

Immunological Mechanisms of Interleukin-2 (IL-2) Treatment in HIV/AIDS Disease

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Abstract: HIV establishes a chronic infection that is marked by the progressive depletion of CD4⁺ T-cells, yet the mechanisms by which this depletion arises are a matter of controversy. Evidence is accumulating that T CD4⁺ depletion is not effected solely by virus-mediated killing and that mechanisms involving T-cell dynamics play a major role in the pathogenesis of HIV infection. Hence antiretroviral therapy, by controlling viral replication alone, invariably fails to achieve the broadest immune reconstitution. This issue has strengthened the rationale to widely explore new adjuvant immunotherapy. Most work has been performed on IL-2, given its potential to correct HIV-driven immune defects, possibly translating in a more effective immune competency.

Important insights stem from the IL-2-mediated immune reconstitution pattern, with a rise in peripheral turnover and thymopoiesis, IL-7 synthesis and functional markers, resulting in the correction of the skewed T-cell immunophenotype and cytokine *milieu*. Combined, these findings suggest that IL-2 has a beneficial effect in correcting the severe disruption in T-cell homeostasis induced by HIV, through the interaction with T-cells and cytokine microenvironment. However, whether or not these immunologic effects translate in an actual immunologic competency and therefore clinical benefit, still awaits demonstration from ongoing large, controlled clinical studies.

Keywords: Immune reconstitution, immunotherapy, interleukin-2.

THE BIOLOGICAL RATIONAL BEHIND IL-2 IN HIV INFECTION

Interleukin-2 (IL-2) was initially described 40 years ago as a factor derived from mixed lymphocyte cultures that was able to induce DNA synthesis from peripheral blood mononuclear cells (PBMCs) [1]. This factor was then called T-cell growth factor as it induces T-lymphocytes to enter the S phase of cell cycle, and some years later was defined IL-2 [2].

IL-2 is a 133 amino-acid protein with a molecular weight of 15.5 kDa produced by activated T-lymphocytes that has a key role in triggering immune responses. IL-2 binds to a specific receptor on the surface of immune cells that is composed by three different subunits called α , β and γ . The $\beta\gamma$ dimer is expressed on resting cells; the α subunit is rapidly expressed upon antigen receptor-mediated T-cell activation. The trimeric complex ($\alpha\beta\gamma$) is characterized by a significant higher affinity for the cytokine, thus amplifying the autocrine effect of IL-2.

The main effect of IL-2 is to induce the clonal expansion of T-lymphocytes after antigen recognition. (Fig. (1)). Amongst other biological effects, IL-2 induces the proliferation of activated B-lymphocytes, boosts natural killers (NK) and cytotoxic T-lymphocyte (CTL)-mediated cytotoxicity (Fig. (1)), stimulates the production of other cytokines including TNF, IFN- γ , and GM-CSF and modulates programmed cell death. [3-5].

The impairment in IL-2 production has been the first functional defect described in HIV-positive patients [6]. Indeed, antigen- and mitogen-stimulated IL-2 production is defective in the majority of HIV-infected, asymptomatic individuals [7-9], and these early and complex defects are predictive for loss of T-cell CD4⁺ counts, clinical progression to AIDS, and death [10, 11]. Most interestingly, recent findings have demonstrated that HIV-infected patients who spontaneously control HIV infection have more CD4 and CD8 T-cells which secrete IL-2 in response to HIV antigens compared with subjects who classically progress in HIV disease [12-14]. Antiretroviral therapy can partially restore IL-2 production in response to soluble antigens, but the defects in the HIV-specific response seem to persist even after long periods of treatment. These observations, together with the realization that HIV replication promptly rebounds in the majority of patients shortly thereafter interruption of

therapy, led to the idea of utilizing IL-2 to boost immune responses in HIV-infected patients. Immunotherapy with IL-2 has thus been investigated in HIV-infected patients with the precise intent to correct HIV-driven immune deficiencies, possibly translating into immunological control over HIV infection [15-19].

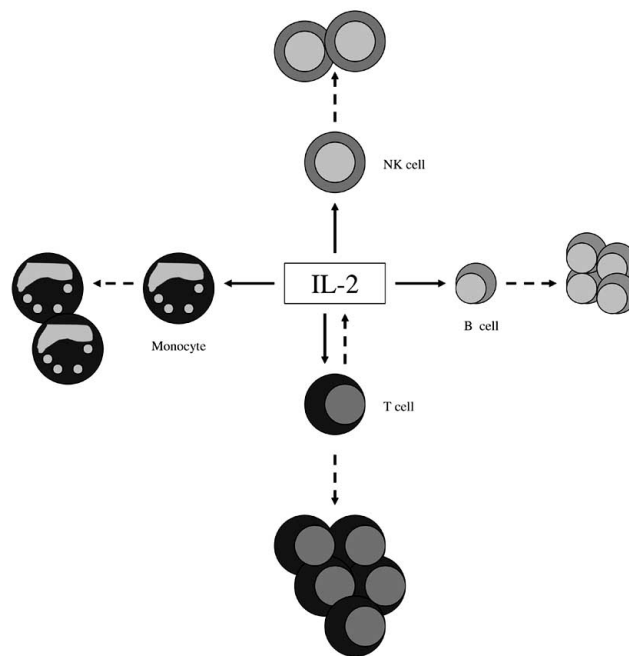


Fig. (1). Effect of IL-2 on the innate and adaptive arms of the immune system.

IL-2 is produced by activated T-cells and induces the proliferation and differentiation of the T-cell compartment. IL-2 also induces the proliferation of B-cells.

IL-2 exerts an effect on the innate arm of the immune system, stimulating NK cells and monocytes.

From a virological standpoint, IL-2 can induce *in vitro* the expression of HIV from latently infected, resting T CD4⁺ cells [20] and has been shown to have a strong proliferative stimulus toward

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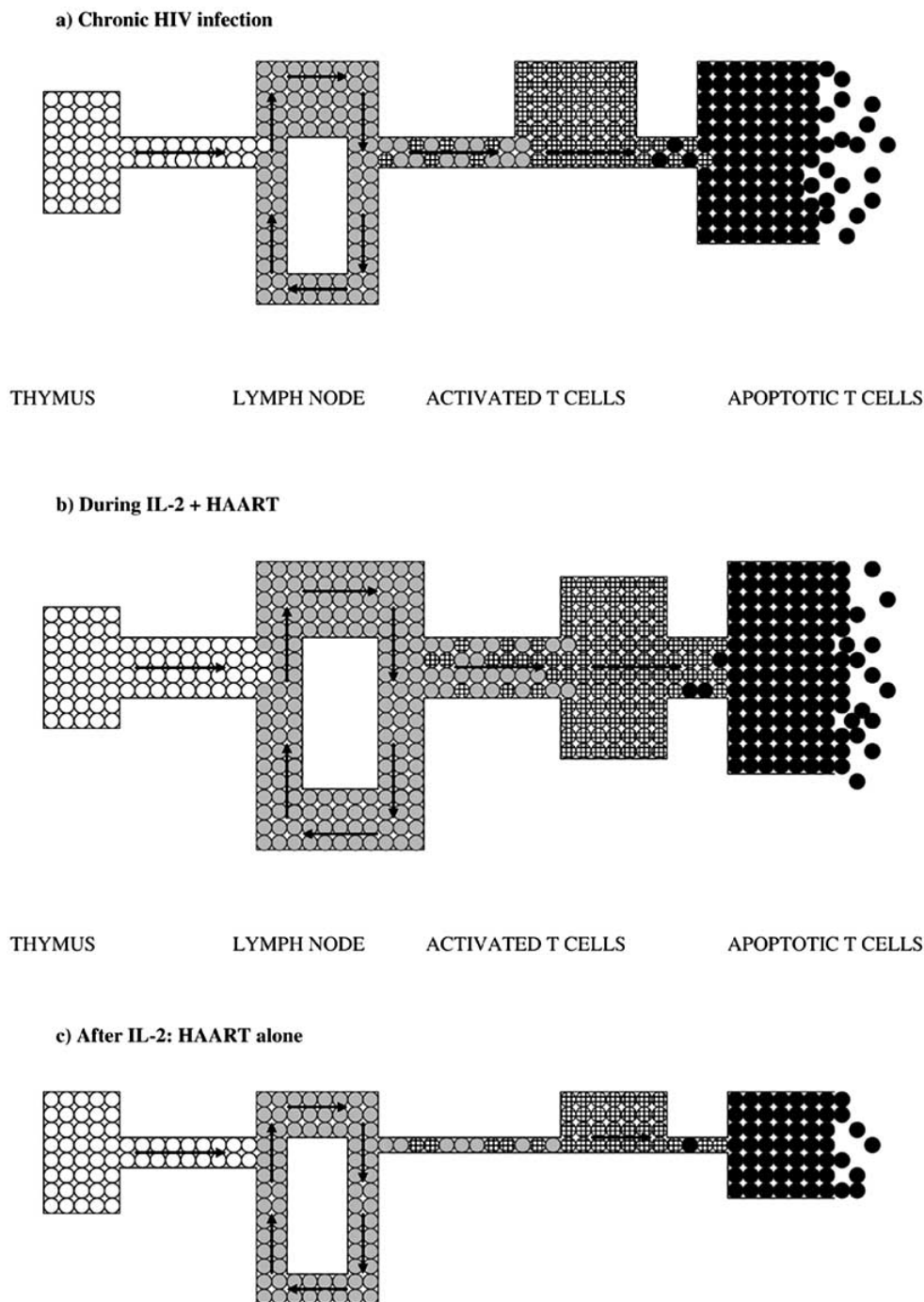


Fig. (2). Immunologic effects of adjuvant therapy with IL-2 in the course of HIV infection.

a) T-cell homeostasis in chronic untreated HIV infection. Naïve T-cells (○) migrate from the thymus to lymph nodes where they encounter antigens and turn into memory T-cells (◐). Memory cells can then circulate within lymph nodes or proliferate and turn into activated (◑) and apoptotic (●), dying cells.

b) T-cell homeostasis in chronic HIV infection during antiretroviral therapy (HAART) and IL-2 cycles.

IL-2 may increase thymic output of T-cells and augments the proliferation of both naïve and memory cells in the periphery. On administration, IL-2 also accounts for increased levels of activation and apoptosis.

c) T-cell homeostasis in chronic HIV infection during antiretroviral therapy, following IL-2 administration. Lower levels of proliferating and activated T-cells have been described in the long term after IL-2 administration, resulting in a net reduction of apoptotic cells.

HIV DNA-bearing cell populations in the periphery [21]. This finding has raised the concern that IL-2 may disrupt virologic control by antiretroviral therapy. Indeed, preliminary studies using IL-2 in the setting of HIV infections have shown that immunotherapy may account for transient increases of HIV viremia during administra-

tion [22, 23]. However, the initial apprehension that IL-2 may trigger HIV viral replication has been set aside by clinical studies demonstrating that the vast majority of IL-2-treated patients do not experience increases in HIV viremia. [24, 25].

THE IMMUNOLOGICAL RATIONAL BEHIND IL-2 IN HIV INFECTION

As shown in (Fig. (2)), the immunological effect of IL-2 adjuvant therapy in HIV-infected patients seems to be composed by different T-cell kinetics in two distinct moments following cycles. On administration, IL-2 acts by expanding the *naïve* and memory cell compartments and increasing T-cell activation and apoptosis. Conversely, in the long-term, IL-2 accounts for decreases in T-cell proliferation, apoptosis and death, resulting in increased survival of existing cells. Despite different pathways, the net result of IL-2 immunotherapy in HIV-infected individuals is a significant expansion of the CD4+ T-cell compartment, thus broadening HAART-induced immune-reconstitution.

Effect of IL-2 on CD4+ T-lymphocyte Growth and Death

Table 1a summarizes the major studies evaluating the effect of IL-2 on T CD4+ lymphocyte growth and death.

Studies *in vitro* have shown that IL-2 is the main T-cell growth factor leading to the proliferation and differentiation of the whole T-lymphocyte compartment [26]. This finding has led to the hypothesis of using IL-2 in the clinical setting for the treatment of HIV disease, with the aim of restoring the depletion of the CD4+ cell subset that characterizes HIV disease. Indeed, the hallmark of IL-2-driven immune reconstitution is characterized by the selective expansion of CD4+ T-cells, however the specific immunological pathways involved in the CD4+ cell expansion have yet to be fully understood. Numerous findings have shown that IL-2 induces peripheral proliferation of CD4+ T-lymphocytes during cycle administration [27-30], resulting in the relative outgrowth of this pool. These findings confirm the efficacy of IL-2 in rapidly increasing CD4+ T-cell numbers and suggest it may indeed be useful in the treatment of HIV infection.

The elaboration of the most efficient IL-2-based immunoadjuvant strategies aimed at the broadest immune reconstitution in the course of HIV infection are hampered by the fact that the precise mechanisms by which the profound loss of CD4+ T-cell arises during HIV infection remain largely unknown. Recent attempts to understand the pathogenesis of HIV have put forward chronic immune activation as a central factor in the exhaustion of the T CD4+ compartment [31, 32]. In particular, a growing body of evidence suggests that the high rates of T-cell death in the chronic phases of HIV disease are the direct consequence of HIV-associated T-cell activation [31]. In this view, IL-2, while directly stimulating peripheral CD4+ proliferation and activation soon after cycle administration, has indeed been proven to exert a major, positive effect in the containment of CD4+ cell proliferation and activation in the long-term [33-35]. In turn, this finding allows us to hypothesize that IL-2 may indeed be efficient in targeting and reversing the HIV-driven generalized immune activation as shown by lower percentage levels of activated T CD8+CD38+ cells in the IL-2 treated population [34-38]. This data sheds light on the precise mechanisms underlying CD4+ rescue in the course of IL-2 immunotherapy, indicating that the rise in CD4 cell counts may be actually sustained by increased survival of existing cells as a consequence of lower T activation, rather than T-cell proliferation in the periphery [28, 34, 35, 37, 39].

In such a context, the effect of IL-2 immunotherapy on peripheral T-cell apoptosis is a question that has been thoroughly addressed. Based on initial *in vitro* evidence supporting a proapoptotic effect of IL-2 [40, 41] various groups then investigated the rates of T-cell apoptosis in HIV-positive patients treated with IL-2. Discordant results were obtained, ranging from data proving increased T-cell apoptosis rates during IL-2 cycles and others showing, at the opposite, reduced apoptotic CD4+ proportions [30, 28, 42, 43]. In this setting, a recent paper by Paiardini *et al.* aiming at the study of

the relationship between lymphocyte cycle dysfunctions, T-cell activation and apoptosis in the course of HIV infection, reported a novel mechanism of exogenous IL-2 in HIV-infected patients. In particular, IL-2 administration had a positive effect in normalizing T-cell cycle perturbations and the exaggerated susceptibility to apoptosis, suggesting a possible new rationale for adjuvant IL-2 therapy in HIV disease [44].

Overall, considering the combined effect of IL-2 on driving peripheral T-cell turnover and expanding CD4+ T-cell numbers, we can hypothesize that elevated death rates accompany and partly correct the heightened T-cell proliferation, still resulting in an overall CD4+ T-cell expansion [31]. However, the observation that IL-2 in the long-term accounts for decreases in T-cell proliferation and activation along with enhanced T-cell survival allows for the speculation that immunotherapy may lead to a parallel long-term reduction in T-cell apoptosis [31].

Despite the intrinsic ability of IL-2 in expanding T CD4+ cells, it must be noted that not all HIV-infected patients adequately respond to immunotherapy with IL-2. In particular, older age and low T CD4+ cell nadir [45] have been considered predictors of non-responsiveness to IL-2; heightened levels of immune activation too may adversely affect CD4+ T-cell rescue after IL-2 administration [46].

Effect of IL-2 on CD4+ T-lymphocyte Production

Table 1b summarizes the major studies evaluating the effect of IL-2 on T CD4+ lymphocyte production.

While evaluating the role of HIV-induced activation in the pathogenesis of HIV disease, one should not overlook the direct effect of the virus in terms of CD4+ T-cell infection and cytopathicity [32]. Activated *naïve* cells are amongst the ones to be particularly sensitive to HIV infection [47]; in addition, the effect of HIV on thymus inhibition renders it increasingly difficult for the latter to keep up with drain on the peripheral *naïve* T-cell compartment caused by the flow of *naïve* T-cells into the memory pools [32].

In this view, immunoadjuvant therapy with IL-2 may represent a useful strategy, as various findings have confirmed that the IL-2-induced CD4+ increases are preferentially in cells of a *naïve* phenotype [48-50]. Unfortunately, studies conducted to shed light on the mechanisms underlying the IL-2-driven expansion have not reached a clear-cut consensus on whether the growth of this particular subset derives from *de novo* T-cell synthesis and/or peripheral expansion. As far as the impact of thymopoiesis on T-cell subsets is concerned, data produced until now are divisive. Some studies suggest that thymic output does not play a major role [27] while others put forward a possible role of IL-2-related thymopoiesis in the *naïve* CD4+ T-cell rise [33, 51].

Along with the effect on peripheral T-cell expansion and thymic development, IL-2 signalling has been proven pivotal in maintaining the suppressive activity of CD4+ CD25+ regulatory T-cells (T regs) [52]. Given the emerging evidence of its primary role in generating and maintaining T reg cells, the major question arises as to the effect of IL-2 therapy on T reg homeostasis and function [53].

The role and function of T regs in the context of HIV infection is currently under investigation, and represents a fascinating matter for speculation: on the one hand IL-2 may support T reg cell suppressor function, translating into the control over several detrimental processes underlying HIV pathogenesis (i.e. activation-induced cell death and anergy, immune-mediated T-cell destruction, and CD4+ susceptibility to productive HIV infection) [54]; on the other, immunotherapy may have a detrimental effect by diminishing the HIV-specific T-cell immune responses *in vivo*, possibly even hastening HIV disease progression [54]. The enlightened understand-

Table 1. Overview of the Major Studies Evaluating the Immunological Role of IL-2 Adjuvant Therapy in the Course of HIV Infection

Ia. Studies Evaluating the Effect of IL-2 on CD4+ T-lymphocyte Growth and Death		
Author	Study Population	Outcome
De Paoli, P. <i>J. Clin. Invest.</i> 1997 [29]	10 pts; CD4+ 200-500;	Increase in the number of CD4+ T-cells; reconstitution of CD4/CD45RA lymphocytes with recovery of the ability to produce IL-2, IL-4, INF- γ
Sereti, I. <i>AIDS</i> 2001 [28]	10 pts; CD4+ 188-884; HIV-RNA <50-2737	Significant rise in CD4+ and CD8+ apoptosis and activation
Natarajan, V. <i>Proc. Natl. Acad. Sci. USA</i> 2002 [27]	6 pts; HIV RNA < 20000 cp/ml	Increase in the percentage of 5-BrdU+ cells and in Ki67 staining in naïve CD4+ T-cells; no substantial shortening of telomeres; decline in TRECs levels
Hengge, U. <i>AIDS</i> 2002 [30]	38 pts; CD4+>400; median HIV-RNA 1000	Significant increase in lymphocyte apoptosis both in peripheral blood and lymph nodes; significant increase in CD4+, CD8+ and CD16/56+ proliferation in peripheral blood and lymph node; no change in HIV-RNA in lymph node
Sereti, I. <i>Blood</i> 2002 [34]	31 HIV+ pts; CD4+ 100-1250; HIV-RNA<50; 16 HIV-controls	Significant expansion of a discrete population of CD4+CD25+ without increased expression of activation and proliferation markers (i.e. CD69, CD95, Ki67)
Kovacs, J. <i>J. Clin. Invest.</i> 2005 [37]	35 pts; cross-sectional study	Significant increase in CD4 and CD8 proliferation and time-dependant half lives

Ib. Studies Evaluating the Effect of IL-2 on CD4+ T-lymphocyte Production		
Author	Study Population	Outcome
Davey, R. <i>J. Infect. Dis.</i> 1999 [49]	49 pts; CD4+ \geq 500; on stable antiretroviral therapy for \geq 6 weeks	Significant and sustained increases in CD4+ cell counts, both of naïve and memory phenotypes
De Paoli, P. <i>Clin. Exp. Immunol.</i> 2001 [50]	22 pts; CD4+>200; HIV-RNA >500	Significant rise in phenotypically naïve CD4+; no change in TRECs levels
Marchetti, G. <i>JID</i> 2002 [33]	22 pts; CD4+ \leq 200; HIV RNA < 50 cp/ml	Significant increases of total and naïve CD4+ cells; increases in TRECs counts
Carcelain, G. <i>AIDS</i> 2003 [51]	72 patients; CD4+<200; HIV RNA <200	By a mathematical model, a linear correlation was found between naïve CD4+ recovery and CD4 TRECs levels
Marchetti, G. <i>Antiviral Ther.</i> 2004 [38]	12 pts; CD4 T-cell count constantly <200; HIV RNA <50 cp/ml after 12 months of HAART	IL-2 induced an expansion in total and naïve CD4+, TRECs and IL-7 plasma levels
Marchetti, G. <i>AIDS</i> 2004 [39]	15 pts; CD4+ <200; HIV RNA < 50 cp/ml after 12 months of HAART	Significant rise in Ki67 proliferating CD4 cells; CD4+ TREC stable and CD8+CD38+ decrease
Sereti, I. <i>J. Clin. Invest.</i> 2005 [55]	40 pts	IL-2 expanded a population of CD4+CD25+; reduced apoptosis; elevated foxp3 expression, but weak suppressive function
Read, S. <i>JAIDS</i> 2006 [63]	230 pts; cross-sectional study	Plasma IL-7 is highest in HIV-infected versus other groups. IL-2-treated pts presented lowest IL-7R CD4 and CD8 expression

NOTE. IL-2, interleukin-2; IL-4, interleukin 4; IL-7, interleukin-7; IL-7/r, IL-7 receptor; INF- γ , interferon- γ TRECs, T-cell receptor excision circles; BrdU, bromodeoxyuridine; HAART, highly active antiretroviral therapy.

ing of the effect of IL-2 in specifically expanding and stimulating T reg cells *in vivo* combined with the most thorough investigation of T reg activity in HIV infection will allow for the most rational clinical manipulation of IL-2 signalling in HIV-infected patients. Indeed, *in vivo* IL-2 administration to HIV-infected patients have been recently shown to lead to peripheral expansion of a population of long-lived CD4+ CD25+ foxp3+ cells with weak suppressive function [55]. On the basis of these specific phenotypic and functional features, distinct from antigen-triggered cells and T regs, a role of this cell population in the maintenance of low T-cell turnover and CD4+ expansion has been speculated.

A major regulator of central T-cell production is represented by the circuit of interleukin-7 and its receptor (IL-7/IL-7R), [56] and higher IL-7 plasma levels have been shown to be independently associated with thymic-function-associated parameters [57].

In vitro data have recently shown an interesting interaction between IL-2 and IL-7. In particular, a potent IL-2 driven down modulation of IL-7 mRNA and protein *via* a phosphatidylinositol 3-kinase/Akt-dependent mechanism, has suggested the existence of a cross-talk between IL-2 and IL-7 [58]. By translating these observations *in vivo*, this would suggest that IL-2 can negatively affect IL-7 functions on T-cell homeostasis.

In the context of HIV/AIDS, a disruption of the IL-7/IL-7R signalling shows increased levels of circulating IL-7 and decreased

proportions of IL-7/R α + T-cells [56, 59-61]. In the attempt of investigating the *in vivo* interactions between IL-2 and the IL-7/IL-7R system [56, 62], discordant results have been produced thus far. Some studies have reported that *in vivo* administration of IL-2 is associated with a rise in IL-7 plasma levels with no changes in the surface expression of IL-7R [38], suggesting that IL-2 accounts for a boost of IL-7 production without down-modulating its specific receptor, thus preserving IL-7-mediated T-lymphocyte regulation. However, the results of a large cross-sectional study involving healthy volunteers and HIV-positive patients treated with IL-2 are in contrast with the above findings, showing overall lower levels of IL-7 in the IL-2 treated population [63].

CONCLUSION AND FURTHER DIRECTIONS

The hallmark of HIV infection is the progressive depletion of CD4+ cells, yet the mechanisms by which this profound loss arises are still unknown. Recent attempts to delineate the pathogenesis of HIV infection have put forward a more complete explanation that, other than the sole effect of HIV-mediated killing, includes T-cell dynamics [34, 25]. Thus, it is easily understood how antiretroviral therapy alone cannot achieve broadest immune reconstitution. In this view, the adjuvant use of IL-2 offers the appealing perspective of correcting the HIV-driven immune deficiencies. Results from Phase I/II studies have attempted to clarify the role of IL-2 in HIV

infection from an immunological standpoint, and have shown that IL-2 associated immune reconstitution is a complex process, consisting in a sequential stimulation and reduction of peripheral T-cell turnover [26-29], apoptosis [28, 42, 43], survival [27, 37, 38] and possibly a boost of neothymopoiesis [33]. An intriguing hypothesis also views a possible role of IL-2 on the IL-7/IL-7R regulatory pathway [38, 63]. However, from the very clinical standpoint, the key question remains whether the IL-2-mediated rise in CD4+ cell counts is indeed associated with an actual clinical benefit in terms of disease progression and death. This issue is currently being addressed in two phase III, international, randomized clinical trials (International Study of Interleukin-2 in people with Low CD4+ T-Cell Counts on Active Anti-HIV Therapy, SILCAAT; Evaluation of Subcutaneous Proleukin in a Randomized International Trial, ESPRIT). While the completion of these large phase III trials will hopefully provide a more direct confrontation of areas of controversy, vis-à-vis IL-2 driven immune expansion and clinical benefit, relevant data on the clinical exploitation of IL-2 need to be thoroughly addressed in pharmacologic and molecular aspects of IL-2 treatment.

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ABBREVIATIONS

AIDS	=	Acquired Immunodeficiency Syndrome
GM-CSF	=	Granulocyte Macrophage Colony Stimulating Factor
HAART	=	Highly active anti-retroviral therapy
HIV	=	Human Immunodeficiency Virus
IFN- γ	=	Interferon- γ
IL-2	=	Interleukin-2
IL-7	=	Interleukin-7
NK cells	=	Natural Killer Cells,
TNF	=	Tumor Necrosis Factor

REFERENCES

- [1] Kasakura, S.; Lownstein, L. A factor stimulating DNA synthesis derived from the medium of leukocyte cultures. *Nature* **1965**, *208*, 794-795.
- [2] Morgan, D. A.; Ruscitti, F. W.; Gallo, R. C. Selective *in vitro* growth of lymphocytes from hormonal bone marrows. *Science* **1976**, *193*, 1700-1800.
- [3] Curfs, J. H.; Meis J. F.; Hoogkamp-Korstanje, J. A. A. A primer on cytokines: sources, receptor, effects, and inducers. *Clin. Microbiol. Rev.* **1997**, *7*, 742-780.
- [4] Kinter, A. Interleukin-2 and human immunodeficiency virus infection: pathogenic mechanism and potential for immunologic enhancement. *Immunol. Res.* **1996**, *15*, 1-15.
- [5] Moll, M.; Snyder-Cappione, J.; Spotts, G.; Hecht, F. M.; Sandberg, J. K.; Nixon, D. F. Expansion of CD1d-restricted NKT cells in patients with primary HIV-1 infection treated with interleukin-2. *Blood* **2006**, *107*, 3081-3083.
- [6] Lane, H. C.; Fauci, A. S. Immunologic abnormalities in the acquired immunodeficiency syndrome. *Annu. Rev. Immunol.* **1985**, *3*, 477-500.
- [7] Giorgi, J. V.; Nishanian, P. G.; Schmid, L.; Hultin, L. E.; Cheng, H. L.; Detels, R. Selective alterations in immunoregulatory lymphocyte subsets in early HIV (human T-lymphotropic virus type III/lymphadenopathy-associated virus) infection. *J. Clin. Immunol.* **1987**, *7*, 140-150.
- [8] Miedema, F.; Petit, A. J.; Terpstra, F. G.; Schattenkerk, J. K.; de Wolf, F.; Al, B. J.; Roos, M.; Lange, J. M.; Danner, S. A.; Goudsmit, J. Immunological abnormalities in human immunodeficiency virus (HIV)-infected asymptomatic homosexual men. HIV affects the immune system before CD4+ T helper cell depletion occurs. *J. Clin. Invest.* **1988**, *82*, 1908-1914.
- [9] Clerici, M.; Stocks, N. L.; Zajac, R. A.; Boswell, R. N.; Bernstein, D. C.; Mann, D. L.; Shearer, G. M.; Berzofsky, J. A. Interleukin-2 production used to detect antigenic peptide recognition by T-helper lymphocytes from asymptomatic HIV-seropositive individuals. *Nature* **1989**, *339*, 383-385.
- [10] Lucey, D. R.; Hensley, R. E.; Ward, W. W.; Butzin, C. A.; Boswell, R. N. CD4+ monocyte counts in persons with HIV-1 infection: an early increase is followed by a progressive decline. *J. Acquir Immune Defic. Syndr.* **1991**, *4*, 24-30.
- [11] Dolan, M. J.; Clerici, M.; Blatt, S. P.; Hendrix, C. W.; Melcher, G. P.; Boswell, R. N.; Freeman, T. M.; Ward, W.; Hensley, R.; Shearer, G. M. *In vitro* T-cell function, delayed-type hypersensitivity skin testing, and CD4+ T-cell subset phenotyping independently predict survival time in patients infected with human immunodeficiency virus. *J. Infect. Dis.* **1995**, *172*, 79-87.
- [12] Pereyra, F.; Addo, M. M.; Kaufmann, D. E.; Liu, Y.; Miura, T.; Rathod, A.; Baker, B.; Trocha, A.; Rosenberg, R.; Mackey, E.; Ueda, P.; Lu, Z.; Cohen, D.; Wrin, T.; Petropoulos, C. J.; Rosenberg, E. S.; Walker, B. D. Genetic and immunologic heterogeneity among persons who control HIV infection in the absence of therapy. *J. Infect. Dis.* **2008**, *197*, 563-571.
- [13] Kannanganat, S.; Kapogiannis, B. G.; Ibegu, C.; Chennareddi, L.; Goepfert, P.; Robinson, H. L.; Lennox, J.; Amara, R. R. Human immunodeficiency virus type 1 controllers but noncontrollers maintain CD4 T-cells coexpressing three cytokines. *J. Virol.* **2007**, *81*, 12071-12076.
- [14] Potter, S. J.; Lacabaratz, C.; Lambotte, O.; Perez-Patrigeon, S.; Vingert, B.; Sinet, M.; Colle, J. H.; Urrutia, A.; Scott-Alagara, D.; Boufassa, F.; Delfraissy, J. F.; Theze, J.; Venet, A.; Chakrabarti, L. A. Preserved central memory and activated effector memory CD4+ T-cell subsets in human immunodeficiency virus controllers: an ANRS EP36 study. *J. Virol.* **2007**, *81*, 13904-13915.
- [15] Kilby, J. M.; Bucy, R. P.; Mildvan, D.; Fischl, M.; Santana-Bagur, J.; Lennox, J.; Pilcher, C.; Zolopa, A.; Lawrence, J.; Pollard, R. B.; Habib, R. E.; Sahner, D.; Fox, L.; Aga, E.; Bosch, R. J.; Mitsuyasu, R. Adult AIDS Clinical Trials Group A5024 Protocol Team. A randomized, partially blinded phase 2 trial of antiretroviral therapy, HIV-specific immunizations, and interleukin-2 cycles to promote efficient control of viral replication (ACTG A5024). *J. Infect. Dis.* **2006**, *194*, 1672-1676.
- [16] Haas, D. W.; Geraghty, D. E.; Andersen, J.; Mar, J.; Motsinger, A. A.; D'Aquila, R.T.; Unutmaz, D.; Benson, C. A.; Ritchie, M. D.; Landay, A. AIDS Clinical Trials Group. Immunogenetics of CD4 lymphocyte count recovery during antiretroviral therapy: An AIDS Clinical Trials Group study. *J. Infect. Dis.* **2006**, *194*, 1098-1107.
- [17] Henry, K.; Katzenstein, D.; Cherng, D. W.; Valdez, H.; Powderly, W.; Vargas, M. B.; Jahed, N. C.; Jacobson, J. M.; Myers, L. S.; Schmitz, J. L.; Winters, M.; Tebas, P. A5102 Study Team of the AIDS Clinical Trials Group. A pilot study evaluating time to CD4 T-cell count <350 cells/mm³ after treatment interruption following antiretroviral therapy +/- interleukin 2: results of ACTG A5102. *J. Acquir Immune Defic. Syndr.* **2006**, *42*, 140-148.
- [18] Levy, Y.; Gahery-Segard, H.; Durier, C.; Lascaux, A. S.; Goujard, C.; Meifredy, V.; Rouzioux, C.; El Habib, R.; Beumont-Mauviel, M.; Guillet, J. G.; Delfraissy, J. F.; Abouker, J. P. ANRS 093 Study Group. Immunological and virological efficacy of a therapeutic immunization combined with interleukin-2 in chronically HIV-1 infected patients. *AIDS* **2005**, *3*, 279-286.
- [19] Vogler, M. A.; Tepler, H.; Gelman, R.; Valentine, F.; Lederman, M. M.; Pomerantz, R. J.; Pollard, R. B.; Cherng, D. W.; Gonzalez, C. J.; Squires, K. E.; Frank, I.; Mildvan, D.; Mahon, L. F.; Schock, B. AIDS Clinical Trials Group 248 Study Team. Daily low-dose subcutaneous interleukin-2 added to single- or dual-nucleoside therapy in HIV infection does not protect against CD4+ T-cell decline or improve other indices of immune function: results of a randomized controlled clinical trial (ACTG 248). *J. Acquir Immune Defic. Syndr.* **2004**, *1*, 576-587.
- [20] Chun, T. W.; Engel, D.; Mizell, S. B.; Ehler, L. A.; Fauci, A. S. Induction of HIV-1 replication in latently infected CD4+ T-cells using a combination of cytokines. *J. Exp. Med.* **1998**, *188*, 83-91.
- [21] Delaguerre, C.; Gourlain, K.; Tubiana, R.; Carcelain, G.; Marcelin, A. G.; Chouquet, C.; Mouroux, M.; Duvuvier, C.; Autran, B.; Costagliola, D.; Katlama, C.; Calvez, V. Increase of HIV-1 pro-viral DNA per million peripheral blood mononuclear cells in patients with advanced HIV disease (CD4<200 cells/mm³) receiving interleukin 2 combined with HAART versus HAART alone (ANRS-082 Trial). *Antivir. Ther.* **2003**, *8*, 233-237.
- [22] Kovacs, J. A.; Baseler, M.; Dewar, R. J.; Vogel, S.; Davey, R. T. Jr.; Falloon, J.; Polis, M. A.; Walker, R. E.; Stevens, R.; Salzman N. P. Increases in CD4 T-lymphocytes with intermittent course of interleukin-2 in patients with human immunodeficiency virus infection. A preliminary study. *N. Engl. J. Med.* **1995**, *332*, 567-575.
- [23] Davey, R. T. Jr.; Chait, D. G.; Piscitelli, S. C.; Wells, M.; Kovacs, J. A.; Walker, R. E.; Falloon, J.; Polis, M. A.; Metcalf, J. A.; Masur, H.; Fyfe, G.; Lane, H. C. Subcutaneous administration of interleukin-2 in human immunodeficiency virus type 1-infected persons. *J. Infect. Dis.* **1997**, *175*, 781-189.
- [24] Mitsuyasu, R.; Gelman, R.; Chern D.W.; Landay, A.; Fahey, J.; Reichman, R.; Erice, A.; Bucy, R. P.; Kilby, J. M.; Lederman, M. M.; Hamilton, C. D.; Lertora, J.; White, B. L.; Tebas, P.; Duliege, A. M.; Pollard, R. B. AIDS Clinical Trials Group 328 Study Team. The virologic, immunologic, and clinical effects of interleukin 2 with potent antiretroviral therapy in patients with moderately advanced human immunodeficiency virus infection: a randomized controlled clinical trial--AIDS Clinical Trials Group 328. *Arch. Intern. Med.* **2007**, *167*, 597-605.
- [25] Go, R.; Steigbiel, R. Interleukin 2 and HIV RNA levels. *Arch. Intern. Med.* **2007**, *167*, 2144-2145.

- [26] Bagby, G.; Heinrich, M. *In Hematology Basic Principles and Practice: Growth factors, cytokines, and the control of hematopoiesis*; Churchill Livingstone: Philadelphia, PA, USA, 2000, pp. 154-202.
- [27] Natarajan, V.; Lempicki, R. A.; Sereti, I.; Badralmaa, Y.; Adelsberger, J. W.; Metcalf, J. A.; Prieto, D. A.; Stevens, R.; Baseler, M. W.; Kovacs, J. A.; Lane, H. C. Increased peripheral expansion of naive CD4+ T-cells *in vivo* after IL-2 treatment of patients with HIV infection. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 10712-10717.
- [28] Sereti, I.; Herpin, B.; Metcalf, J. A.; Stevens, R.; Baseler, M. W.; Hallahan, C. W.; Kovacs, J. A.; Davey, R. T.; Lane, H. C. CD4 T-cell expansions are associated with increased apoptosis rates of T-lymphocytes during IL-2 cycles in HIV infected patients. *AIDS* **2001**, *15*, 1765-1775.
- [29] De Paoli, P.; Zanussi, S.; Simonelli, C.; Bortolin, M. T.; D'Andrea, M.; Crepaldi, C.; Talamini, R.; Comar, M.; Giacca, M.; Tirelli, U. Effects of subcutaneous interleukin-2 therapy on CD4 subsets and *in vitro* cytokine production in HIV+ subjects. *J. Clin. Invest.* **1997**, *100*, 2737-2743.
- [30] Hengge, U. R.; Borchar, C.; Esser, S.; Schroder, M.; Mirmohammadsadegh, A.; Goos, M. Lymphocytes proliferate in blood and lymph nodes following interleukin-2 therapy in addition to highly active antiretroviral therapy. *AIDS* **2002**, *16*, 151-160.
- [31] Grossman, Z.; Meier-Schellersheim, M.; Sousa, A. E.; Victorino, R. M.; Paul, W. E. CD4+ T-cell depletion in HIV infection: are we closer to understanding the cause? *Nat. Med.* **2002**, *8*, 319-323.
- [32] Douek, D.; Picker, L.; Koup, R. T-cell dynamics in HIV-1 infection. *Annu. Rev. Immunol.* **2003**, *21*, 265-304.
- [33] Marchetti, G.; Meroni, L.; Varchetta, S.; Terzieva, V.; Bandera, A.; Mangano, D.; Molteni, C.; Trabaton, D.; Fossati, S.; Clerici, M.; Galli, M.; Moroni, M.; Franzetti, F.; Gori, A. Low-dose prolonged intermittent interleukin-2 adjuvant therapy: results of a randomized trial among human immunodeficiency virus-positive patients with advanced immune impairment. *J. Infect. Dis.* **2002**, *186*, 606-616.
- [34] Sereti, I.; Martinez-Wilson, H.; Metcalf, J. A.; Baseler, M. W.; Hallahan, C. W.; Hahn, B.; Hengel, R. L.; Davey, R. T.; Kovacs, J. A.; Lane, H. C. Long-term effects of intermittent interleukin 2 therapy in patients with HIV infection: characterization of a novel subset of CD4(+)/CD25(+) T-cells. *Blood* **2002**, *100*, 2159-2167.
- [35] Sereti, I.; Anthony, K. B.; Martinez-Wilson, H.; Lempicki, R.; Adelsberger, J.; Metcalf, J. A.; Hallahan, C. W.; Follmann, D.; Davey, R. T.; Kovacs, J. A.; Lane, H. C. IL-2-induced T-cell expansion in HIV-infected patients is associated with long-term decreases in T-cell proliferation. *Blood* **2004**, *104*, 775-780.
- [36] Marchetti, G.; Franzetti, F.; Gori, A. Partial immune reconstitution following highly active antiretroviral therapy: can adjuvant interleukin-2 fill the gap? *J. Antimicrob. Chemother.* **2005**, *55*, 401-409.
- [37] Kovacs, J. A.; Lempicki, R. A.; Sidorov, I. A.; Adelsberger, J. W.; Sereti, I.; Sachau, W.; Kelly, G.; Metcalf, J. A.; Davey, R. T. Jr.; Falloon, J.; Polis, M. A.; Tavel, J.; Stevens, R.; Lambert, L.; Hosack, D. A.; Bosche, M.; Issaq, H. J.; Fox, S.D.; Leitman, S.; Baseler, M. W.; Masur, H.; Di Mascio, M.; Dimitrov, D. S.; Lane, H. C. Induction of prolonged survival of CD4+ T-lymphocytes by intermittent IL-2 therapy in HIV-infected patients. *J. Clin. Invest.* **2005**, *115*, 2139-2148.
- [38] Marchetti, G.; Meroni, L.; Molteni, C.; Taskaris, G.; Gazzola, L.; Galli, M.; Clerici, M.; Moroni, M.; Franzetti, F.; Gori, A. IL-7/IL-7 receptor system regulation following IL-2 immunotherapy in HIV-infected patients. *Antivir. Ther.* **2004**, *9*, 447-452.
- [39] Marchetti, G.; Meroni, L.; Molteni, C.; Bandera, A.; Franzetti, F.; Galli, M.; Moroni, M.; Clerici, M.; Gori, A. Interleukin-2 immunotherapy exerts a differential effect on CD4 and CD8 T-cell dynamics. *AIDS* **2004**, *18*, 211-6.
- [40] Lenardo, M.; Chan, K. M.; Hornung, F.; McFarland, H.; Siegel, R.; Wang, J.; Zheng, L. Mature T-lymphocyte apoptosis-immune regulation in a dynamic and unpredictable antigenic environment. *Annu. Rev. Immunol.* **1999**, *17*, 221-253.
- [41] Ku, C. C.; Murakami, M.; Sakamoto, A.; Kappler, J.; Marrack, P. Control of homeostasis of CD8+ memory T-cells by opposing cytokines. *Science* **2000**, *288*, 675-678.
- [42] Caggiari, L.; Zanussi, S.; Bortolin, M. T.; D' Andrea, M.; Nasti, G.; Simonelli, C.; Tirelli, U.; De Paoli, P. Effects of therapy with highly active antiretroviral therapy (HAART) and IL-2 on CD4+ and CD8+ lymphocyte apoptosis in HIV+ patients. *Clin. Exp. Immunol.* **2000**, *120*, 101-106.
- [43] Pandolci, F.; Pierdominici, M.; Marziali, M.; Livia Bernardi, M.; Antonelli, G.; Galati, V.; D'Offizi, G.; Aiuti, F. Low-dose IL-2 reduces lymphocyte apoptosis and increases naive CD4 cells in HIV-1 patients treated with HAART. *Clin. Immunol.* **2000**, *94*, 153-159.
- [44] Paiardini, M.; Galati, D.; Cervasi, B.; Cannavo, G.; Galluzzi, L.; Montoni, M.; Guetard, D.; Magnani, M.; Piedimonte, G.; Silvestri, G. Exogenous interleukin-2 administration corrects the cell cycle perturbation of lymphocytes from human immunodeficiency virus-infected individuals. *J. Virol.* **2001**, *75*, 10843-10855.
- [45] ESPRIT Research Group; Fox, Z.; Antunes, F.; Davey, R.; Gazzard, B.; Kilmas, N.; Labriola, A.; Losso, M.; Necton, J. D.; Phillips, A. N.; Ruxrungtham, K.; Staszewski, S.; Weiss, L.; Lundgren, J. D. Predictors of CD4 count change over 8 months of follow up in HIV-1-infected patients with a CD4 count ≥ 300 cells/microL who were assigned to 7.5 MIU interleukin-2. *HIV Med.* **2007**, *8*, 112-123.
- [46] Sereti, I.; Sklar, P.; Ramchandani, M. S.; Read, S. W.; Aggarwal, V.; Imamichi, H.; Natarajan, V.; Metcalf, J. A.; Kovacs, J. A.; Tavel, J.; Davey, R. T.; Dersimonian, R.; Lane, H. C. CD4+ T-cell responses to interleukin-2 administration in HIV-infected patients are directly related to the baseline level of immune activation. *J. Infect. Dis.* **2007**, *196*, 677-683.
- [47] Douek, D. C.; Brenchley, J. M.; Betts, M. R.; Ambrozak, D. R.; Hill, B. J.; Okamoto, Y.; Casazza, J. P.; Kuruppu, J.; Kunstman, K.; Wolinsky, S.; Grossman, Z.; Dybul, M.; Oxenius, A.; Price, D. A.; Connors, M.; Koup, R. A. HIV preferentially infects HIV-specific CD4+ T-cells. *Nature* **2002**, *417*, 95-98.
- [48] Kovacs, J. A.; Vogel, S.; Metcalf, J. A.; Baseler, M.; Stevens, R.; Adelsberger, J.; Lempicki, R.; Hengel, R. L.; Sereti, I.; Lambert, L.; Dewar, R. L.; Davey, R. T. Jr.; Walker, R. E.; Falloon, J.; Polis, M. A.; Masur, H.; Lane, H. C. Interleukin-2 induced immune effects in human immunodeficiency virus-infected patients receiving intermittent interleukin-2 immunotherapy. *Eur. J. Immunol.* **2001**, *31*, 1351-1360.
- [49] Davey, R. T. Jr.; Chait, D. G.; Albert, J. M.; Piscitelli, S. C.; Kovacs, J. A.; Walker, R. E.; Falloon, J.; Polis, M. A.; Metcalf, J. A.; Masur, H.; Dewar, R.; Baseler, M.; Fyfe, G.; Giedlin, M. A.; Lane, H. C. 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.
- [50] De Paoli, P.; Bortolin, M. T.; Zanussi, S.; Monzoni, A.; Pratesi, C.; Giacca, M. Changes in thymic function in HIV-positive patients treated with highly active antiretroviral therapy and interleukin-2. *Clin. Exp. Immunol.* **2001**, *125*, 440-446.
- [51] Carcelain, G.; Saint-Mezard, P.; Altes, H. K.; Tubiana, R.; Grenot, P.; Rabian, C.; de Boer, R.; Katlama, C.; Debrè, P.; Autran, B. IL-2 therapy and thymic production of naive CD4 T-cells in HIV-infected patients with severe CD4 lymphopenia. *AIDS* **2003**, *17*, 841-850.
- [52] Furtado, G. C.; Curotto de Lafaille, M. A.; Kutchukhidze, N.; Lafaille, J. J. Interleukin 2 signaling is required for CD4(+) regulatory T-cell function. *J. Exp. Med.* **2002**, *196*, 851-857.
- [53] Nelson, B. H. IL-2, regulatory T-cells, and tolerance. *J. Immunol.* **2004**, *172*, 3983-3988.
- [54] Kinter, A. L.; Hennessey, M.; Bell, A.; Kern, S.; Lin, Y.; Daucher, M.; Planta, M.; McGlaughlin, M.; Jackson, R.; Ziegler, S. F.; Fauci, A. S. CD25(+)CD4(+) regulatory T-cells from the peripheral blood of asymptomatic HIV-infected individuals regulate CD4(+) and CD8(+) HIV-specific T-cell immune responses *in vitro* and are associated with favorable clinical markers of disease status. *J. Exp. Med.* **2004**, *200*, 331-343.
- [55] Sereti, I.; Imamichi, H.; Natarajan, V.; Imamichi, T.; Ramchandani, M.S.; Badralmaa, Y.; Berg, S. C.; Metcalf, J. A.; Hahn, B. K.; Shen, J. M.; Powers, A.; Davey, R. T.; Kovacs, J. A.; Shevach, E. M.; Lane, H. C. *In vivo* expansion of CD4CD45RO-CD25 T-cells expressing foxP3 in IL-2-treated HIV-infected patients. *J. Clin. Invest.* **2005**, *115*, 1839-1847.
- [56] Fry, T. J.; Connick, E.; Falloon, J.; Lederman, M. M.; Liewehr, D. J.; Spritzler, J.; Steinberg, S. M.; Wood, L. V.; Yarchoan, R.; Zuckerman, J.; Landay, A.; Mackall, C. L. A potential role for interleukin-7 in T-cell homeostasis. *Blood* **2001**, *97*, 2983-2990.
- [57] Ruiz-Mateos, E.; de la Rosa, R.; Franco, J. M.; Martinez-Moya, M.; Rubio, A.; Soriano, N.; Sanchez-Quijano, A.; Lissen, E.; Leal, M. Endogenous IL-7 is associated with increased thymic volume in adult HIV-infected patients under highly active antiretroviral therapy. *AIDS* **2003**, *17*, 947-954.
- [58] Kovacs, J. A.; Vogel, S.; Albert, J. M.; Falloon, J.; Davey, R. T. Jr.; Walker, R. E.; Polis, M. A.; Spooner, K.; Metcalf, J. A.; Baseler, M.; Fyfe, G.; Lane, H. C. Controlled trial of interleukin-2 infusions in patients infected with the human immunodeficiency virus. *N. Engl. J. Med.* **1996**, *335*, 1350-1356.
- [59] Napolitano, L. A.; Grant, R. M.; Deeks, S. G.; Schmidt, D.; De Rosa, S. C.; Herzenberg, L. A.; Herndier, B. G.; Andersson, J.; McCune, J. M. Increased production of IL-7 accompanies HIV-1-mediated T-cell depletion: implications for T-cell homeostasis. *Nat. Med.* **2001**, *7*, 73-79.
- [60] Llano, A.; Barreira, J.; Gutiérrez, A.; Blanco, J.; Cabrera, C.; Clotet, B.; Esté, J. A. Interleukin-7 in plasma correlates with CD4 T-cell depletion and may be associated with emergence of syncytium-inducing variants in human immunodeficiency virus type 1-positive individuals. *J. Virol.* **2001**, *75*, 10319-10325.
- [61] Mastroianni, C. M.; Forcina, G.; d'Ettorre, G.; Lichtner, M.; Mengoni, F.; D'Agostino, C.; Vullo, V. Circulating levels of interleukin-7 in antiretroviral-naïve and highly active antiretroviral therapy-treated HIV-infected patients. *HIV Clin. Trials* **2001**, *2*, 108-112.
- [62] Schluns, K. S.; Kieper, W. C.; Jameson, S. C.; Lefrançois, L. Interleukin-7 mediates the homeostasis of naïve and memory CD8 T-cells *in vivo*. *Nat. Immunol.* **2000**, *1*, 426-432.
- [63] Read, S. W.; Higgins, J.; Metcalf, J. A.; Stevens, R. A.; Rupert, A.; Nason, M. C.; Lane, H. C.; Sereti, I. Decreased CD127 expression on T-cells in HIV-1-infected adults receiving antiretroviral therapy with or without intermittent IL-2 therapy. *J. Acquir Immune Defic. Syndr.* **2006**, *42*, 537-544.