of IL-2 are associated with low saturation rates of low-affinity IL-2 receptors, they may still be able to saturate high-affinity IL-2 receptors found on most T-lymphocytes and a small number of NK cells. High-dose in-

intermittent therapy tends to be associated with severe leukopenia toward the end of each cycle, followed by transient increases in various immunologic cell populations. Low-dose continuous therapy, on the other hand, is associated with steady increases in the NK-cell population, at a rate of 2.5 cells/day for approximately two months (approximately 200 NK cells/mm³ above baseline after eight weeks of therapy) (Smith, 1999). While CD4+ cell increases are much slower using low-dose continuous therapy—compared to more robust CD4+ cell increases seen in patients receiving intermediate- and high-dose IL-2—the increase of 0.35 CD4+ cells/day is still considerably more robust than the 0.14 cells/day increase in CD4+ counts seen in patients receiving HAART alone.

Another possible advantage of low-dose IL-2 therapy is its clinical utility in patients with advanced HIV disease. According to one study conducted in Barcelona, initiated in collaboration with Dr. Clifford Lane at the NIH, six cycles of IL-2 injections in combination with HAART appeared to be well tolerated and improved immunologic surface markers in HIV-positive patients with fewer than 200 CD4+ cells/mm³ (Arnó, 1999). Twenty-five study volunteers with a mean average of 150 CD4+ cells/mm³, all of whom had been receiving HAART for at least 24 weeks and had undetectable HIV-RNA levels at the time of randomization, were evenly divided either to continue HAART alone or to receive concomitant IL-2 therapy (3 million IU sc injections twice-daily for five days every four weeks). Due to the high rate of adverse events upon initiating therapy—10/13 (80%) of patients receiving IL-2/HAART experienced one or more grade 3 toxicity—the dose of IL-2 was decreased to 3 million IU sc injections once daily.

After the dose reduction, the side effects reported were generally mild (grade 2) and were easily controlled using adjunctive anti-inflammatory therapies. CD4+ cell counts increased significantly in the IL-2 group after six months of therapy, compared with mean baseline values (+105 cells/mm³), whereas a significant difference was not reported in the control group. Memory T-cells initially contributed to the CD4+ cell increase at week four, and naive T-cell increases, when compared to baseline levels, became statistically significant after 24 weeks of therapy. NK-cell data were not analyzed or reported.

Similar data were reported recently by

Dr. Christine Katlama and her colleagues at the 7th European Conference on Clinical Aspects and Treatment of HIV-Infection, held last October in Lisbon (Katlama, 1999). According to Dr. Katlama's report, 68 patients receiving HAART—none of whom achieved CD4+ count levels greater than 200

cells/mm³ while on therapy—were randomized to receive several cycles of intermediate-dose IL-2 therapy (4.5 million IU sc twice-daily for five days every eight weeks) or placebo. Three of the 34 patients randomized to receive IL-2 discontinued because of adverse effects, with an additional five patients switching to a lower dose of the drug. Improvements in lymphocyte proliferative responses (LPRs) to CMV—but not to tuberculosis, tetanus toxoid, or p24 antigen—were observed in the patients receiving IL-2. Increases in CD4+ counts were also reported, the bulk of which were naive cells.

While the clinical benefit of IL-2 has not yet been determined, a few clinical endpoint studies employing intermediate-dose IL-2 regimens are currently under way or in the final stages of development. One such study (SILCAAT), launched recently by Chiron, will randomize 1,400 patients with CD4+ counts between 50 and 299 cells/mm³ and currently on a stable antiretroviral regimen to receive either six cycles of intermediate-dose, subcutaneous IL-2 for five days every 8 weeks with HAART or HAART alone. According to Dr. Smith, the study will evaluate whether the intermediate dose (i.e. 9 million IU/day) intermittent administration of IL-2, which induces improvements in circulating CD4+ cell numbers and function, actually results in better clinical outcomes and/or prolonged antiretroviral effectiveness of HAART. More information about this study can be obtained on the World Wide Web at <http://www.silcaat.com> or by calling toll-free 1-800-CHIRON-8.

Structured Treatment Interruptions

ACCORDING TO DR. BRUCE WALKER AND HIS colleagues, HIV-specific CD4+ cells are rapidly destroyed early in the course of HIV in-

Table 1: Doses Used for Cancer and HIV Infection

Dose	Bioactivity (IU x 100,000)	Protein (mg)	Regimen
Ultra-high	150	10	3 to 5 days
High	15	1	5 days every 8 weeks
Intermediate	9	0.6	5 days every 4 weeks
Low	2	0.133	Daily

Source: Smith, 1999. (*The AIDS Reader*: scp Communications).

fection, rendering cytotoxic T-lymphocytes and other components of the immune system virtually unable to effectively control viral replication (see "Effects of Antiviral Therapy on Immune Responses in HIV Infection," in Volume IV, Number 3 of *The PRN Notebook*). However, Dr. Smith pointed out that a study conducted at the University of Texas, Southwestern Medical Center, recently demonstrated that HIV-specific CD4+ cells are very much present and active in chronically infected individuals (Picker, 1999). The study, using flow cytometry to detect antigen-induced intracellular cytokines, indicated the presence of HIV-gag-specific CD4+ memory cells in patients with both non-progressive infection (median frequency: 0.40%) and active/progressive (median frequency: 0.12%) HIV disease. While the median frequencies of these cells were considerably higher in the long-term non-progressive patients (LTNPs), there was substantial overlap between the two populations studied (median frequency ranges: 0 to 0.66% and 0.10 to 1.7% for patients with progressive and non-progressive disease, respectively).

Not surprisingly, continuous HIV-RNA suppression using antiretroviral therapy was associated with a time-dependent reduction in median frequencies of these HIV-specific CD4+ cells. Thus, while HAART maintains control of HIV-replication, it appears that other therapies will be necessary to boost the participation of these limited HIV-specific CD4+ cells if the long-term control of viral infection and prevention of virologic relapse is to be achieved. IL-2 can be used to promote the proliferative expansion of HIV-specific T-cells, both CD4+ and CD8+, but for IL-2 to be effective, antigen must be present in order to promote IL-2 receptor signaling, particularly those of existing HIV-specific CD4+ cells. And for this to be possible, exogenous HIV antigens must be provided or

HIV-RNA must be permitted to rebound in order to awaken these cells from their quiescent slumber.

To examine the possibility of promoting HIV immunity using IL-2, Dr. Smith and his colleagues conducted a non-randomized, open-label structured HAART interruption study in patients who received continuous IL-2 therapy plus maximally suppressive antiretroviral therapy for at least three months. In order to be considered for entry, patients were also required to have achieved normal CD4+ counts (approximately 600 cells/mm³) and elevated levels of both CD8+ cells and NK cells. [Editor's Note: Dr. Smith and his colleagues used Amgen's IL-2 formulation in this study, employing a dose of 250,000 IU/daily, which is roughly equal to the Chiron formulation at a dose of 2 million IU/daily.]

According to data presented by Drs. Smith and his colleagues at the 7th Conference on Retrovirus and Opportunistic Infections (CROI), nine individuals have opted to cease antiretroviral therapy while at the same time continuing their daily course of subcutaneous IL-2 (Smith, 2000). No surprise to Dr. Smith, HIV-RNA levels rebounded to detectable levels almost immediately, with peak levels reported within two weeks. After this peak, the viral loads fell precipitously and lingered at a "steady state" level for the next few weeks. The mean peak viral load was approximately 375,000 copies, which decreased to a mean HIV-RNA level of approximately 31,000 copies/mL, with a maximum decrease of 0.94 log.

Coincident with the rise in plasma HIV concentration, there was a marked increase in circulating CD8+ T-cells. A likely reason for this finding is that these CD8+ cells are HIV-specific CTLs, stimulated by antigen-specific CD4+ cells mediated by IL-2. "After HAART is discontinued," Dr. Smith explained, "continuation of IL-2 therapy should promote the proliferation of CTLs, and also enhance the CTL reactivity, along with that of NK cells, which together should contain virus replication. And based on these data, this might very well be the case."


In the absence of a control group, it was difficult to determine the exact benefit of concomitant IL-2 as a part of structured treatment interruptions. But the data are promising nonetheless and warrant additional investigation.

IL-2 and Eradication: An Update

USED AT HIGH DOSES, IL-2 THERAPY IS ASSOCIATED with a cascade of cytokine events that may purge proviral DNA from latent memory CD4+ cells, thus exposing the virus to antiretroviral therapy (see "Studies of HIV Latency: Implications for Treatment and Virus Eradication" in Volume IV, Number 2 of *The PRN Notebook*). To assess this potential role of recombinant IL-2, therapy was recently stopped by 18 patients enrolled in a study being conducted at the NIH (Davey, 1999b). All patients had been receiving HAART for a minimum of 12 months; the average time on HAART was actually 137 weeks. The median duration of undetectable HIV-RNA levels (<500 copies/mL) was 108 weeks, with all patients presenting with undetectable HIV-RNA levels using ultrasensitive testing (<50 copies/mL) at the time of treatment cessation.

Twelve of these patients had previously received IL-2. As reported by Dr. Tae-Wook Chun at a PRN meeting in early 1999, one patient had undetectable HIV-DNA in PBMCs and three had undetectable nested gag-RNA. And a high proportion of patients (39.9%), five of whom had taken IL-2, had levels of latently infected resting CD4+ cells that were below assay detection.

Nevertheless, viral rebound occurred in all patients following withdrawal of HAART. The rate of increase of HIV-RNA was approximately 0.2 log₁₀ per day, reaching > 50 copies/mL within 11 days, and > 100 copies/mL in 18 days. There was no difference in the observed rate of viral rebound between IL-2-treated and untreated persons. Reappearance of detectable HIV-RNA was delayed in the patient with undetectable HIV-DNA at baseline, occurring at week 7. And another patient—jokingly dubbed "the Bethesda Patient"—had only low-level (> 50 but < 500 copies/mL) viral rebound, which has been maintained through six months of follow-up.

From these data, and those reported by Dr. Smith, it appears that HAART is effective therapy for HIV, suppressing replication so that plasma virus becomes undetectable. However, HAART does not cure the infection, as evidenced by the rapid return of detectable plasma HIV if HAART is discontinued. Therefore, a new strategy is needed to ultimately control any residual latent HIV provirus, one that augments the function of the immune system. 

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