REVIEW

Interleukin-7 (IL-7): immune function, involvement in the pathogenesis of HIV infection and therapeutic potential

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ABSTRACT. Interleukin 7 (IL-7), which is constitutively produced particularly by stromal cells from the bone marrow and thymus, plays a crucial role in T cell homeostasis. This cytokine is implicated in thymopoiesis since it sustains thymocyte proliferation and survival. It regulates peripheral naive T cell survival by modulating the expression of the anti-apoptotic molecule Bcl-2, and sustains peripheral T cell expansion in response to antigenic stimulation. Infection by the human immunodeficiency virus (HIV) leads to severe T lymphopenia and general immune dysfunction. Increased IL-7 plasma levels are generally observed in HIV-infected patients. The existence of an inverse correlation between IL-7 plasma levels and the CD4+ T cell count suggests that a direct feedback mechanism is working to restore peripheral T cell counts in lymphopenic patients. Here, IL-7 plasma levels are a good predictive marker of CD4+ T cell restoration during therapy. Combinations of antiretroviral treatments considerably slow disease progression. They drastically decrease the viral load and, in most patients, significantly increase peripheral CD4+ T cell counts. However, despite their usual ability to reduce viral replication, such treatments often fail to reverse lymphopenia and do not restore specific antiviral immune responses. IL-7, based therapy, combined with efficient antiretroviral treatment, may be beneficial to HIV-infected patients by promoting thymic output, sustaining naive T cell counts and increasing memory T cell activation.

Keywords: IL-7, HIV, CD4+ T cells, thymopoiesis, homeostasis, immunotherapy

INTRODUCTION

Human IL-7 has been identified as a 25 KDa protein [1]. The human IL-7 gene, which is located on chromosome 8q12-13 [2], is composed of six exons leading to multiple transcripts. Substantial homology between human and mouse sequences (around 80%) make it a conserved protein. This protein contains six cysteine residues and two glycosylation sites, which explain why the mature protein has a molecular weight of 25 KDa rather than the 17.5 expected from its sequence. IL-7 is constitutively produced by stromal cells from bone marrow and thymus [3], dendritic follicular cells [4], keratinocytes [5], dendritic cells [6], liver [7] and intestinal epithelium [8].

The interleukin 7 receptor (IL-7R), which is a member of the hematopoietin family, consists of an α-chain, which confers specificity (IL-7Rα or CD127) [9], and an γc-chain, which is common to several cytokines (IL-2, IL-4, IL-9, IL-15, IL-21) [10, 11] *(figure 1)*. Human T-cell lineages and macrophages express the receptor [12]. IL-7R expression is regulated during T cell differentiation and T cell activation: surface down-regulation is observed when the receptor is complexed with its ligand [13]. Signal transduction by IL-7 requires both IL-7Rα and IL-7Rγc and involves the recruitment of intracellular kinases. The pathways involve Janus kinase 1 and 3 (Jak1; Jak3) [14], STAT activators of transcription [15], phosphatidylinositol 3-kinase (PI3-kinase) [16] and Src tyrosine kinases [17]. Binding of IL-7 to its receptor results in the transduction of multiple signals such as proliferation, anti-apoptotic and activation *(figure 1)*. Despite the fact that IL-7 and IL-7R were discovered many years ago, their physiological roles are far from being completely elucidated. Furthermore, the picture has been complicated by the characterization of TSLP-1, a cytokine with a similar structure and function to that of IL-7 *(figure 1)*.

Outstanding review articles have been published concerning the immune functions of IL-7 and its involvement in immune responses against infectious agents and cancer [18]. Similarly, the involvement of IL-7 in various pathological disorders and in bone marrow transplantation [19, 20] have also been discussed. In this review we shall concentrate on the immune functions of IL-7 directly relevant to the physiopathology of HIV infection and on the therapeutic potential of IL-7 as an alternative treatment option for this infection.
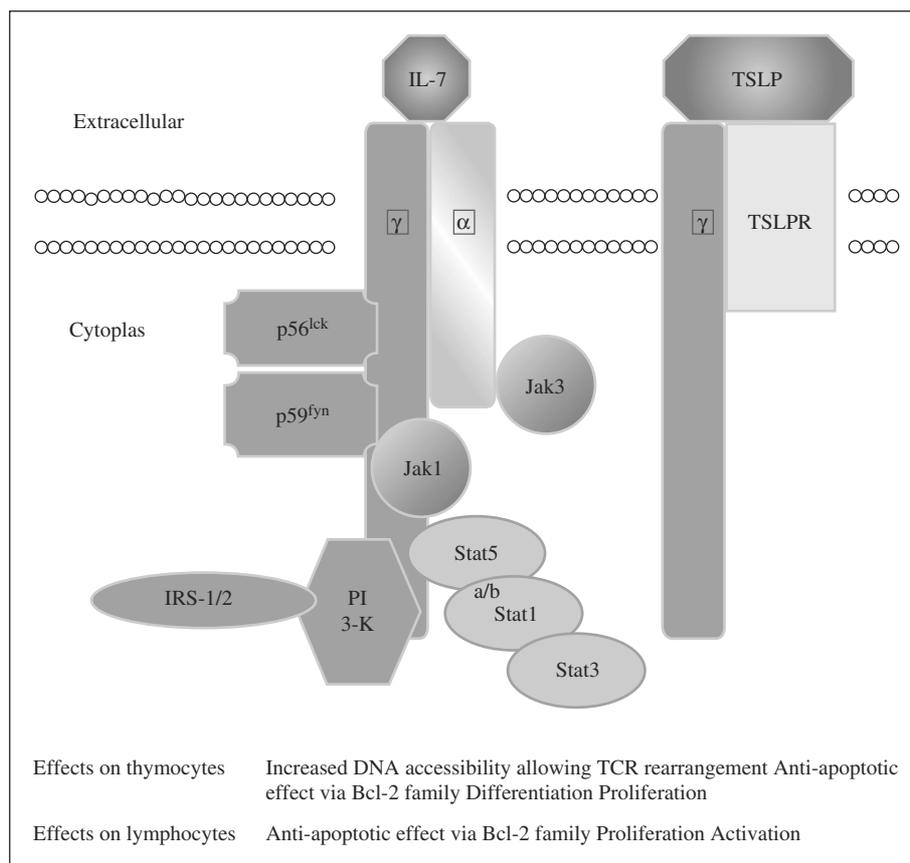
Figure 1

IL-7R – associated signal transduction molecule and physiological effects. IL-7R shares the γc chain with IL-2, IL-4, IL-9, IL-15, IL-21 and IL–7Rα with a new, IL-7-like cytokine called "thymic stromal-derived lymphopoietin" (TSLP). IL-7 induces both Jak1 and Jak3, which then interact with IL-7Rα and γc respectively, activating transcription factors Stat3, Stat5a and Stat5b. Stat5 has been shown to facilitate DNA locus accessibility for TCR rearrangement. Activated IL-7Rα also binds phosphatidyl inositol-3'-OH kinase (PI3-K), which ultimately leads to proliferation. IL-7 can also activate non-receptor protein tyrosine kinases of the Src family: p59fyn and p53lck. IL-7 signaling is important for both thymocytes and mature lymphocytes, leading to proliferation, cell survival and differentiation.

IL-7 – A CYTOKINE WITH CENTRAL AND PERIPHERAL ACTIVITY

IL-7 was initially characterized in 1988 as a mouse pre-B cell growth factor [21]. In humans, its best established role concerns T-cell immunobiology. IL-7 is a crucial, pleiotropic cytokine controlling T-lymphopoiesis and T-cell peripheral expansion. The properties of IL-7 are summarized in *figure 2*.

Role of IL-7 during lymphopoiesis

Analysis by knockout animals (table 1)

IL-7 knockout mice show a profound reduction in thymic and peripheral lymphoid cellularity affecting both T and B cell lineages [22, 23]. The studies conducted in these KO animals suggest that IL-7 plays a key role in both T-cell and B-cell maturation. T-cell development is blocked at an early stage before CD4 and CD8 surface expression is acquired and before rearrangement of the TCRβ chain, at the CD3-CD4-CD8- stage. However, lymphopoiesis is not entirely blocked and some mature B and T cells are found in peripheral lymphoid organs. The partial maturation of T cells in these mice models can be explained by the presence of a cytokine called thymic stromal lymphopoietin (TSLP1), which possesses overlapping properties with

IL-7 and uses IL-7Rα as its receptor [24]. TSLP1 is produced by thymic stromal cells and can also induce immature thymocyte proliferation. It can be hypothesized that TSLP1 is able to overlap IL-7 functions during thymopoiesis, but in a very weak manner as only 1% of pre-T cells become mature T-cells in IL-7 -/- mice [22].

Murine KO animals have also been useful in dissecting the molecular mechanism of action of IL-7. The thymocytes of IL-7Rα -/- mice show a low level of Bcl-2 expression [25], suggesting that one of the roles of IL-7 during thymopoiesis is to sustain the survival of immature T cells. Furthermore, enforced expression of the Bcl-2 transgene in IL-7Rα -/-mice may completely rescue T-cell development and T-cell function [26, 27]. Some development may proceed in IL-7Rα -/- mice, but mature CD4+ and CD8+ cells are relatively unresponsive to mitogenic and antigenic activation [27]. *In vitro* studies have shown that IL-7 can maintain the expression of rag-1 and rag-2 genes, which are known to control TCR rearrangement [28]. IL-7 is implicated in the D-J rearrangement of the TCRß chain [29] in T cell progenitors, presumably by sustaining the survival of these cells [30]. This action is probably exerted by up-regulation of Bcl-2 [27]. In this context, it should be noted that IL-7 KO animals show a complete lack of mature TCRγδ T-cells [25, 31, 32].
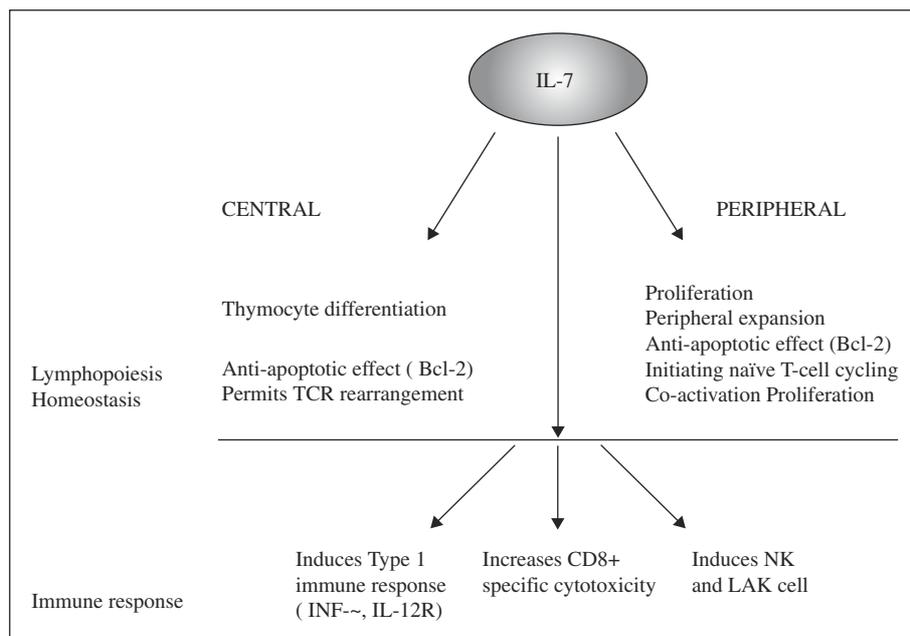
Figure 2

IL-7 effects on central and peripheral compartments. IL-7 is a potent pleiotropic cytokine implicated in both thymopoiesis and peripheral homeostasis. In the thymus, its role is to support the viability and the expansion of early thymocytes. At the peripheral level, IL-7 co-stimulates T- lymphocytes, thereby initiating their proliferation and survival. IL-7 also affects the immune response: IL-7 induces a type 1 immune response, specific cytolytic effects and LAK activity.

Implication of IL-7 in human lymphopoiesis

Highly purified human CD34+ stem cells differentiate to form mature T cells when seeded into foetal thymic lobes of severe combined immunodeficient (SCID) mice [33]. In this model, the use of neutralizing antibodies against human IL-7 or human IL-7Rα to block of IL-7 binding results in a profound reduction in thymic cellularity and blocks early T-cell development.

A common feature in X-linked, severe combined immunodeficiency disease (XSCID), with mutations in the gene coding for the γc chain, is a profound defect in T cell development. Patients possess few or no T or NK cells but have normal or increased numbers of B cells [34]. Furthermore, the cause, in two SCID patients with a deficiency in T-cell maturation, was found to be an IL-7Rα deficiency [34]. Both showed a point mutation in the IL-7Rα gene preventing the production of functional IL-7Rα (in one patient a null mutation was responsible whereas a missense mutation leading to impaired ligand binding was found in the other) [35]. This firmly established that IL-7Rα-dependent signaling plays a major role in T cell development in humans and probably explains the reduced

T cell count. Moreover, these results suggest that the T cell defect observed in XSCID and in JAK3-deficient SCID patients can be largely if not entirely attributed to defective IL-7 signaling. Therefore, as in mice, IL-7 is crucial for the maturation of human pre-T cells *(table 1)*. In humans, IL-7 is critical for the development of the T lineage but not the B lineage [35].

IL-7 as a peripheral homeostatic regulator

IL-7 regulates homeostasis in lymphopenic and athymic mice

Because a complete lack of IL-7 has dramatic effects on T cell development, the action of IL-7 on mature T cells *in vivo* has been difficult to test. Many murine models have been developed to study the potential of IL-7 in controlling homeostasis.

When IL-7 is injected into mice made lymphopenic by prior injection of cyclophosphamide, it accelerates lymphoid regeneration. This acceleration in organ cellularity occurs in the spleen, lymph nodes and peripherally, but not in bone marrow or thymus. The populations expanded were CD4 and CD8 mature T cells, and their normal levels

Table 1

Phenotype of mice deficient in one of the components of the IL-7 transduction pathway, and correlation with human phenotype

Knockout mouse	phenotype	human correlate
γc and JAK3	Impaired T- and B-cell development, absence of IEL, γδ T cells, NK cells, normal thymus architecture	No T cells or NK cells, normal B cells T-B+NK-SCID phenotype
IL-7	Impaired T- and B-cell development, Absence of γδ T cells, normal NK cells	Not yet observed
IL-7Rα	Similar to IL-7 knockout with more severe block in T- and B-cell development	No T cells normal NK cells and B cells T-B+NK+SCID phenotype

A common feature of severe combined immunodeficiency disease (SCID) is a profound defect in T cell development and/or function. Mice made deficient for γc, Jak3 IL-7 or IL-7Rα show a profound defect in B cell development whereas humans with SCID caused by Jak3, γc or IL-7Rα deficiency have a normal number of B cells. Forms of SCID resulting from genes required for the functioning of the IL-7/IL-7R signalling pathway lead in humans to a profound defect in T cell development.

were significantly exceeded. Transgenic expression of IL-7 in nude mice restores T cell populations and enhances both IL-2 production and their response to mitogens [36]. IL-7 is required for the homeostatic expansion of both naive CD8+ and CD4+ T cells in lymphopenic hosts by maintaining T cell survival and inducing proliferation [13]. Thymectomized mice that have been depleted of their T cells by treatment with cyclosporine, can be reconstituted by exogenous T cells. Treatment of these cells with rhIL-7 before replenishment increases Bcl-2 expression and enhances the peripheral expansion of mature T cells. Moreover, IL-7 restores recall and primary immune responses in these mice [37]. This model shows that IL-7 has the capacity to restore immune competence in the absence of the thymus, solely by peripheral expansion. A complementary experiment showed that thymectomized hosts reconstitute T cells primarily by antigen-driven peripheral expansion [19]. This suggests that the homeostatic properties of IL-7 in normal mice are due to action on both thymopoiesis and peripheral expansion.

Implication of IL-7 in human homeostasis

Tsark *et al.* developed a functional model of the human immune system in mice (beige/nude/xid/human) [38]. They studied the development of extrathymic human T lymphocytes in an *in vivo* system, hypothesizing that IL-7 might have the same properties in human cells as on mice cells. They showed that endogenous IL-7 is necessary to sustain a xenograph and the mice produced equivalent levels of CD4 and CD8 T cells. They also showed that IL-2 production in response to a stimulus, was restored. Once again, IL-7 showed its capacity to enhance peripheral T cell expansion.

In vitro, IL-7 can induce proliferation of human adult CD45RA+ T cells without switching the phenotype to CD45RO+ [39]. IL-7 can maintain the viability of naive T cells *in vitro* by augmenting Bcl-2 expression for at least 15 days, and also maintains longer telomeres [39]. After antigenic stimulation, naive CD4+ T cells lose detectable IL-7R expression and also their ability to respond to cytokine [13, 39]. These observations suggest that IL-7 participates in the extrathymic expansion of neonatal CD45RA+, and may also contribute to the maintenance of the CD45RA+ T cell pool, thus playing a key role in maintaining the pool of naive circulating CD4+ T cells.

Role of IL-7 in immune responses

IL-7 and Th1/Th2 responses

Antigenic stimulation leads to the development of CD4+ T-helper cells capable of producing a broad spectrum of cytokines. The types of cytokine secreted determine the profile of the immune response: T helper cells type 1 (Th1) produce INF-γ and control cell-mediated immunity, T helper cells type 2 (Th2) produce IL-4 and participate in controlling humoral immunity. IL-12 promotes the Th1 response and enhances INF-γ secretion [40]. IL-7 acts by up-regulating the IL-12 receptor and synergizes with IL-12 to increase INF-γ production [41, 42]. IL-7 also enhances INF-γ production in co-stimulation with αCD3/αCD28. IL-7 also up-regulates IL-4 mRNA synthesis but to a lesser extent than INF-γ and in a more transitory manner [42]. IL-7 modulates INF-γ and IL-4 expression by transcrip-

tional and post- transcriptional mechanisms in activated human T lymphocytes [42]. IL-7 induction of INF-γ production is consistently more pronounced than that of IL-4, although it is less potent than IL12, suggesting that IL-7 is an intermediate cytokine in the Th1 immune response.

IL-7 induces CTL responses

Several cytokines are able to exert regulatory effects on the different steps involved in the process that results in an effective cytotoxic response. CTLs constitute a major part of the cell-mediated response and CTLs play a critical role in recovery from primary viral infections by limiting viral replication and eradicating virus-infected cells. Studies have been conducted to assess the generation of a virus-specific CTL *in vitro* in human PBMCs. Addition of IL-7 to human influenza A virus-stimulated PBMCs at the start of the culture strongly and specifically enhanced cytotoxicity against influenza A virus-infected autologous targets [43]. *In vitro,* a dose-dependent increase in the cytolitic activity of human T-cell is accompanied by an increase in total cell numbers. This cytotoxicity is specifically IL-7-induced and its enhancement was mediated primarily by CTL precursors rich in the CD8+ subpopulation [44]. IL-7 generation of CTL activity shows similar potency but occurs a little later in comparison with IL-2. It may be hypothesized that IL-2 is a potential mediator of the IL-7 effect because it has been shown that IL-7 can induce the expression of IL-2Rα and γ [45]. However, the enhancement of IL-7 activity in its generation of CTL was only partially inhibitable by antibodies that neutralize IL-2, and no synergy was observed with IL-2 [44], suggesting that the role played by IL-7 in the proliferation and differentiation of CTL precursors is at least partially independent of IL-2.

The increase in CTL activity by IL-7 was also associated with an increase in total serine esterase activity per cell (present in the cytolytic granules) [43] and in T-cell-pore-forming protein, a major lytic protein that increases levels of cytotoxic molecules such as INF-γ [42].

IL-7 induces human NK activity

The LAK phenomenon has been describes as the ability of IL-2-activated PBL to kill fresh tumor cells in a non-MHC-restricted manner [46]. Several cytokines may synergize with IL-2 in LAK development, generating LAK activity directly or through endogenous IL-2 production. IL-7 was found to generate LAK activity in bulk PBMCs, but with substantially less potency than IL-2 (10- to 20-fold lower) in both human and mouse models [47, 48]. Although IL-7 is a weak inducer of LAK cells compared to IL-2, IL-7 potentiates the capacity of IL-2 to induce LAK cells [47, 48]. IL-7 generates higher LAK activity in purified NK cells (CD56+) than in bulk PBMCs with a rate comparable to or slightly lower than the LAK activity induced by IL-2 [49]. CD56+ NK cells seem to be the predominant LAK cells in response to IL-7 [47, 49, 50]. The properties of IL-7 are summarized in *figure 2*.

IL-7 – IN THE PHYSIOPATHOLOGY OF HIV INFECTION

Infection by the human immunodeficiency virus (HIV) leads to severe T lymphopenia and general immune dys-

function. Several mechanisms, including direct and indirect T cell killing, disrupted peripheral homeostasis and impaired, central, *de novo* production certainly contribute to this lymphopenia.

Mechanisms of CD4 depletion

A direct mechanism may explain part of the CD4 decrease observed during HIV infection, i.e. the cytopathogenic effect of the virus. Here, active viral replication induces CD4 T cell destruction, and the infection is accompanied by elevated viremia that reflects the continuous round of virus infection and the ongoing destruction of infected cells [51, 52]. The rate of CD4+ T cell loss has been estimated to be approximately $1x10^6$ cells per day. However, the direct action of HIV on infected cells cannot explain the high T cell death rate. The absolute number of infected CD4 T cells is too small in contrast to the number of apoptotic cells. Although the direct cytopathic potential of HIV is well established, indirect mechanisms have been proposed to explain the CD4 depletion. The virus may induce apoptosis in both infected and uninfected cells [53]. Uninfected T cell death involves both CD4 and CD8 cells, and may be due to chronic activation of the immune system, which exhausts the T cells [54]. Other observations suggest that the CD4 T cell decline results from the inability of the infected host to adequately replace CD4 T cells [55]. In this model, HIV interferes with the effective lymphocyte proliferation necessary to compensate for on-going T cell destruction [56]. Suppression of bone marrow and thymic function, and damage to peripheral lymphoid tissue are also important factors contributing to the inability of the infected patient to maintain lymphocytes populations [57, 58].

IL-7 and CD4 depletion in HIV-infected and HAART-treated patients

Recently, a study was conducted into human immunodeficiency infection in newborns and older children [59]. Serum IL-7 concentrations were significantly increased in HIV-infected children, correlating with a reduced CD4 count. Adult HIV-positive patients show higher IL-7 levels than the healthy population. In fact, the same strong negative correlation between IL-7 levels and the CD4 count that seen in children is observed in adults [60]. Both HAART-treated and untreated HIV-infected patients show higher IL-7 levels than the healthy population. Higher IL-7 levels are associated with depletion of both memory/effector and naive T-cells. Another association may be mentioned in adult HIV-positive patients since increased IL-7 levels correlate positively with a greater viral load [6]. This association persists throughout all the stages of the disease. Patients who respond well to HAART i.e. with a viral load decrease accompanied by a CD4 T-cell increase, show lower plasma IL-7. Despite an increased CD4 count, IL-7 levels do not return to a normal level [61].

Two different mechanisms have been put forward to explain the up-regulation of IL-7 levels. First, a decrease in the T-cell count could diminish IL-7R availability, thereby increasing circulating IL-7. In HAART, increases in the CD4 count do not result in a rapid return to normal IL-7 levels as would be expected if IL-7 levels are regulating

solely by the binding of the cytokine to its receptor. The beginnings of an answer may be provided by the fact that IL7R expression on CD8+ T cells is reduced by HIV infection, and can only be partially restored with effective antiretroviral therapy [62]. The mechanism by which HIV infection causes IL-7R down-regulation remains a matter of speculation, and the simple reduction of IL-7R expression cannot explain the lack of complete IL-7 clearance. In the second model, IL-7 elevation is due to massive IL-7 secretion in response to T-cell lymphopenia. In support of this, IL-7 production indeed seems to be increased in depleted lymphoid tissues [6]. This suggests that the system has the capacity to detect a reduction in the T-cell count of a microenvironment, and can react by enhancing IL-7 secretion. IL-7 levels are expected to return to normal once the lymphopenia is resolved. The reason why circulating IL-7 remains high in HAART patients after restoration of CD4 to normal levels remains unclear [61]. In support of this second hypothesis, IL-7 levels have been found to increase in different diseases characterized by marked T cell depletion.

In vitro *IL-7 boosts anti-HIV cytolytic activity*

An analysis was made of the capacity to generate an HIV-specific CTL response *in vitro* in PBMCs taken from asymptomatic HIV+ individuals. As expected, IL-7 induced a proliferative response and an increase in the cytotoxic activity of the CD8+ cell subset. This CTL activity is HIV-1 specific and can replace IL-2 as an efficient factor for the conversion of resting CD8+ into effector CTL cells *in vitro* [63]. IL-7 can act directly on the CD8+ subset to augment specific viral cytotoxicity, and this without the presence of helper cells. IL-7 in PBMCs taken from HIV-infected patients significantly augments the proliferative response to gp120 *in vitro,* with a predominant effect in individuals who have been primed by exposure to an antigen using an HIV-1 env subunit vaccine [64]. Donors who previously tested negative for HIV-specific CTL did not convert to a positive CTL response subsequent to *in vitro* stimulation by IL-7. This lack of response was accompanied by a significant reduction in IL-7R expression by CD8+ T cells. *In vitro,* IL-7 is unable to induce a specific primary response against HIV if the CD8+ T cells are not primed by antigen *in vivo* [65]. However, patients who initially tested positive for HIV-specific CTL showed an increased response in the presence of exogenous IL-7 [63].

Another system consisting of the use of autologous PB-MCs acutely infected with vaccinia/HIV-1 expression vectors, provides a far more efficient means of achieving antigen-specific activation of CTL precursors than activation by conventional mitogens in the presence of IL-7 [66]. This specific-antigen activation is variable. In conclusion, it has been shown *in vitro*, under various experimental conditions that IL-7 increases both the antigendependent proliferation and the specific cytolytic activity of CD8+ CTL against virus-cell infected, including against HIV-1.

IL-7 induces HIV replication in circulating T-cells

Relatively little is know about the mechanisms that activate HIV replication in HIV-1 infected PBMCs. Several

cytokines such as IL-2, IL-4, IL-6, IL-12, IL-15 and TNF-α potentiate HIV replication *in vitro*. IL-7 has also been reported to augment HIV production *in vitro* [67, 68]. Exogenous IL-7 increases HIV-1 production by soluble αCD3-stimulated PBMCs from HIV-infected donors. A 10 to 20-fold increase in p24 production and *tat* RNA induction are observable [67] with naturally infected PBMCs, CD8-depleted in culture. In response to cross-linking of CD3 and CD28, which mimics physiological signals, naïve T-cells do not support HIV replication, while memory T-cells support active replication [69]. The memory CD4 T-cell population seems to be preferentially infected by HIV-1 [70]. This suggest that IL-7 may act on CD45RO+ and CD4+ subsets to enhance viral replication. IL-7 alone, or in combination with other intracellular stimuli, induces cellular pathways that specifically enhance virus replication without driving cell proliferation [67]. Moreover, IL-7 induces the production of secondary cytokines from monocytes (IL-1, IL-6, TNF-α) [47] that regulate T-cell differentiation and increase HIV production *in vitro*. IL-7-mediated virus replication is completely inhibited in the presence of CD8+ cells in PBMC culture. IL-7 also enhances expression of the chemokine receptor CXCR4 [71], a cofactor for HIV entry into T cells [72]. Recently, it has been shown that CD45RO+ memory T-cells can be divided into two populations, in particular one that expresses CXCR4. In agreement with previous arguments whereby HIV preferentially infects memory cells, it can be hypothesized that IL-7 may increase the number of targets for HIV, rendering entry easier for the virus. It is interesting to note that IL-2, IL-4, IL-7 and IL-15, which all bind to the γc chain, induce HIV replication and CXCR4-expression on memory T-cells [71]. Taken together, these data suggest that IL-7 promotes HIV replication in the CD4 subset, making effective viral production more likely and increases viral targets for *de novo* infection by the virus produced under IL-7 stimulation.

IL-7 induces HIV replication in thymocytes

In the SCID-hu mice model, with infection by a CXCR4-tropic molecular clone of HIV-1, the viral load observed in thymocytes after biopsy is increased by IL-7 injections [73]. *In vitro,* mature SP CD4+ and immature human thymocytes are cellular targets of HIV infection [74, 75]. IL-7 regulates HIV replication in immature lymphocytes by protecting them from apoptosis [76] and by inducing differentiation and expansion of mature CD27+ thymocytes [74]. IL-7 also increases cell surface expression of CXCR4 in mature CD4+, thus promoting X4 strain virus replication compared with intermediate thymocytes [77]. The IL-7 interactions with the physiopathology of HIV infection are summarized in *figure 3*.

IL-7 – THERAPEUTIC POTENTIAL

IL-7 and CD4 count restoration

Infection by the human immunodeficiency virus (HIV) leads to CD4 lymphopenia [78]. With HAART, viral replication is generally brought under control. No viruses are detectable and the T-cell count increases. However, pro-
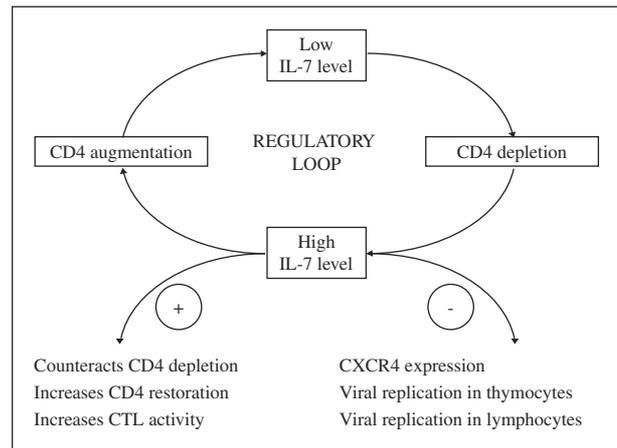

Figure 3

IL-7 in the physiopathology of HIV infection. Several studies have shown an inverse correlation between plasma IL-7 level and the CD4 count in HIV-infected patients. This IL-7 elevation is also observed in other cases of lymphopenia such as after chemotherapy or during leukemia. These observations suggest that IL-7 is implicated in a regulatory loop that aims to restore the CD4 count. High plasma levels of IL-7 in HIV-infected patients before antiretroviral treatment contribute to improved CD4 restoration during HAART. In the SIV-infected simian model, which mimics HIV infection, IL-7 injections counteract CD4 depletion.

longed antiviral therapy leads to drug resistance and hence to a resurgence in viral replication that correlates with a decrease in the peripheral lymphocyte count. In such cases, the patients develop complex metabolic abnormalities [79]. In some cases, the CD4+ T cell count increases very slowly even if viral replication is controlled. To counteract this lymphopenia, IL-7 injections may be effective by acting in two ways: firstly by controlling thymopoiesis [33-35] and secondly by inducing peripheral expansion [80]. Moreover, CD4 T-cells appear to survive for longer when cultured in the presence of IL-7. Here, by inhibiting programmed T-cell death (acting via Bcl-2) [26], IL-7 may maintain the peripheral CD4 count at a certain level, at least by increasing cell survival. Also, IL-7 injections in normal mice increase the CD4 count without activation [81], in the same manner as in T-cell, depleted hosts [36, 37]. This suggests that IL-7 could have the same peripheral proliferative effect in human lymphopenic patients *(table 2)*.

IL-7 and the generation of an increased T cell repertoire

The diversity of the T cell receptor repertoire is produced by rearrangements of gene elements in immature thymocytes. In HIV infection, the capacity to respond specifically to the virus is lost. The lack of HIV-specific, CD4 responses and CD8 cytotoxicity is only partially overcome by antiretroviral therapy. If IL-7 controls thymic production by acting as a survival factor that promotes accessibility to the DNA for TCR rearrangements, we would expect IL-7 treatment in HIV-infected patients to result in the output of new cells with a larger repertoire generating the capacity to recognize HIV antigens *(table 2)*. It is therefore very important to evaluate the new T-cell repertoire in the thymic output of patients who received IL-7 treatment.

Table 2
Arguments for and against the use of IL-7 in immunotherapy

Arguments **in favor** of IL-7 treatment for HIV-positive patients	Arguments **against** IL-7 treatment for HIV-positive patients
Central actions	**Supports viral replication**
Induces proliferation of immature thymocytes (could increase thymic output)	Directly
Implicated in TCR rearrangement (could increase the repertoire during AIDS)	Induces production of cytokines which induce viral replication
Induces Bcl-2	
	Increases potential number of targets
Peripheral actions	Increases CXCR4 expression
Co-mitogenic effect on mature circulating T cells (could increase peripheral expansion)	Promotes "X4" replication in the thymus
Induces Bcl-2	
Immune response induction	
Increases CD8+ specific cytotoxicity against viral antigens	
Increases INF-γ secretion	
Promotes Th1 response	
Initiates viral replication in resting T cells (could purge the system, diminishing reservoirs)	

In view of its properties, IL-7 is an immunotherapeutic candidate that brings hope to HIV patients. The unwanted effects noted, such as the induction of viral replication *in vitro*, would not be a problem in patients receiving antiretroviral therapy. Moreover, recent studies have demonstrated that IL-7 does not increase viral load *in vivo*. IL-7 immunotherapy could increase the CD4 count (by stimulating thymopoiesis and enhancing peripheral expansion) and promote a specific anti-HIV response in infected patients.

New technology called TREC quantification can help evaluate whether IL-7 is able to act during thymopoiesis. Here, T cell receptor rearrangement circles (TREC) were used to quantify thymic activity [57, 82]. TRECs are considered as markers of naive cells, and these disappear with the cell division subsequent to their activation. TREC values are therefore the result of both thymic production and the peripheral proliferation of T-cells. TREC values diminish with ageing as the thymus begins to atrophy [83]. If IL-7 injections enhance peripheral expansion without any effect on the thymus, the TREC value should diminish. By contrast, if the TREC value remains constant or increases, this indicates that IL-7 injections have a central renewal effect (increase in the number of naive cells, TRECs stability despite an intense peripheral activation). The preliminary tests conducted with IL-7 injections in nonhuman primates confirmed this hypothesis, with IL-7 significantly increasing the CD4 count in normal and in SIV-infected monkeys [84, 85]. The action of IL-7 on thymopoiesis in both healthy and SIV-infected animals [84] was clearly crucial, but its role in the diversity of the repertoire remains to be demonstrated.

However, in this matter, very few techniques are available to evaluate the diversity of the repertoire. ELISPOT assays examine the ability of CD4 or CD8 cells to secrete INF-γ in response to antigen-specific stimulation. The level of INF-γ secretion is correlated with the presence of specific anti-HIV clones. The use of peptides-HLA tetrameric complexes to stain antigen-specific CD8 T cells based on the ability of their TCR to interact specifically with a relevant peptide could also be a marker of cytotoxicity.

IL-7 and peripheral immunostimulation

In the peripheral immune system, IL-7 may be a crucial cytokine in the response to HIV infection: first by by acting in the determination of the Th1 profile during the infection by its capacity to increase both antigen-dependent proliferation and the specific cytolytic activity of CD8+ CTL against virus-infected cells, including against HIV-1 [42, 86]. Given the importance of cellular immunity in the

control of HIV infection, it is essential to consider the potential of a cytokine such as IL-7 which increases the antigen-specific response and T cell proliferation. This has considerable implications for HIV infection, where a decrease in CTL activity often corresponds with a deterioration in clinical condition. The use of a lymphokine such as IL-7 to increase or sustain a specific CTL response may be beneficial in combination with antiviral drugs, slowing disease progression. Moreover, many experiments have highlighted the potential of IL-7 to regulate the homeostatic proliferation of both naive and memory T-cells and its role in immune reconstitution in lymphopenic recipients [13, 39, 87-89]. Its profound stimulating and co-stimulating effects on T lymphocytes make it a logical candidate for clinical evaluation as a lymphoproliferative and antiviral agent.

Possible side effects of IL-7 immunotherapy

The first obvious negative action of IL-7 injection is its potential for direct and indirect induction of viral replication [67, 77] *(table 2)*. Secondly, by initiating the cycling of resting cells, IL-7 could increase the number of viral targets [68, 90].

A recent study [91] in healthy carrier Sooty Mangabeys (SMs) infected by SIV, raised the possibility that part of the explanation for their non-development of AIDS is that their immune system presents a Th2 pattern of cytokine production. This pattern may follow SIV infection and could be one of the components explaining their resistance to the disease. This observation could contraindicate IL-7, which would probably direct the immune response into a Th1 pattern when a Th2 pattern could be more beneficial [42].

Another undesirable effect is the possibility that the cytokine could actually potentiate progression of some leukemias or lymphomas, given that IL-7 transgenic mice develop lymphomas and thymomas [92, 93]. IL-7 injections could lead to autoimmunity since IL-7 transgenic mice develop severe dermatitis with alopecia or ulcerative colitis [94, 95]. However, the non-physiological levels of IL-7

in the bodies of these IL-7 transgenic mice may explain the toxicity observed. More recently, in an *in vivo* experiment of IL-7 injections used to reconstitute CD4-T cells after autologous transplantation, a monkey showed a GVHD-like disorder and prolonged diarrhea [96]. The IL-7 in this study was injected for a prolonged period (four weeks) at very high levels. In our view, these observations and the fact that the regulation of IL-7R is not well understood suggest that before IL-7 is used in a clinical trial, its dosing regimen should be thoroughly explored. It is clear that low but efficient levels of rhIL-7 should be administered over short periods. The possible side effects of IL-7 immunotherapy are reported in table 2.

Pre-clinical results

IL-7 plasma levels as a predictive marker of CD4+ T cell restoration during therapy

In the same manner as other research groups, we found higher plasma IL-7 levels in HIV patients naive to antiretrovital treatment and a decrease during HAART [61, 97]. Also, an inverse correlation was observed between circulating IL-7 levels in HIV patients and their CD4 count, suggesting that IL-7 plays a role in regulating homeostasis during HIV infection. We demonstrated a new, positive correlation between pre-HAART IL-7 levels and the CD4 restoration noted during HAART. Pre-HAART IL-7 levels in HIV-infected patients are predictive of the CD4 restoration after HAART. The higher the IL-7 levels, the more marked the CD4 restoration during HAART, suggesting that IL-7 levels may reflect the functional capacity of the lymphoid organs to restore the CD4 subset [61, 97]. These results suggest that IL-7 production is part of a homeostatic response to T-cell depletion and that IL-7 plays a role in homeostasis during HIV infection.

IL-7 role in CD4+ T cell restoration during IL-2 immunotherapy

Recent work in the laboratory has shown that IL-2 immunotherapy in CD4-LR patients increases the levels of circulating IL-7 (Beq S. *Interleukin-7 and FLT-3L plasma levels are increased during HAART-associated IL-2 therapy*. AIDS. In press). This observation raises the question of the role played by IL-7 in increasing the CD4 count during IL-2 immunotherapy. IL-7 may participate in increasing the CD4 count by inducing thymic production and output. In a recent clinical trial with IL-2, thymic involvement in naive T-cell recovery was suggested after quantifying TRECs [98]. In addition, the IL-7 increase may, in conjunction with IL-2, induce or extend the expansion and survival of peripheral CD4 T cells.

An *in vivo model of IL-7 immunotherapy*

In vivo models are currently under development to evaluate the effects of rhIL-7 injections on immune reconstitution in healthy or SIV-infected juvenile macaques. Preliminary results are encouraging as rhIL-7 has been seen to induce a substantial but reversible increase in total CD4 and CD8 T cell counts. The naive T-cell count was increased in both healthy and infected animals, suggesting that rhIL-7 is able to boost thymic production [84, 85]. An increase in T cell proliferation and activation was also observed [84]. Given the capacity of IL-7 to enhance HIV replication and potentially to increase the number of tar-

gets, its use in immunomodulating therapy could be a problem. However, *in vitro* data may not exactly reflect the effects *in vivo*. In a simian model, where IL-7 was used as an immunostimulant in SIV infection - thereby mimicking what could happen in humans - IL-7 injections did not modify the viral load in the blood, lymph nodes or thymus [84]. These results suggest that treatment with IL-7 in HIV disease will potently T-cell-replete, without increasing the viral load. These recent results are encouraging for the use of IL-7 as an immunotherapeutic agent to restore CD4 counts in HIV-infected patients.

CONCLUDING REMARKS

As demonstrated above, IL-7 is a crucial cytokine which can use both central and peripheral mechanisms to regulate homeostasis *(figure 2)*. IL-7 administration in HIV-positive patients might be beneficial in stimulating the recovery of the CD4 cell count, as suggested by preclinical data. The manner in which a single IL-7 injection might reconstitute the CD4 pool has not been elucidated, but both thymic and peripheral actions probably participate, as suggested by the initial results obtained in SIV-infected monkeys. One of the current challenges in the treatment of HIV-1 is to restore the immune response to the virus. IL-7, with its ability to restore both recall and naive responses after T-cell depletion, has the potential to be an excellent adjuvant in this setting. Moreover, IL-7 may be a critical factor in maintaining or inducing a CTL response against HIV thanks to its ability to enhance memory T-cell expansion and thus promote the Th1 response and increase specific CD8+ cytotoxicity against the virus. Its properties make it a logical candidate for clinical evaluation as an antiviral agent *(table 2)*. The role played by IL-7 in specific immunity remains to be assessed *in vivo*. Further studies are needed to assess the role played by this cytokine in HIV infection in combination with a better antiretroviral regimen. In order to remain as close as possible to the effect of such therapy in human beings, the IL-7 injection regimen in the macaque model should be similar to that proposed for use in humans.

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