Effects of Intermittent Interleukin-2 Therapy on Plasma and Tissue Human Immunodeficiency Virus Levels and Quasi-Species Expression

Joseph A. Kovacs,1 Hiromi Imamichi,1 Susan Vogel,2 Julia A. Metcalf,2 Robin L. Dewar,3 Michael Baseler,3 Randy Stevens,3 Joseph Adelsberger,1 Laurie Lambert,3 Richard T. Davey, Jr.,2 Robert E. Walker,2,4 Judith Falloon,2 Michael A. Polis,2 Henry Masur,1 and H. Clifford Lane2

To characterize the effects of intermittent interleukin (IL)-2 therapy on human immunodeficiency virus (HIV), 11 patients underwent detailed virological evaluation during a year of IL-2 therapy. Six patients showed a >0.5 log increase in plasma HIV during at least 1 IL-2 cycle, with 2 experiencing an increase in >50% of cycles. Three of the remaining 5 patients had a >0.5 log decrease during at least 1 IL-2 cycle, and the remaining patients exhibited <0.5 log changes. No changes in lymphoid (tonsil) levels of HIV were seen during the year. Quasi-species analysis in a separate cohort demonstrated that the virus induced by IL-2 most commonly resembled pre-IL-2 plasma quasi species. Thus, intermittent IL-2 does not result in sustained increases in either plasma or tissue levels of HIV and does not result in sustained expression of a previously silent quasi species.

Controlled trials have shown that intermittent administration of interleukin (IL)-2 [1–4] to patients infected with human immunodeficiency virus (HIV) can result in substantial CD4 cell count increases, and follow-up studies have shown that these increases can be sustained for over 5 years [1, 5, 6] (J.A.K., unpublished observations). In a proportion of patients, IL-2 can induce transient increases in plasma HIV load [5], which raises a concern that long-term therapy with IL-2 could result in sustained increases in viremia. However, in a recent randomized, controlled trial of IL-2, no statistically significant differences in plasma virus load between the 2 groups were noted during the 14 months of study [1]. In fact, the control group demonstrated a significant increase in virus burden over time, whereas the IL-2 group showed no change in virus burden during the study [7]. In certain situations, activation of viral replication may be potentially beneficial. For example, recent studies have suggested that resting CD4 cells may serve as a reservoir for latent HIV infection [8–10]. Activation of these resting cells in the presence of highly active antiretroviral therapy, perhaps by IL-2 and the cytokine milieu that it induces, could potentially facilitate reduction of this reservoir [11, 12].

To better understand the short-term effects of IL-2 on HIV, the current study was undertaken to examine in detail the virological changes that occur during and immediately after IL-2 therapy.

Methods

The study enrolled 11 patients with HIV infection and CD4 cell counts >200 cells/mm3, who had never received IL-2, had no history of a prior acquired immunodeficiency syndrome (AIDS)-defining opportunistic infection, and had received no corticosteroids, cytotoxic chemotherapy, or experimental therapy in the previous 4 weeks. The study was designed to obtain frequent measurements of immunological and virological parameters in the context of 5-day infusions of IL-2 every 8 weeks. For HIV quasi-species analysis, 8 additional patients who had enrolled in previously reported IL-2 trials were studied [1, 5]. Three of these latter 8 patients had a transient increase in plasma HIV RNA levels (>0.5 log) during IL-2 therapy, whereas the other 5 did not. For the quasi-species analysis, 2 HIV type 1 (HIV-1)-infected patients who had not received IL-2 or antiretroviral drugs during the sampling period were used as control patients.

IL-2 (Chiron, Emeryville, CA) was diluted in D5W containing 0.1% albumin and administered by continuous infusion at a starting dose of 18 million IU per day for 5 days, as described elsewhere [1], with dose reductions of 3–6 million IU per day as needed for management of clinical or laboratory toxicities. IL-2 cycles were
administered approximately every other month for 1 year. Virol-
ological parameters were evaluated daily for 10 days after beginning
each IL-2 cycle and at monthly follow-up visits. Pre- and post-IL-
2 values were compared with a paired, 2-sided Student’s t test (SPSS
version 6.1 for Macintosh; SPSS, Chicago). P < .05 (2-sided) was
considered significant.

All patients received approved antiretroviral drugs (limited to zi-
dovudine, didanosine, zalcitabine, or stavudine when the study
was conducted) either alone (2 patients in each cohort) or in 2-drug
combination regimens (the remaining patients). Antiretroviral therapy
could be changed during the course of the study, except during IL-
2 administration. The specific regimen was determined by the patient
and referring physician, with input from the study team.

Particle-associated plasma HIV RNA levels were determined dur-
ing the study with the branched DNA assay (lower limits of detection,
10,000 HIV RNA copies/mL; Chiron) [13]. For patients with plasma
levels <10,000 copies/mL, frozen samples from the first cycle in which
adequate stored plasma was available were evaluated with a modi-
fication of the Amplicor HIV-1 Monitor kit, in which 1 mL, rather
than 200 μL, of plasma was used for pelleting HIV (lower limits of
detection, 50 HIV RNA copies/mL; Roche Molecular Systems,
Branchburg, NJ) [14, 15]. Tonsillar biopsy samples were processed
for measurement of HIV RNA levels as follows: samples were
weighed and then were homogenized in cold 8 M guanidine thio-
sulfate/0.5% sodium sarcosyl (1 mL per 25 mg tissue). After pelleting,
the RNA was precipitated from the supernatant by use of ethanol, re-
suspended in water, and used in the branched DNA assay.

For quasi-species analysis, HIV-1 RNA was isolated from 140
μL of plasma by use of the QIAamp Viral RNA Mini kit (Qiagen,
Valencia, CA) and reverse transcribed to cDNA with the cDNA
Cycle kit (Invitrogen, Carlsbad, CA) with the following primers: 5’-
AGTGGTCTCTGCTGCTCCAAAGA-3’ (corresponding to nu-
cleotides 7788–7811 of HIV-1 HXB2; GenBank accession no.
K03455). DNA was prepared from peripheral blood mononuclear
cells or lymph node cells with use of the PUREGENE DNA iso-
lation kit (Gentra Systems, Minneapolis, MN). The HIV envelope
V1–V5 region was amplified from cDNA or genomic DNA by use
of nested polymerase chain reaction (PCR) with a mixture of Klen-
taq1 (Ab Peptides, St. Louis, MO) and pfu (Stratagene, La Jolla,
CA) and the following primer pairs: initial PCR, forward primer
(nucleotides 6342–6360, HIV-1 HXB2) 5’-TATGGGGTACCT-
GTGTGGA-3’ and reverse primer (nucleotides 7720–7743) 5’-
GCACCACTCTTCTTTGGCTTGG-3’; nested PCR, forward
primer (nucleotides 6565–6582) 5’-AAAGCCTAAAGCCTATGG-
3’ and reverse primer (nucleotides 7645–7668) 5’-CCTTCTCC-
AATTGTCCTCCATAT-3’. The following PCR amplification cy-
cles were used for both reactions: cycle 1, 30 s at 99°C, 30 s at
67°C, and 15 min at 68°C; cycles 2–30, 2 min at 94°C, 2 min at
60°C, and 10 min at 72°C. Ten microliters of the PCR product
from the first reaction were used in the nested amplification. The
final PCR product was purified with the QIAquick PCR Purifi-
cation Kit (Qiagen), ligated into pCR II vector (Invitrogen), and
sequenced with an Applied Biosystems 377 automated sequencing
system (Perkin-Elmer, Foster City, CA).

For each patient, sequences of the V1–V5 region of the HIV-1
envelope were determined from simultaneously obtained plasma viral
RNA and cellular proviral DNA for 3 serial times: before IL-2 in-
fusion (day 0), immediately after a 5-day course of IL-2 (days 5–7;
proviral DNA was obtained for 3 patients) and ~1 month after the
IL-2 infusion (days 21–28). In one patient, proviral DNA from a
lymph node biopsy obtained 6 days before the IL-2 infusion began
was also studied. An average of 12 clones per sample were sequenced.
Nucleotide sequences were aligned by CLUSTAL W (ftp://ftp/
ebi.ac.uk/pub/software) [16] with default settings and were subse-
quently edited by hand. Phylogenetic relationships were estimated
with the neighbor-joining method [17] with the program PAUP* (ver-
sion 4.0.0b1; Sinauer Associates, Sunderland, MA). The appro-
priate model of evolution was determined by the likelihood ratio
tests of model of evolution with the program Modeltest (http://
bioag.byu.edu/zoology/crandall_lab/modeltest.htm) [18]. Gaps were
ignored for affected pairwise comparisons. Statistical support for
various nodes in the neighbor-joining tree was obtained by 1000
replications of the bootstrap procedure [19].

Results

Eleven men (mean age, 35 years; range, 31–42 years) were
enrolled in the longitudinal study. Six patients received the
scheduled 6 cycles of IL-2. Three patients (baseline CD4 cell
counts, 191–242 cells/mm3) left the study after 3 cycles, because
their CD4 cell counts did not demonstrate a sustained increase.
One of these patients died of AIDS-related complications ~13
months after his last IL-2 cycle. Among 2 patients, IL-2 was
held after 3 and 4 cycles, respectively, because their CD4 cell
counts had increased to >1500 cells/mm3. Mean CD4 cell
count for the group increased significantly at 6 months (11 patients;
from 371 to 803 cells/mm3; P = .026) and at 12 months (8
patients; from 427 to 991 cells/mm3; P = .001), with 8 of the
patients demonstrating an increase in CD4 cell counts ≥50%.
Side effects related to IL-2 administration were similar to those
reported in previous studies and resulted in decreases in IL-2
dosage in all patients [1, 3, 5, 6]. The average dose received
during the first year of the study was 46 million IU by contin-
uous intravenous infusion per 5-day cycle.

The geometric mean virus load before the study was 14,549
HIV copies/mL. There was an ~0.2 log increase in virus load
(P = .064) during the study. Antiviral therapies available dur-
ing the study were limited to nucleoside analogues. To examine
the kinetics of this virus load change in greater detail, plasma
viral RNA levels were measured daily for 10 days by the branched
DNA assay or the Amplicor assay. Figure 1 shows changes in virus
load during and immediately after a 5-day IL-2 infusion, as well as
the simultaneously determined CD4 cell counts, during a represent-
tative cycle for each patient (cycle 2 for 9 patients, and cycles 1 and 4 for 1 patient each). Of the
11 patients, 6 showed a >0.5 log increase in virus load during
≥1 cycle of IL-2. Among 2 of these patients, an increase was
seen in ≥50% of the cycles. Among the 5 patients in whom no
increase was seen, 3 had a >0.5 log decrease in virus load during
at least 1 cycle. Among patients with an increase or decrease in
plasma virus load during IL-2 therapy, levels generally re-
turned to baseline by day 10, although, on occasion, they did
Figure 1. Changes in plasma virus load (▲) and CD4 cell count (□) during and after a cycle of interleukin (IL)-2. Results for a representative cycle for each patient are graphed (patient no., lower right corner of each panel), as well as the geometric mean for the group (bottom right). Determinations were made during the second IL-2 cycle for all patients, except for patients 8 (cycle 4) and 9 (cycle 1). Bars indicate the 5-day period during which IL-2 was administered. Straight line in each graph indicates the lower limit of detection used for virus load determination for that patient (10,000 human immunodeficiency virus [HIV] RNA copies/mL for the branched DNA assay and 50 HIV RNA copies/mL for the modified Amplicor assay).
Figure 2. Changes in tissue virus load during a year of interleukin (IL)-2 therapy. Tonsillar biopsies were obtained from 8 patients with visible tonsillar tissue before the first cycle (pre) immediately before (Mo. 4 [pre]) and after (Mo. 4 [post]) the third cycle of IL-2 and 11 months after starting the study. RNA was extracted and measured by the branched DNA assay. Results are expressed per gram of tonsillar tissue. Results for individual patients are shown (solid lines). Geometric mean for the group is also indicated (dashed line).

Table 1. Plasma human immunodeficiency virus (HIV) levels and CD4 cell counts at the time of quasi-species analysis in 8 patients receiving interleukin (IL)-2.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Time, in days a</th>
<th>HIV RNA, copies/mL</th>
<th>CD4+ cells, cells/μL</th>
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<td>27</td>
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<td>0</td>
<td>726</td>
<td>476</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3948</td>
<td>2630</td>
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<tr>
<td></td>
<td>27</td>
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<td>464</td>
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a Days after beginning a 5-day cycle of IL-2. Day 0 sample was obtained immediately before beginning the IL-2 cycle.

Discussion

Although IL-2 can induce a transient increase in plasma virus load in some HIV-infected patients [5], such an increase is not universally induced, even in patients with measurable plasma virus. Although transient increases were consistently seen in 2 not return to baseline until the 1-month follow-up visit. All patients showed a decrease in CD4 cell count during the administration of IL-2, followed by a marked increase immediately after its discontinuation, presumably due in part to trafficking effects of IL-2 on lymphocytes. There was no clear relationship between changes in virus load and changes in CD4 cell count.

To examine the effects of IL-2 on tissue reservoirs of HIV, 8 patients had sequential tonsillar biopsies performed before the IL-2 therapy began, at month 4 (before cycle 3 of IL-2), and at month 11 (6 patients). Five of these patients also had biopsies performed at the end of the third IL-2 cycle. As shown in figure 2, no consistent changes in tissue virus load were seen either during the course of the study or during a cycle of IL-2. There was a mean decrease of 0.4 log (P = .24) in tonsillar virus load during the course of the study. Similar results were seen when data for only those patients who completed a year of therapy were examined.

HIV quasi-species analysis was undertaken in a separate cohort of 8 patients receiving IL-2 to try to identify the source of the virus transiently induced by IL-2. Table 1 summarizes the virus load and CD4 cell count changes during and after a cycle of IL-2. In 6 patients, the virus seen immediately after a 5-day cycle of IL-2 was indistinguishable from the viral quasi species seen at day 0 and at 1 month (table 1, patients 1, 2, 4–7; figure 3A and 3B). However, in 2 patients (patients 3 and 8, neither of whom demonstrated an increase in plasma HIV with IL-2 therapy), the virus present immediately after IL-2 therapy showed unique clustering in the phylogenetic tree (figure 3C). In one of these cases, this cluster resembled the sequences found in proviral DNA obtained from peripheral blood mononuclear cells at all 3 times, whereas in the second it represented a unique cluster. In both cases, the viral quasi species seen 1 month after IL-2 was closely related to the day 0 quasi species and not to the day 5–7 virus. It is noteworthy that in all patients, the proviral DNA sequences tended to cluster together and separately from the plasma viral sequences, except as noted above. In the single patient in whom lymph node mononuclear cells were analyzed, the proviral sequences present in the lymph node clustered with the plasma viral sequences, especially with the sequences that were seen immediately after IL-2 therapy (figure 3B) and not the peripheral blood mononuclear cell proviral DNA. For the control patients, no shift in quasi species was seen during the month in which serial samples were studied (data not shown).
Figure 3. Phylogenetic relationships among the sequences of the V1–V5 region of the human immunodeficiency virus type 1 (HIV-1) envelope in 3 patients (A–C; patients 1–3 in table 1) who received interleukin-2 therapy. For each phylogenetic tree, the appropriate model of evolution was determined by the likelihood ratio tests of model of evolution. Bootstrap percentile values from 1000 bootstrap replications are shown at nodes that define major groupings of sequences. Branch lengths are shown proportional to the amount of change along the branches. Each patient’s 6 closest sequences found in GenBank were included in the analysis (shown as GenBank accession nos.) and were used as outgroups. Isolates obtained from plasma virion RNA are shown (○), as are isolates obtained from peripheral blood mononuclear cell (PBMC) proviral DNA (□) and isolates obtained from lymph node provirus (+). Each time point is color coded.
of 11 patients in the current study, among 5 patients, plasma HIV levels remained unchanged or declined during or immediately after IL-2 therapy. As shown in randomized, controlled trials of IL-2, there does not appear to be any long-term detrimental effect associated with this transient viral activation [1, 3]. Thus, the transient viral burst does not result in more rapid development of resistance or escape from antiretroviral control.

Examination of lymphoid tissue during the study supports these observations. No changes were seen in HIV levels in sequential tonsil biopsies, and qualitative evaluation of sequential biopsies by in situ hybridization similarly showed no effect (data not shown). Thus, even in patients with measurable virus loads, IL-2 can result in substantial immunological changes that are independent of effects on the virus.

The mechanism behind the increases in plasma HIV levels is uncertain, but it may be a result of direct activation by IL-2 of cells latently infected by HIV or activation of uninfected cells that can serve to amplify the ongoing HIV replication. Quasi-species analysis demonstrated that in the majority of patients, the quasi species that is induced by IL-2 is similar to the quasi species that is present in the plasma immediately before, as well as 1 month after, IL-2 therapy. This suggests that IL-2 is inducing a viral burst primarily by amplifying the ongoing replication process, rather than inducing production from a silent reservoir. Previous observations that the addition of potent antiretroviral drugs, either nonnucleoside reverse transcriptase inhibitors or HIV protease inhibitors, can prevent the viral burst further suggest that ongoing replication is the primary mechanism, because these drugs would not prevent activation of chronically or latently infected cells [20]. Among 2 patients, however, the predominant plasma viral species present immediately after a cycle of IL-2 appeared to form a unique cluster that was more closely related to peripheral blood mononuclear cell-associated proviral DNA than to plasma HIV RNA sequences, which suggests that, in some patients, activation of latent infection may be occurring. However, in both of these patients, the quasi species at 1 month resembled the day 0 rather than the day 6–7 sample, which demonstrates that IL-2 therapy did not result in the permanent emergence of a unique quasi species.

Other cytokines may also be playing a role in the transient activation of HIV, since cytokines such as tumor necrosis factor-α and granulocyte-macrophage colony-stimulating factor, both of which are induced during IL-2 therapy (J.A.K., unpublished observations), can lead to stimulation of viral replication in vitro [21–23]. Stimulation of HIV-specific immune responses (e.g., CD8-mediated responses) or induction of cytokines (e.g., interleukin-10, which is also induced during IL-2 therapy) that have been shown to have an anti-HIV effect in vitro may account for the declines or lack of increase in plasma HIV levels seen in some patients [24–26]. Because of the differences seen among the patients, the outcome may depend on the relative contribution of these various parameters in an individual patient.

These experiences with antiretroviral regimens that are less effective than the currently available combination regimens leave the possibility that improved responses may be seen when IL-2 is used in combination with more active antiviral regimens that include potent protease inhibitors or nonnucleoside analogue reverse-transcriptase inhibitors. Preliminary results suggest that improved CD4 cell responses are seen in these settings [20, 27, 28]. However, it is noteworthy that substantial CD4 cell increases can occur with IL-2 therapy even in patients with plasma virus loads >10,000 copies/mL [1].

As the experience with highly active antiretroviral regimens has increased, substantial side effects as a result of long-term therapy are being reported with increasing frequency, and patient tolerance of these adverse effects, as well as the need for strict adherence, is decreasing [29, 30]. Identification of alternative therapeutic approaches to the long-term management of HIV-infected patients is clearly needed. Because opportunistic complications occur primarily after a decrease in the CD4 cell count to <200 cells/mm³, administration of IL-2 to maintain a CD4 cell count above that level (which may require IL-2 administration as infrequently as once every 6–12 months once a CD4 cell count increase has been induced) may confer clinical benefit even in patients with detectable plasma HIV levels. Concerns that long-term administration of IL-2 may lead to sustained increases in virus load in this setting have not been supported by any of the controlled trials conducted to date [1–4, 31]. The current study helps to better understand this observation by demonstrating that IL-2–induced increases in plasma virus load are not invariably, that when the increases occur they are transient, and that, although the increases may be associated with expression of a novel quasi species, this expression is transient and does not result in a permanent shift in the dominant quasi species. Controlled trials to evaluate the clinical benefit of IL-2 therapy, which are needed to help define the role of IL-2 in the management of HIV-infected patients, are currently being conducted.

Acknowledgments

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References


