HOT TOPICS

The rise and fall of intermittent interleukin-2 therapy in HIV infection

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ABSTRACT. In 1995, a breakthrough paper showed that intermittent cycles of interleukin-2 (IL-2), together with suboptimal ART, caused an unprecedented, stable increase in $CD4^+$ T cell counts, without altering the steady state levels of viremia. At the time, this was somewhat obscured by the first successes of combination antiretroviral therapy (cART). However, since then, numerous studies have confirmed this basic finding, opening up a new perspective in the long-term management of chronic HIV infection. One of the benchmarks of this experimental treatment is the expansion of $CD4^+CD25^+$ T lymphocytes probably including T regulatory cells (Tregs). Based on these encouraging findings, two major phase III clinical trials, ESPRIT and SILCAAT, involving thousands of patients worldwide, were launched and continued over several years. Unfortunately, they both resulted in the highly unexpected, yet unequivocal, outcome of a lack of a protective effect of IL-2-expanded $CD4^+$ T cells on HIV disease progression towards the acquired immunodeficiency syndrome (AIDS) or death. In addition, there was the suggestion of an increase in certain deleterious effects on treated patients in terms of cardiovascular and inflammatory events. While IL-2 therapy is unlikely to be studied any further in the context of HIV infection, other cytokines, such as IL-7, are still being tested in the hope of more promising results.

Keywords: HIV-1, IL-2, JAK-STAT, immune reconstitution, ART

Interleukin-2 (IL-2) was described by Ruscetti, Morgan and Gallo as "T cell growth factor" [1], thus following chronologically, IL-1 in the new classification system. This cytokine has been widely studied both for its pleiotropic effects and its involvement in a variety of diseases, including inflammatory diseases, cancer, and infectious diseases. It was later recognized that IL-2 belongs to a larger group of molecules sharing a similar strategy for delivering their modulatory signal to cells expressing specific receptors. Today, this family encompasses cytokines sharing a "common γ chain", together with a β chain and an α chain, conferring cytokine specificity; both the intermediate affinity (βγ) and high affinity (αβγ) receptors are competent for signal transduction and cell activation [2]. Cytokines sharing the common γ chain activate the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) pathways, and several of them (IL-2, IL-7, IL-9, and IL-15) phosphorylate STAT5a or STAT5b *via* activation of JAK1 and JAK3, while IL-4 and IL-13 activate STAT6 [2], and IL-21 mediates its signal *via* STAT1 and STAT3 [3].

IL-2 has pleiotropic effects on several types of cells of the immune system including $CD4^+$ and $CD8^+$ T lymphocytes, B lymphocytes, natural killer (NK) cells and mononuclear phagocytes, generally resulting in their activation. In this regard, the expression of the IL-2 α chain receptor, also known as CD25, conferring high binding affinity to the cytokine, characterizes both activated $CD4^+$ T cells and a subset of $CD4^+$ T cells with suppressive functions on immune activation known as T regulatory cells (Tregs) [4]. Unlike activated T cells, Tregs proliferate poorly, express CD25 at a high surface density and in the absence of other activation markers such as CD69, and express the protein Forkhead box P3 (Fox P3) at the intracellular level. In addition to these naturally occurring Treg cells, other cells exerting a similar, suppressive function have also been described [5].

This short review will investigate the role of IL-2 in HIV infection and, in particular, will summarise the "rise and fall" of its clinical experimental use as adjunctive therapy in infected individuals.

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IL-2 AND HIV INFECTION *IN VITRO*

Together with interferon-α (IFN-α), IL-2 is probably the first cytokine to have been investigated in the context of HIV infection and replication. Isolation of HIV (the discoverers of this as the etiological agent of the acquired immunodeficiency syndrome, AIDS, were awarded the Nobel prize in 2008) was (and still is) achieved using T cell blasts (*i.e.* peripheral or cord blood leukocytes stimulated with a mitogen and IL-2) from allogeneic donors, the expansion and survival of which is highly dependent upon a supply of exogenous IL-2: the "T cell growth factor" [6, 7].

While this earlier culture system privileges the expansion of HIV strains using CXCR4 as entry co-receptor (X4 strains), together with CD4 (primary viral receptor), the stimulation of leukocytes with just IL-2, in the absence of a mitogen, allows the infection and replication of both CCR5-dependent (R5) and X4 strains. In this latter system, IL-2 promotes T cell proliferation, while virus propagation is largely dependent on the release of endogenous pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), IL-1, IL-6 and IFN-γ [8]. As discussed later, a cytokine storm, triggered by IL-2 stimulation is responsible for the several unwanted side effects, such as a flu-like syndrome, that follow the administration of "high dosages" (*i.e.* several millions of international units, MIU, per day) to infected individuals.

Interestingly, IL-2 exerts profound effects on the activation of $CD8^+$ T cells of infected individuals in terms of stimulating both their lytic potential as cytotoxic T lymphocytes (CTL), and their non-lytic, suppressive function on HIV replication. This may be explained, in part, by the release of CCR5-binding chemokines [9]. Similar effects are exerted by IL-2 on NK cells from infected individuals [10]. Indeed, when leukocytes were isolated either from peripheral blood or lymph nodes of HIV^+ individuals and were then stimulated in culture in the presence of IL-2, minimal or no virus replication was observed unless $CD8^+$ cells were removed [11, 12]. These results are consistent with a dominant, HIV-suppressive effect exerted by $CD8^+$ T cells and probably NK cells, on HIV-spreading among $CD4^+$ cells (T lymphocytes and monocyte-macrophages). Furthermore, administration of IL-2 to *ex vivo*, cultivated $CD8^+$ T cells from infected individuals reversed their constitutive uncoupling of the ζ chain of their T cell receptor (TCR), restoring their function [13]. In addition, it has been reported by different authors that HIV-1 infection leads to a progressive loss of IL-2 production, linked to an impairment of $CD4^+$ Th1 immune responses, along with a loss of $CD4^+$ T cell proliferative capacity *in vitro*, in turn linked to aberrant cell death following TCR stimulation. This dysfunctional profile has been linked to activation-induced death (AICD) of $CD4^+$ Th1 cells that can be prevented by IL-12 [14, 15]. Conversely, IL-2 has been shown to rescue the propensity of T lymphocytes from HIV-infected individuals to undergo AICD, although under other conditions, IL-2 may favor AICD [16]. All of these *in vitro* and *ex vivo* findings (and possibly many others), although showing the complexity of using a cytokine for the *in vivo* manipulation of a perturbed immune system as in the case of HIV-infected individuals, overall supported the hypothesis that administration of IL-2 to infected individuals could result in a significant restoration of immune functions, complementing and enhancing those achieved with combination antiretroviral therapy (cART).

IL-2 AND HIV INFECTION *IN VIVO*

Defective IL-2 expression was described early, and was interpreted as one of the hallmarks of the immunodeficiency associated with advanced HIV disease. It was the first rationale for administering IL-2 to infected individuals. Indeed, IL-2 was administered to infected individuals shortly after the initial description of AIDS and of the immunodeficiency associated with the infection. The first protocols mostly explored modalities that could be summarised as "continuous administration of low doses", usually given subcutaneously, with "low dose", operationally defined as < 1-2 MIU/day. This approach was associated with an increase in NK cell activity tested *ex vivo*, and with no significant side effects [17]. However, it did not result in any obvious beneficial effects for infected individuals.

In contrast, in 1995, a study by Joe Kovacs, Cliff Lane and colleagues, showed that administration of relatively high doses (several MIU/day) of IL-2 for five days followed by four to eight weeks of no treatment but in the presence of suboptimal ART, caused a significant increase in circulating $CD4^+$ T lymphocytes that was significantly greater than that induced by ART alone [18]. IL-2-boosted $CD4^+$ T cell levels remained elevated for several weeks after IL-2 infusion (originally studied intravenously, but then subcutaneously in follow-up studies and showing equivalent effects and fewer side effects) [19]. In most individuals, a type of "ladder" effect was observed, *i.e.* at the time of the subsequent administration of the cytokine, the absolute number of circulating $CD4^+$ T cells was superior to that observed before the previous cycle. Typically, after three cycles of IL-2 administration, the number of $CD4^+$ T cells in most individuals at least doubled, a result not observed for a long time, even with effective cART regimens that were frequently associated with a "ceiling" effect in terms of $CD4^+$ T cell recovery, and even in the face of complete suppression of viremia. Under intermittent IL-2 therapy, several individuals indeed normalised their $CD4^+$ T cell counts after 3-6 cycles [19]. An attractive feature of intermittent IL-2 therapy was its durability, *i.e.* after having reached normal or near normal levels of circulating $CD4^+$ T cells, IL-2 could be discontinued for several months and even years before there was a significant loss of peripheral $CD4^+$ T cells (in the presence of cART). Several phase II studies confirmed this original observation while trials administering IL-2 alone, in the absence of ART, were also conducted and resulted in only modest increases in $CD4^+$ T cell counts, inferior to those observed in the presence of antiviral agents [20]. In France, intermittent IL-2 was given to many "immunologically discordant" individuals, *i.e.* patients who responded to cART in terms of suppression of their viremia without however, significant persistence in the number of circulating $CD4^+$ T cells [21].

A second, attractive feature of intermittent IL-2 therapy was that it did not have a significant impact on steady state viremia levels. In the original paper by Lane and Kovacs, viral "blips" occurred frequently after administration of IL-2, but they did not result in increased steady state levels of viremia. However, increased virus replication was observed in advanced disease patients (< 200 $CD4^+$ T cells/μL) [18], although subsequent studies, conducted in the presence of potent cART regimens, showed the feasibility of administering IL-2 to individuals with advanced disease. Studies investigating the potential impact of IL-2 therapy on HIV DNA load indicated that the cytokine did not increase, and indeed eventually decreased, the number of circulating cells carrying HIV proviruses [22, 23]. Since this also occurred in individuals with incomplete control of viremia, as in our trial [22], this suggested that IL-2 therapy might be an exception to the so called "-predator-prey" model, *i.e.* that an expansion of $CD4^+$ T cells in the absence of fully controlled viral replication would inexorably result in increased viremia due to an increased availability of target cells, as observed in trials involving exclusively antiretroviral agents [24]. This overall, favourable profile of IL-2 administration not resulting in increased viremia (even in the absence of ART) has been interpreted as the result of the increased capacity of $CD8^+$ T cells and NK cells to exert lytic and non-lytic antiviral effects *in vivo* as observed during *in vitro* infection, or by cultivating leukocytes isolated from infected individuals. However, the role of IL-2-stimulated apoptosis or of Tregs activation could not be excluded.

Finally, meta-analysis studies of the results of several phase II trials indicated that individuals receiving IL-2 therapy showed fewer adverse events, while the occurrence of opportunistic infections or tumours was observed in those individuals who, although treated with IL-2, had low $CD4^+$ T cell counts compatible with the emergence of these diseases [25].

In conclusion, all *in vitro* and *in vivo* studies supported the hypothesis that intermittent IL-2 therapy was probably beneficial to HIV-infected individuals in the perspective of their long-term management, perhaps including a period of antiretroviral wash-outs or strategies for sparing regimens for certain classes of antiretroviral agents, to prevent or delay the emergence of multi-drug-resistant viruses.

PHASE III CLINICAL TRIALS OF INTERMITTENT IL-2 ADMINISTRATION A RUDE AWAKENING!

On the basis of the favourable studies summarised above, two, large efficacy clinical trials ("Evaluation of subcutaneous proleukin in a randomized international trial, ESPRIT" and "Subcutaneous, recombinant, human interleukin 2 in HIV-infected patients with low CD4 counts under active antiretroviral therapy, SILCAAT"), were designed at the end of the '90s to address the question of whether intermittent IL-2 administration was indeed beneficial to infected individuals with chronic HIV infection. The two studies differed as regards a number of variables, the most important being the inclusion of individuals with less advanced disease in ESPRIT *versus* SILCAAT. ESPRIT enrolled > 4,000 patients with circulating $CD4^+$ T cells > 300/μL, while SILCAAT enrolled approximately 1,700 patients with circulating $CD4^+$ T cells < 300/μL, at entry. The primary endpoints were death by any cause, and opportunistic infections, while the secondary endpoints encompassed severe (grade 4) adverse events. The control arm received ART without IL-2 administration, in a simple 1:1 ratio [26].

These studies involved thousands of patients worldwide and more than seven years of observation on average, in order to achieve a statistically meaningful result. However, both studies were completed, and produced identical and unequivocal result: there was no benefit to HIV^+ individuals in receiving IL-2 in association with ART regimens, in spite of a confirmed superior increase in circulating $CD4^+$ T cells compared to individuals receiving only ART. Furthermore, undesired effects, sometime severe, particular of an inflammatory and cardiovascular nature, were more strongly associated with IL-2 than with ART alone [26]. This clear-cut conclusion, from a long history of clinical investigations, highlights a number of important issues, the resolution of which could be important for future trials of immune-based therapeutics in HIV infection and, possibly, for other clinical conditions. Why did IL-2-expanded $CD4^+$ T cells not confer protection? Why was the negative outcome not predictable on the basis of the several phase II studies and by their meta-analysis? Is there a dosage of IL-2 that could minimise the negative side effects, while retaining some protective activity? Are there other cytokines or immunologically active compounds that could take over from IL-2 or should the future management of infected individuals be based exclusively on antiretroviral agents?

Some of these questions have been partially answered. For example, the $CD4^+$ T cells expanded by IL-2 administration, are characterised by a peculiar phenotype resembling (or encompassing) that of natural Tregs [27]. A fraction of IL-2-expanded $CD4^+$ T cells express CD25 (the α chain of the IL-2 receptor), without expressing common activation markers such as CD69 and HLA-DR *(figure 1)*. In addition, we reported earlier that IL-2 therapy caused an increase in cells expressing a C-terminally truncated variant of STAT5 [28], acting as a dominant, negative of full length STAT5 [29]. If these cells, whatever their composition or functional role [27, 30], are ineffective in protecting HIV^+ individuals from clinical progression towards AIDS and death, future trials using cART or novel immunologically-based compounds should monitor carefully the phenotype and function of the expanded $CD4^+$ T cells. In this regard, ongoing studies are exploring the potential benefit of administering IL-7, another common γ chain cytokine, rather than IL-2, in conjunction with cART. Overall, these early studies indicate that IL-7 is well tolerated, causes no or fewer side effects than IL-2, and promotes expansion of several leukocyte subsets, including $CD8^+$ and $CD4^+$ T cells, an effect that remains relatively stable for weeks after the administration of the

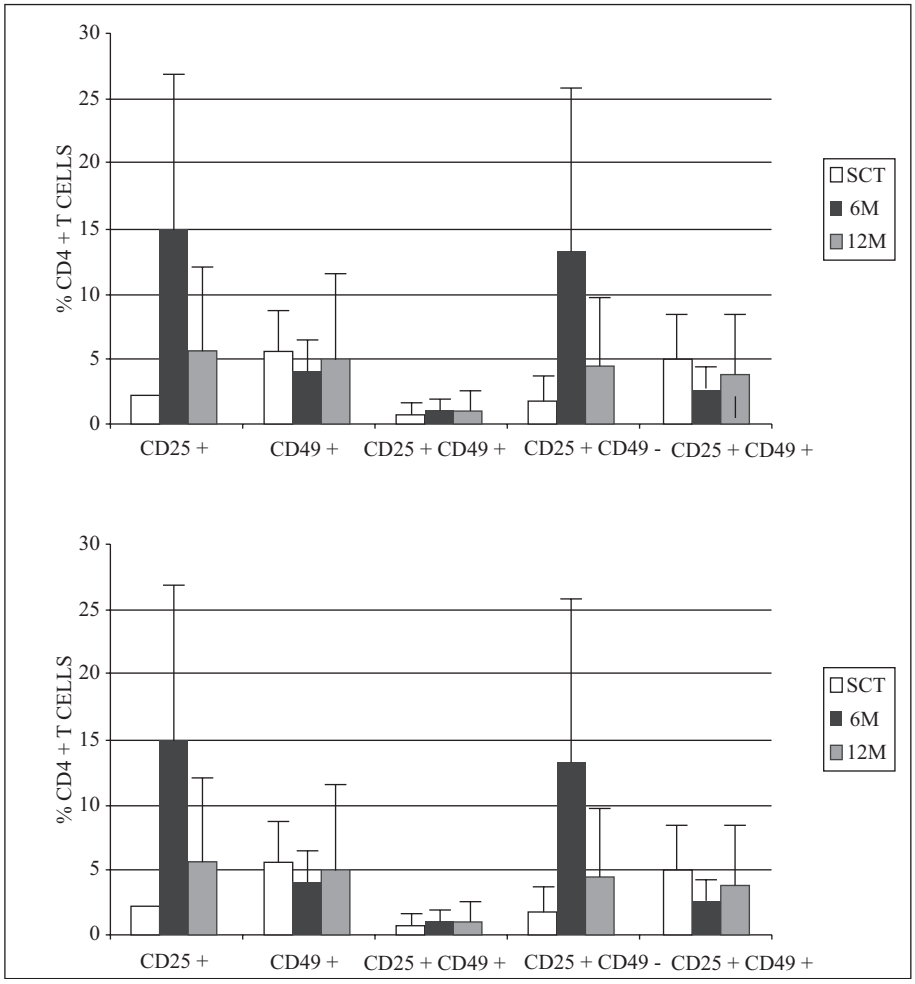

Figure 1

Percentage of circulating $CD4^+$ T cells co-expressing activation markers. A three-colour staining, using anti-CD4, -CD25 and -CD69 monoclonal Abs, was performed on frozen PBMC from certain patients enrolled in a phase II study of intermittent IL-2 administration together with ART *versus* ART alone arms, at the screening, and then at the 6^{th} and 12^{th} month of IL-2 treatment (22). The data are expressed as mean ± SD (screening v*ersus* 6^{th} month, and 6^{th} month *versus* 12^{th} month).

cytokine [31]. There are, as yet, no detailed studies on the nature of the increased levels of $CD4^+$ T cells following IL-7 administration, but the overall effect of this cytokine seems to be substantially different from that of IL-2.

Whether lower dosages of IL-2 could have resulted in a better clinical outcome than that described in ESPRIT and SILCAAT will remain unanswered since no more large, phase III clinical to investigate intermittent IL-2 administration in HIV disease are planned. In this regard, we have recently reported that individuals infected with an R5 HIV-1 and receiving IL-2 therapy at lower dosages than those of the phase III trials, have a greater tendency to maintain this viral phenotype than subjects undergoing exclusively ART [32]. Therefore, a theoretical scenario might involve the combination of IL-2 (or a similar agent) and CCR5 inhibitors, with a lower chance of observing viral escapes due to extension or switch of the viral co-receptor usage to CXCR4, an event that is a predictor of disease progression, independent of $CD4^+$ T cell counts and viremia levels [33].

In conclusion, the saga of IL-2 therapy has a bitter conclusion for all who have invested time and energy in attempting to improve the long-term therapeutic management of infected individuals, and for the general idea of controlling an important viral disease by harnessing the immune system rather than attacking the virus directly. Nonetheless, if we learn from this failure, we might be able to refine our strategies and ultimately succeed in identifying molecules that would allow better long-term control of HIV disease progression, and not rely exclusively on antiretroviral agents.

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