



Review

The Immunological Effects of Interleukin 2 Therapy in HIV⁺ Patients

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Abstract. Human immunodeficiency virus (HIV) infection produces a profound impairment of immune functions that antiretroviral therapy is unable to restore. Because of its immuno-enhancing properties, interleukin 2 (IL-2) has been used as a therapeutic tool in HIV⁺ subjects. IL-2 produces an increase of CD4 and CD8 lymphocyte absolute counts that is preferentially due to the expansion of the “naive” cells. In addition, IL-2 increases cytokine production from the cells of the immune system and is able to up-regulate the expression of cytokine receptors, such as the chemokine receptors CCR-5 and CXCR-4. Less informations on the IL-2 activity on the CD8 subset are available at the moment. The advent of highly active antiretroviral therapy has changed this scenario, making the IL-2 effects less clear-cut than previously hypothesized. We suggest that the ongoing studies will define the precise role of IL-2 in the therapy of HIV infection.

Key words: HIV infection; interleukin 2; CD4 lymphocytes; CD8 lymphocytes; immunological function.

The peculiar hallmarks of the human immunodeficiency virus (HIV) infection are the progressive loss of CD4⁺ T lymphocytes and the functional inability of these cells to perform a normal immunologic response. Since at this moment there is experimental evidence indicating that lymphopenia could depend on either accelerated T cell destruction or from decreased CD4 T cell production by the virus, the primary cause of CD4 T cell lymphopenia remains still unclear^{17, 24, 26, 56, 58}.

One of the major factors contributing to the immunological derangement present in HIV⁺ subjects is the inability of CD4⁺ lymphocytes to produce interleukin 2 (IL-2)^{6, 57}; although low IL-2 mRNA levels have been found in the PBMCs of HIV⁺ subjects¹⁵, it is still a matter of debate whether a numerical reduction of IL-2 producing cells or a switch from the Th1 to the Th2 phenotype is responsible for this phenomenon^{6, 12, 22, 30}.

IL-2 has a central position in the immune system; secretion of IL-2, in fact, is the final event following various second-messenger intracellular pathways that integrate signals from surface receptors with the micro-environment. Then, T lymphocyte antigen proliferation is initiated via the TCR complex that triggers IL-2 and IL-2 receptor (IL-2R) production and the subsequent autocrine IL-2/IL-2R interaction allows T cell proliferation. In addition, IL-2 exerts pleiotropic effects on almost all the cells of the immune system. As far as HIV infection is concerned, *in vitro* experiments have demonstrated that the addition of IL-2 to cultures of mononuclear cells from HIV⁺ subjects restored some of the defective immune parameters, suggesting that lymphocytes from these patients maintain their capacity to respond to IL-2 and that the ability to transduce signals of the IL-2 receptor complex is preserved.

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The obvious therapeutical strategies in HIV disease resides in the suppression of viral replication. Although there is a wide consensus in clinical practice and in literature on the remarkable progress made in the last years in producing potent drugs that allow a significant suppression of HIV replication, it is also clear that even the most aggressive drug combinations neither eradicate the virus nor permit a complete reconstitution of the immune system^{18, 19, 38, 43, 62}. For these reasons, IL-2 has been chosen as an additional tool to improve the efficacy of current antiretroviral regimens.

The aim of this review is to summarize the immunological data that have been obtained until now in IL-2 treated HIV⁺ individuals.

Since the mid-eighties, several clinical trials used IL-2 in HIV disease according to very different protocols; therefore, it is impossible to compare all the results obtained, since the type of IL-2 administration and dosage, the length of the treatment, the HIV disease stage, the number of CD4 cells and the associated antiretroviral regimen deeply influence the immune parameters in treated patients.

IL-2 has been primarily studied as continuous intravenous infusion, but the demonstration that this type of administration produced alterations in the clearance of antiretroviral drugs and that subcutaneous injections of IL-2 resulted in adequate absorption in HIV⁺ subjects, permitting treatment in an out-patient regimen and associated with less severe side effects, provided important reasons to switch to this type of treatment^{10, 13, 32, 46, 47}. A general point obtained from these clinical trials is that the immunological effects derived in patients are dose-dependent and are similar irrespective of the chosen type of IL-2 administration. In this review we will therefore not make distinctions between data obtained using the intravenous or subcutaneous regimens.

Concerning the influence of initial CD4 cell counts on the efficacy of treatments including IL-2, an appreciable amount of data is available at this moment only for patients with CD4 ≥ 200 cells/mm³. These patients, because of the partial impairment of their immune responses, still have a functional reservoir that may be boosted by cytokine therapy. Patients having a profound degree of immunodeficiency (i.e. <50 – 100 CD4⁺ cells/mm³) may be completely different in the extent of their immunological reservoir and in their ability to respond to IL-2 treatment. Therefore, the usefulness of IL-2 administration in these patients could be established only after specific clinical trials.

Due to difficulty in standardization and to the inability to finely dissect the immunological defects pres-

ent in HIV infection, only a limited number of studies evaluated IL-2 activity on immunological parameters *in vivo*, such as delayed hypersensitivity responses or specific immunoglobulin production after immunization. One study, in particular, suggested that there is a trend towards an increase in antigen response *in vivo* after pneumococcal vaccination of IL-2 treated patients³². In contrast, many studies on *in vitro* parameters have become available in the last few years and, therefore, these data constitute the basis for this discussion regarding the efficacy of IL-2 when used in clinical trials.

IL-2 and CD4 Subsets and Functions

The pioneering studies on IL-2 used this cytokine in combination with a suboptimal antiretroviral therapy, including various combinations of nucleoside inhibitors. At that time, it was clear that several months of treatment with various nucleoside analog combinations produced only a very limited increase of CD4 cell counts^{11, 23}. In contrast, the studies including IL-2 administration clearly demonstrated a dramatic increase of CD4⁺ percentages and cell counts as compared to the negligible effects of antiretrovirals alone^{8, 9, 10, 13, 31, 32, 50}. The therapeutical use of IL-2 has been, therefore, considered a remarkable option in pursuing immune reconstitution in HIV disease.

Issues concerning the origin and immunological function of the expanded CD4 T cell pool after IL-2 therapy are relevant to further progress. CD4⁺ T lymphocytes, in fact, are a heterogeneous population composed of CD45RA⁺/CD62L⁺ naive cells that have no previous antigenic exposure and CD45RO memory cells generated after antigenic stimulation. Naive cells respond to mitogenic stimuli with greater calcium flux and proliferative activity, while memory cells are rather characterized by a broader cytokine profile and by their antigenic specificity. Although controversy exists whether HIV-1 targets a particular subset of CD4 T cells, *in vitro* evidence suggests that naive T cells are more refractory to HIV^{41, 51, 59}. Previous studies also concentrated on the possible effects of IL-2 therapy on the remodeling of the CD4 T cell populations, showing a preferential restoration of the CD4⁺ CD26⁺ and of the CD4⁺/CD45 "naive" T cells^{9, 13}. The importance of this fact was further substantiated by the inverse correlation observed between total proviral load and the number of CD4 naive cells after 6 cycles of IL-2 therapy⁶⁰. An acute up-regulation of membrane-associated and of soluble CD25 in response to each cycle of IL-2 injections was also demonstrated; therefore, the persistence of cel-

lular responsiveness to IL-2 also after repeated exposures provides the rationale for planning multiple treatment cycles in HIV⁺ patients^{9, 10, 12, 31, 50}.

The availability of protease inhibitors has radically changed the therapy of HIV infection by producing a more severe inhibition of HIV replication and a consistent, although incomplete, recovery of CD4 cell counts. Several studies have demonstrated that the early rise in CD4 memory cells after initiation of therapy may depend on redistribution of these cells from the lymphoid compartment to the peripheral blood^{3, 20, 43}. A slow recovery of naive CD4 T cells and a treatment-dependent decline of activated CD4⁺/HLA-DR⁺ T cell values were observed after 4–6 months of highly active antiretroviral therapy (HAART)^{3, 20}.

Immediately after the availability of protease inhibitors to treat HIV infection, several clinical trials investigated the safety and usefulness of IL-2 administration in association with HAART. Despite the limited amount of data available on the immunological effects of such therapeutical combination, it has been demonstrated that the synergistic effects of adding IL-2 are less evident when compared to the previous experiences including nucleoside inhibitors.

The most favourable effects of IL-2 treatment were present in the study of DAVEY et al.¹⁰, where CD4 cells doubled (from 600 to 1200 cells/ μ l) after 36 weeks of treatment. HENGGE et al.²⁵ demonstrated a more limited effect consisting of a median increase of 100 CD4 cells/ μ l after 1 year of IL-2 treatment. In two recent papers we have suggested that the addition of HAART to IL-2 delayed its beneficial effects on CD4 counts, since after 6 months of treatment the increase of CD4⁺ cells is similar in IL-2 plus HAART as compared to HAART treated subjects, while CD4 cell counts still increase after this time until 1 year of treatment only in the IL-2 group^{12, 61}. Furthermore, an analysis of the lymphoid tissue in these groups of patients showed that the percentages of CD4⁺ cells and the CD4/CD8 ratios do not differ in the lymphoid compartment and in the peripheral blood of treated patients, suggesting that the expansion of CD4 cells is present in several districts of the immune system. Furthermore, the demonstration that CD4 “naive” cells are peculiarly increased not only in the peripheral blood but also in the lymphoid tissue of IL-2 treated subjects⁶¹ suggests that this increase is not due to the redistribution of cells from the lymphoid tissue to peripheral blood, but rather to the proliferation or to *de novo* generation of lymphoid cells from thymic or extrathymic anatomical sites. It is, therefore, conceivable that the immune system is not irreversibly damaged by HIV infection and that IL-2 therapy may

substantially contribute to a better reconstitution of the immune response.

HIV infection results in a progressive depletion of CD4 cells and in a disruption of the CD4 T cell repertoire. It is, therefore, important to determine whether “immune-based” therapies are able to restore these holes in the immune system. CONNORS et al.⁹ have provided evidence that the increase of CD4 cells seen is polyclonal and that the CD4 T cell pool is not immediately restored after IL-2 therapy. Although IL-2 seems to be unable to recruit specific antigen responsiveness after it has been lost, it is conceivable that the expansion of naive T cells induced by IL-2 may be used to induce *de novo* memory cells after specific immunization with selected pathogens.

It is widely accepted that, besides producing CD4 T cell depletion, HIV is responsible for compromising CD4 T cell functions. In general, human PBMCs respond *in vitro* to antigens that have been seen *in vivo* in the recent past; for this reason, *in vitro* T cell proliferation to recall or HIV-specific antigens does not completely cover the T cell repertoire capacity. Despite these limitations, there is an overall consensus in considering HIV-infected subjects defective in their response to HIV-specific and non-specific antigens. CD4 T cell dysfunction is also dependent on a switch of cytokine production from a Th1 to a Th2 profile^{6, 49} and from an abnormally elevated cellular apoptosis that may contribute to accelerated CD4 destruction and reduced immune responsiveness^{1, 21}.

When compared to previous treatments, the use of HAART produced an obvious improvement of most of these functions, although the best results are obtained in patients who had a certain degree of immune responsiveness before treatment, while patients who did not have a specific response before treatment were generally unable to respond *de novo* to antigen stimulation^{32, 37}. In order to clarify the effects obtained in HIV⁺ patients treated with nucleoside analogs and IL-2, we demonstrated that HIV infection was characterized by a rather generalized impairment of cytokine production rather than a Th1 to Th2 switch and that this therapeutical combination produced a dramatic increase of IL-2 and interferon γ production¹³. Accordingly, LEVY et al.³² demonstrated that a similar therapeutic schedule produced an improvement of lymphocyte proliferative response to anti-CD3/anti-CD28 or to recall antigen (PPD, candidin, tetanus toxoid) stimulations after 30 weeks of treatment. Because the *in vitro* experiments gave contradictory results, it is still a matter of debate whether HIV-infected subjects are able to respond *in vitro* to HIV-specific antigens, such as p24 or gp120/160^{32, 37, 48}.

Increasing HIV-specific immune response represents a fundamental goal of HIV disease treatment and, therefore, this topic should be the subject of extensive investigations in the near future.

At this moment, a description of CD4 immunological functions after combination therapy including HAART and IL-2 is not available. We have observed, in a very limited number of patients, that cytokine production is moderately improved also by HAART alone and that IL-2 does not significantly overboost this effect, probably because, when viremia is reduced to levels that are undetectable with current assays, the diminished antigenic stimulation lowers the sustained CD4 T cell activation, making these cells less prone to IL-2 engagement¹².

An increased rate of programmed cell death (apoptosis) has been included among the mechanisms explaining the loss of CD4 T cells in HIV-infected subjects^{1, 33}. An obvious question concerns the effects that the suppression of HIV replication by antiretroviral drugs produces on apoptosis. It has been shown that CD4 T cell apoptosis is reduced in treated patients^{5, 27}. We have also investigated whether the additional use of IL-2 could potentiate the effects of HAART and we found that the percentages of spontaneous apoptosis was reduced in CD4 T cells after 4 weeks of HAART, but remained significantly higher when compared to healthy subjects. Furthermore, the use of IL-2 did not have synergistic effect on apoptosis (CAGGIARI et al., submitted).

IL-2 and CD8 Subsets and Functions

There is a general consensus that IL-2 administration increases CD8 counts only for a very limited amount of time and that, at the end of therapy, CD8 cell numbers are not modified when compared with pretreatment values. We have performed an extensive immunophenotypic evaluation of CD8 subsets after IL-2 therapy^{34, 61} and found that the proportions and absolute numbers of CD8/CD28⁺ lymphocytes, a subset that has been suggested to play a role in the control of HIV replication through a non-cytotoxic response, were not modified after treatment. The contemporaneous reduction observed in the expression by CD8⁺ lymphocytes of CD38 and C1.7, antigens associated with cellular activation and cytotoxicity, was exclusively dependent on antiretroviral therapy and was probably related to the abrogation of antigenic stimulation after the suppression of viral replication^{45, 61}. Although HIV elaborates strategies to evade the host immune response,

virus-specific CTL activity is generally considered an important candidate for controlling viral replication^{2, 44}. Mechanisms involved in CTL protection include the elimination of infected cells and the production of suppressive soluble factor(s)^{2, 34}. The direct measurement of specific cytotoxic activity against HIV is realized by two assays: a classical *in vitro* CTL activity against histocompatible HIV-infected target or a flow cytometric assay that uses HLA-tetrameric complexes^{2, 28, 39, 40}. In particular, the latter allowed establishing a significant correlation between the frequency of HIV-specific CTL and plasma RNA-load⁴⁰. More recently, the same authors demonstrated rapid fluctuations of CTL activity after the initiation of HAART, followed by an exponential decay of this activity until 20 months of treatment. An implication of this study is that the loss of circulating CTL activity during treatment may weaken immune control and favor the emergence of resistant viral strains. Boosting the immune system by immunological therapy may, therefore, be an important aid to antiretroviral drugs. LEVY et al.³² performed a very important study that investigated the cytotoxic T lymphocyte activity specific to HIV-1 antigens during a combined therapeutical approach including reverse transcriptase inhibitors and IL-2. At week 30, this activity disappeared in the IL-2 treated patient, while was still present in 4 of 5 of the patients in the control group; the reasons and the influence of these findings on the clinical outcome are at this moment unknown.

Because of the IL-2 dependent ability to suppress *in vitro* HIV replication²⁹, we have also investigated whether the *in vivo* administration of this cytokine was able to up-regulate IL-16, a well known anti HIV soluble factor. Our results suggests that IL-2 injections acutely increased the production of IL-16 and that CD8⁺ cells were responsible for this phenomenon¹². These observations raise the possibility that IL-2 could not only correct defective CD4 counts, but also may promote anti-HIV activities by CD8 lymphocytes.

IL-2 and Natural Killer Cell Numbers and Activity

Natural immunity is also damaged by HIV infection. The HIV-induced loss of natural immunity includes defects in IFN-stimulated natural killer (NK) activity and in LAK cell activity^{53, 54}. Both these factors have been considered important in the pathogenesis of HIV-disease and the role of natural immunity in protection against HIV may be further reinforced by recent observations showing the production by NK cells of

cytokines, such as IL-15, or chemokines, such as MIP1 α and β , that have an important protective role in HIV infection^{16, 55}.

While clinical trials using high doses of IL-2 did not investigate NK and LAK activities, those trials that used low dose IL-2 gave particular attention to these activities. These trials, in fact, showed that NK cells having a bright membrane expression of CD56 sharply increase after IL-2 therapy³⁵. All the patients demonstrated high levels of lymphokine-activated killer and NK cell activities, presumably because the frequency of IL-2 responsive cells increased from abnormally low to levels above normal⁵².

IL-2 and Chemokine Receptor Expression and Chemokine Production

The role of chemokines and chemokine receptors in HIV infection has been recently highlighted^{4, 36}. Chemokines, in fact, selectively block *in vitro* HIV infection by interacting with their receptors that are necessary as coreceptors for HIV entry into target cells. Furthermore, the protection of individuals highly exposed *in vivo* to HIV infection correlated with the production of high chemokine levels⁷. The effects of IL-2 on the immune system may also modify the pool of target cells by triggering chemokine receptor expression or may alter the immune response to the virus by increasing or suppressing chemokine production. It has been recently demonstrated that IL-2 therapy increases the expression of CXCR-4 and CCR-5 that act, respectively, as receptors for entry by lymphocyte-tropic and monocyte-tropic viruses⁴. It has been also suggested that IL-2 up-regulated the *in vitro* synthesis of RANTES, while chemokine production is not significantly affected at the end of IL-2 therapy¹³.

Conclusions

Interleukin-2 has become a part of therapeutic schedules for HIV infection. Many data have accumulated in the last few years showing the immunological and virological consequences of such a treatment. This topic is still a matter of great discussion and, in my opinion, some questions are still open: first, should IL-2 be considered as a part of the routine treatment of HIV-infection or rather only considered only in selected categories of patients? Secondly, which IL-2 schedule gives the best improvements with reasonable side-effects and good compliance by the patients? Thirdly, could

IL-2 be beneficial for its antiviral activity? Fourthly, what are the long-term effects of IL-2 therapy?

Since many clinical and laboratory data are becoming available on IL-2 and HIV disease, the answers to the above questions will be reasonably available in the near future.

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