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MINIREVIEWS

Immunological Effects of Interleukin-2 Therapy in Human Immunodeficiency Virus-Positive Subjects

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A variety of immune-based therapies are under consideration to improve immunological functions in human immunodeficiency virus (HIV)-infected patients. Whereas several therapeutic approaches have failed to improve immunological parameters or provide a clear clinical benefit, the use of interleukin-2 (IL-2)-based therapies has been supported by several in vitro and in vivo studies (23, 59). IL-2 is a cytokine that is produced by antigen or mitogen-activated T cells and that has been shown to exert a key role in the immune system. Production of IL-2 by these cells is the final event following various second-messenger intracellular pathways that integrate signals from surface receptors. In fact, antigen-induced T-lymphocyte proliferation is initiated via the engagement of the T-cell receptor complex that triggers IL-2 and IL-2 receptor production, and the following autocrine interaction of IL-2 and IL-2R allows T-cell proliferation to occur. This is permitted by a switch in T cells from the G_1 phase to proliferative phases of the cell cycle (60). In addition, IL-2 plays a complex immunoregulatory role by inducing activated cells to enter a preapoptotic phase, increasing the levels of production of proinflammatory cytokines, and influencing T-cell differentiation (8, 45, 60). These well-established in vitro effects of IL-2 have prompted the in vivo use of this cytokine as a therapeutic agent for the treatment of cancer patients (38, 54, 63). Several functions mediated by IL-2 suggest that it might be useful as an anticancer agent; IL-2 supports the growth of cytotoxic T cells, enhances the cytotoxicity of NK cells, and is essential for the induction of lymphokine-activated killer cells (38, 41, 63).

Although the precise mechanisms of its antitumor activity are not completely understood, it has been suggested that IL-2 may act through the activation of killer cells (41). On the basis of the results obtained in clinical trials involving cancer patients, it has been hypothesized that the enhancing activity of IL-2 could also be used to improve the functions of the immune system in HIV-infected subjects. The major goals of IL-2-based therapy for HIV disease have been different from those for anticancer therapy and can be summarized as follows: (i) a better control of HIV disease obtained by increasing CD4-lymphocyte counts and controlling the deleterious effects of HIV-induced cytokine dysregulation, (ii) the potentiation of the patient's native and acquired immunity against opportunistic microorganisms, and (iii) the reduction of the pool of cells latently infected with HIV by the activation of resting CD4 T lymphocytes.

IL-2 was initially administered through a continuous intravenous infusion, but the demonstration that this route of administration produced alterations in the clearance of antiretrovirals agents, together with the fact that subcutaneous injections of IL-2 resulted in adequate absorption and less severe side effects, provided the rationale to switch to this type of therapeutic modality. The pharmacokinetic profiles of recombinant cytokines are characterized by a nonlinear disposition and may be altered by several processes (47). For this reason, knowledge of the pharmacokinetics of IL-2 after its administration to HIV-positive subjects seems necessary for the proper design of immunotherapeutic regimens. Piscitelli et al. (48) have demonstrated that the concentrations of IL-2 in serum reach a peak within the first 24 h after administration and show a time-dependent decline over the following days. Those investigators suggested that this systemic clearance is likely to be the result of a receptor-mediated mechanism in which the decline of IL-2 concentrations may be related to IL-2 receptor overexpression as a consequence of the activation of the immune system.

The mechanisms underlying the immunological effects of IL-2 therapy have been widely studied in the last few years. The findings suggest that this cytokine acts through several, sometimes conflicting, mechanisms that are deeply influenced by the host immunological status, the concomitant antiretroviral therapy adopted, and the nature of the target cells. A general conclusion drawn from clinical trials is that the immunological effects measured in treated patients are similar, irrespective of the route of IL-2 administration chosen. In this minireview, I will therefore not make any distinctions between the data obtained by using the subcutaneous or intravenous regimen but will outline and discuss the major findings related to the immunological effects of IL-2 treatment.

IL-2 AND CD4 AND CD8 COUNTS

When antiretroviral therapies were still not available, pioneering studies used IL-2 as a therapeutic agent for AIDS patients (34, 65). In those studies the patients received IL-2 doses ranging from 2.5×10^2 to 2×10^6 units. Treated patients did not show either appreciable clinical responses or changes in immunological status after treatment. These results are not surprising, since it has been established by more recent studies that the association of IL-2 with an effective antiretroviral therapy is necessary to achieve biological and clinical benefits.

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TABLE 1. Therapeutic and immunological characteristics of relevant clinical trials including IL-2 for the treatment of HIV-positive patients

Investigators (reference)	Baseline CD4 count (no. of cells/mm^3)	Treatment	CD4 increase [no. of cells/mm^3 (%)] after:	
			ART[a]	IL-2 treatment
Simonelli et al. (58)	200–500	RTIs	+60	+256
Davey et al. (13)	>200	RTIs	ND	>200 (44), 0–200 (33)
Emery et al. (19)	100–500	RTIs	+153	+368
Levy et al. (36)	250–550	RTIs	+55	+564
Davey et al. (14)	200–500	HAART	+64	+384
Ruxrungtham et al. (55)	≥350	HAART	+42	+252
Abrams et al.[c]	≥300	HAART	+22	+276

[a] ART, antiretroviral therapy.
[b] ND, not determined.
[c] Addendum Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. L11, p. 24, 2000.

In 1991, Schwartz et al. (57) treated patients with IL-2 doses ranging from 1.5×10^6 to 12×10^6 IU/m^2 in the presence of zidovudine and demonstrated a significant improvement in CD4 counts in nine patients available for long-term follow-up. A few years later, a greater interest in the therapeutic use of IL-2 was aroused by an article by Kovacs et al. (31) that appeared in the *New England Journal of Medicine*. That paper described a sharp increase in CD4 counts with a concomitant stable number of CD8 cells in 25 patients treated with IL-2, suggesting that IL-2 may permit the preferential recovery from the HIV-dependent CD4 T-cell depletion. Randomized, controlled studies conducted in many countries have provided definite evidence of a significant recovery of CD4 counts after IL-2 administration (14, 16, 19, 36, 55). The type of treatment and the immunological improvements obtained in some relevant clinical trials with IL-2 are reported in Table 1. The net increase in the number of CD4 T cells compared to the increase obtained by use of antiretroviral agents alone was remarkable, although some data suggested that compared to the effects obtained when reverse transcriptase inhibitors (RTIs) were used, the synergistic effects of IL-2 were less evident when highly active antiretroviral therapy (HAART) was used (14, 16). Until recently, it was not clear whether the increase in CD4 counts may translate into a clinical benefit in the cohort of patients studied. Emery et al. (19) have recently proposed a preliminary answer to this question. They analyzed data from three randomized, controlled trials of IL-2 therapy. Their analysis of the pooled data revealed a nonsignificant, but clinically relevant, 43% reduction in the risk of disease progression and death in patients receiving IL-2 compared to the risk for those who were randomized to receive antiretroviral therapy alone. Moreover, they proposed that the differences observed in the study originated from the long-term preservation of HIV-specific, CD4-cell-mediated immune function in the IL-2 group.

Although the effects of IL-2 in patients with CD4 counts <200/mm^3 have been widely investigated, little information is available for patients with advanced HIV disease. In a French study (C. Katlama, C. Duvivier, and C. Choquet, 7th Eur. Conf. Clin. Aspects Treatment HIV Infect., abstr. 1205, 1999), IL-2 was given to patients with <1,000 copies of HIV RNA/ml and with CD4 counts in the range of 50 to 200 cells/mm^3. The median change in CD4 counts after 24 weeks were +65 cells/mm^3 in the IL-2 group and +18 cells/mm^3 in the control group, providing encouraging data for the use of IL-2 also in patients with reduced baseline CD4 counts and an advanced stage of disease. Several studies also investigated the effects of IL-2 therapy on CD8 counts. There is general consensus about the capacity of IL-2 to increase CD8 counts and the expression of perforin and granzyme B (two enzymes contained in the granules of cytotoxic cells) only during cytokine administration, while prolonged observations clarified that CD8 counts have the tendency to diminish with time (58, 68, 70).

IL-2 AND CD4 AND CD8 SUBSETS IN PERIPHERAL BLOOD AND LT

The lymphoid tissue (LT) is a major reservoir of virus in patients with HIV infection and is a major site of virus-immune system interactions. LT and peripheral blood differ in their cellular compositions because LT hosts a greater proportion of naive cells and its lymphocytes have increased expressions of adhesion and activation molecules. This peculiarity may deeply influence the interactions between the virus and the cells present in LT (24). The immunological differences between the lymphoid and the peripheral blood compartments are further evidenced by the observations that there is a reduction in the proportion of CD4$^+$ T cells in the peripheral blood compared to the proportion in LT in patients with advanced HIV disease. Moreover, considering the greater reduction of CD4 counts observed in the peripheral blood, the total number of CD4 T cells in LT is higher than expected (24). Recent investigations focused on the effects of HAART on CD4-lymphocyte trafficking and on the subsequent variations in CD4 counts in peripheral blood. A conclusion drawn from those studies was that the initial increase in CD4 T-cell numbers observed in HAART-treated patients after 3 to 4 weeks may depend upon a redistribution from the LT to the peripheral blood (44). Later, a net proliferation is predominant, resulting in newly produced CD4 T cells in the peripheral blood as well. IL-2 treatment may profoundly affect the redistribution-proliferation model mentioned above by altering cellular proliferation and the expression of adhesion molecules and by changing the microambient situation through the production of immunoregulatory cytokines and other soluble factors. The immunological changes induced by IL-2 in the peripheral blood compared to those observed in the lymphoid system were analyzed in a recent study (68). Nasopharyngeal tissue has been chosen for determination of the cellular immune composition of the host LT because it is considered a T-cell organ involved in cellular responses rather than a B-cell-specific area (32). For

TABLE 2. Relevant results of clinical trials investigating immunological functions in IL-2 treated, HIV-positive patients[a]

Reference	Cytokine production	Recall antigen response	HIV-specific response	Apoptosis	Thymic function
De Paoli et al. (15)	IL-2 ↑, IL-4 ↑				
Blanco et al. (5)	Chemok. =				
Kelleher et al. (30)		TT =, SS =	Gag =, Pol =		
Levy et al. (36)		PPD ↑	CTL =		
Caggiari et al. (9)				AICD =	
De Paoli et al. (submitted)					TRECs =

[a] Symbols and abbreviations: =, not modified; ↑, increased; AICD, activation-dependent cellular death; TT, tetanus toxoid; SS, streptokinase-streptodornase; PPD, purified protein derivative; Chemok., chemokine; CTL, cytotoxic T lymphocyte.

this reason, the nasopharyngeal tissue could more appropriately reflect the interactions of HIV type 1 (HIV-1) with the lymphoid immune system. My findings suggest that the use of IL-2 plus HAART increased after 24 weeks the percentage of the naive ($CD26^+$) CD4-cell subset in the peripheral blood of treated patients, confirming my previous observations of the selective and precocious activity of IL-2 on the naive-cell subpopulation. The results of immunophenotypic analysis of the nasopharyngeal lymphoid system mirrored the results obtained with peripheral blood, suggesting that IL-2 produced an overall expansion of naive cells (68). In view of the inner resistance of naive cells to productive HIV infection (67), the ability of IL-2 to expand a naive CD4 population that is probably not infected with HIV may contribute to a delay in HIV disease progression.

In general, T-cell activation is reflected by increased levels of production of membrane CD25 and soluble CD25 (sCD25) as a result of stimulation by IL-2. For this reason, although the IL-2 receptor is a complex of three distinct polypeptide chains, the CD25-related markers have been considered surrogates for IL-2 (20, 59). For clinical purposes, it was therefore essential to establish whether the amount of surface CD25 or sCD25 expression could be used to predict the IL-2 reactivity and the IL-2-dependent increase in CD4 cell numbers in IL-2-treated, HIV-infected subjects. With this aim, colleagues and I performed two studies; the first one included IL-2, and two RTIs and the second one included IL-2 and HAART (16, 58). Irrespective of the type of treatment, an acute up-regulation of surface CD25 and sCD25 in response to each cycle of IL-2 injection was measured, demonstrating the persistence of cellular responsiveness to IL-2 after repeated exposures and providing the rationale for planning multiple treatment cycles in HIV-positive patients (58). The fact that the clinical trial with RTIs showed a relatively more evident increase in the percentage of CD4 cells compared to that in the HAART study (58), while the absolute numbers of CD4 and CD25 cells and the serum sCD25 levels were similar in the two studies, brought us to the conclusion that the two parameters may not accurately reflect the IL-2-dependent increase in the percentage of CD4 cells.

An immunophenotypic study conducted with the $CD8^+$ subset in the peripheral blood and in the LT of HIV-positive patients did not show appreciable variations in the expression of CD38 compared to that after HAART alone after 24 weeks of IL-2 therapy (68). The proportions and absolute numbers of CD8 and $CD28^+$ lymphocytes, a subset that has been suggested to play a role in the control of HIV replication through a noncytotoxic response (35), were also not modified after treatment (68).

The effects produced by IL-2 treatment also include improvements to selected immunological functions, like chemokine production, cytokine production, lymphocyte proliferation, and de novo generation of cells of the immune system. The most relevant results concerning these immunological functions are discussed in the following paragraphs and are summarized in Table 2.

IL-2 AND CHEMOKINE RECEPTOR EXPRESSION AND CHEMOKINE PRODUCTION

Chemokine receptors play a key role in the immunopathology of HIV infection by acting as coreceptors of HIV-1 entry into target cells (18). In vitro studies have demonstrated that IL-2 increased CXCR-4 and CCR-5 expression on CD4 T cells, while it down-modulates CCR-5 expression in monocytes (6, 33). For this reason, it has been suggested that IL-2 may modify the pool of HIV-1 target cells by triggering the expression of chemokine receptors. In vivo, IL-2-treated patients showed increased levels of CXCR-4 expression in the CD4 T-cell pool, while no modifications were seen in the cells from the monocytic lineage (5). The increased levels of CXCR-4 expression by CD4 T cells might favor the in vivo replication of the more pathogenic T-cell-tropic isolates of HIV-1, but the limited extent of this increase and the strong control of HIV replication exerted by HAART made this possibility unlikely (5). In conclusion, the data presently available generate doubts about the existence of evident IL-2 effects that favor the in vivo spread of HIV.

Chemokines selectively block in vitro HIV infection, and several in vivo studies have demonstrated an association between higher levels of chemokine production from activated peripheral blood lymphocytes and a more favorable clinical status in HIV-positive individuals (53, 62). Taken together, these results suggest that an enhancement of chemokine production by therapeutic regimens could be of additional benefit for patients. Because IL-2 administration did not show that chemokine levels had appreciable effects in vivo, chemokine receptor up-regulation and cellular chemokine production are probably independently regulated in treated patients (5, 15).

IL-2 AND CYTOKINE PRODUCTION

The immunopathological processes consequent to HIV infection cause a dysregulation in the cytokine network. These alterations contribute to increased levels of viral replication and to a decreased ability of the immune system to mount an appropriate immune response (12). In particular, HIV-in-

fected subjects have a reduced capacity to produce immunoregulatory cytokines, such as IL-2, IL-12, and gamma interferon (IFN-γ), indicating a defect in the Th1 limb of the immune response (10, 66). Whether the Th1 defect is associated with a shift to a Th2 pattern or, rather, to a Th0 state is still a matter of controversy (12, 52). Intracellular cytokine detection has been used not only to measure the degree of immunosuppression or immune system dysregulation (39, 64) but also to investigate the differentiation status of CD4 cells. This technique allowed establishment of the fact that unprimed lymphocytes produce IL-2 but not IFN-γ, while the opposite profile (i.e., no production of IL-2 but production of IFN-γ) characterized fully differentiated CD4 cells (46, 61). The measurement of the levels of cytokine production in culture supernatants and in cells may provide complementary information, allowing establishment of the degrees of immune recovery and cellular differentiation during treatments for HIV infection. Aiming to define the effects of IL-2 on cytokine production and on cellular differentiation, colleagues and I analyzed these parameters in patients treated with IL-2 and RTIs. This trial suggested that while a suboptimal antiretroviral therapy did not restore cytokine production, appreciable increases in the levels of IL-2, IL-4, and IFN-γ production in vitro were obtained in IL-2-treated subjects. The reconstitution of CD4 counts in IL-2-treated patients was very rapid, while the effects of therapy on cytokine production were slow but were persistent for up to 24 weeks (15). It was very difficult to reconcile these data with the simple notion that a Th1-Th2 switch parallels the progression of HIV infection. In fact, HIV-positive patients showed a nonselective pattern of reduced levels of cytokine production before therapy; moreover, because of the influence of IL-2 on cellular growth and survival, it is difficult to establish the importance of IL-2 itself in the priming of T lymphocytes to develop into Th1 or Th2 cells (60). In a second trial, colleagues and I investigated cytokine production and cellular differentiation in patients treated with IL-2 in association with HAART (16, 68). The use of HAART alone moderately restored cytokine production in treated patients, while the contemporary administration of IL-2 in conjunction with HAART temporarily depressed the number of IL-2-producing cells and the total level of IL-2 production, possibly because of a feedback inhibition by IL-2 infusion. Since HAART suppresses HIV viremia to below detectable levels, it may also be possible that the consequent reduction in the level of antigenic stimulation lowers the level of sustained CD4 T-cell activation, making these cells less prone to engagement with IL-2.

As mentioned above, the therapeutic use of IL-2 is associated with a preferential expansion of CD4 cells expressing CD25, the alpha chain of the IL-2 receptor. $CD4^+$ and $CD25^+$ cells express high levels of activation molecules and genes and contain, in patients with untreated infection, HIV molecular forms different from those present in $CD4^+$ and CD25-negative cells (7, 51). It is therefore important to investigate accurately the effects of IL-2 treatment on cells expressing the IL-2 receptor and compare them to those exerted on the CD25-negative $CD4^+$ counterpart. Our data suggest that IL-2 alone inhibits the differentiation of the $CD25^+$ cells, while it strongly activates the proliferation of this subset. On the contrary, IL-2 effectively helps the differentiation of resting (i.e., CD25-negative) CD4 T cells, but the IL-2-dependent effect on the proliferation of this subset is minimal (L. Caggiari et al., submitted for publication).

IL-2 AND T-CELL PROLIFERATION

A reproducible finding of IL-2 therapy is the significant increase in CD4/CD8 ratios compared with those for patients treated with antiretroviral agents alone (13, 16, 22). A recent trial confirmed that this effect on T-cell homeostasis is actually dependent on IL-2 (3), but no explanation for the mechanism(s) involved in this finding has been produced. Colleagues and I therefore postulated that a more pronounced rate of proliferation of the $CD4^+$ subset compared to that of the reciprocal $CD8^+$ subset could be involved in increased CD4/CD8 ratios during IL-2 treatment. The lymphocyte proliferation rate in IL-2-treated patients was investigated by measuring the expression of Ki67, an antigen present in cells in the late G_1, S, G_2, and M phases of the cell cycle but not in cells in the G_0 phase. Longitudinal measurements of Ki67 positivity in CD4 and CD8 T lymphocytes of HIV-positive patients showed that IL-2 rapidly stimulated CD4 proliferation, while CD8 proliferation was minimally affected. The selective expansion of the CD4 subset was significantly correlated with a sustained increase in CD4/CD8 ratios (Caggiari et al., submitted). We also confirmed the previous observation demonstrating that HAART alone suppressed both CD4 and CD8 T-cell proliferation (27). Although T-cell recovery in HIV-positive subjects results from several combined mechanisms, our data suggest that CD4 proliferation is predominant in the early CD4 rise during IL-2 treatment. On the contrary, high levels of T-cell stimulation produced with OKT3 and IL-2 cause proliferation mostly of the CD8 T cells and reinforce the notion that coregulation, i.e., a unique density-dependent mechanism regulating CD4 and CD8 T cells at the same time, is a key to understanding the effects of immune activation treatments (22).

IL-2 AND ANTIGEN-SPECIFIC IMMUNE RESPONSE

The increased susceptibility to opportunistic infections in patients with HIV disease results from the loss of memory CD4 T cells. Ideally, an optimal therapeutic approach should be able not only to increase CD4 T-cell numbers but also to restore T-cell reactivity against microbial antigens. Previous studies have shown that HAART produced a partial recovery of CD4 T-cell reactivity against recall antigens in vitro, although flow cytometric data suggest that the HIV-specific response declines with the use of antiretroviral therapy (37, 43, 49). The effects of IL-2 on the specific response to recall antigens were investigated in two different clinical trials. In the first one, the lymphoproliferative response to streptokinase and streptodomase, tetanus toxoid, and HIV Gag and Env antigens was measured. That study did not demonstrate improvements in lymphoproliferative responses after 48 weeks of intravenous or subcutaneous IL-2 administration (30). On the contrary, the proliferative responses to tetanus toxoid, candidin, and tubercolin were increased in frequency and magnitude in IL-2-treated French patients (36). The researchers also investigated cytotoxic T-lymphocyte activity specific to HIV-1 antigens by a chromium release assay using as targets autolo-

gous lymphoblastoid cell lines expressing HIV-1 proteins. Cytotoxic lymphocyte activity was detectable in 19 of 25 patients at the baseline and disappeared at week 30 in all the patients in the IL-2 group, while it was detectable in a proportion of the subjects in the control group. Taken together, the results suggest that IL-2 has a limited potential to preserve or potentiate antigen-specific responses in patients with HIV disease. However, the conclusions drawn from the available literature are far from being definitive because the in vivo immune response network is very complex and difficult to dissect and the available in vitro methods used to investigate memory cell functions are not completely adequate and may not entirely reflect the in vivo situation.

IL-2 AND CD4 LYMPHOCYTE APOPTOSIS

Apoptosis, a programmed cell death, constitutes an important mechanism of CD4 T-cell depletion in HIV infection (2). In particular, due to its correlation with the viral burden in plasma, spontaneous apoptosis may contribute to the advance of HIV disease (56). Activation-induced cell death may also contribute to the pathogenesis of AIDS, and cytokines are key regulators of this process (2, 11). Initial studies of apoptosis in patients with HIV disease focused mainly on the ability of HAART to influence the functions of the immune system and came to the conclusion that the level of peripheral blood lymphocyte apoptosis was reduced in treated patients (29, 42). In vitro experiments suggested that increased levels of apoptosis of HIV-infected cells were related to a down-regulation of Bcl-2 expression and that the addition of IL-2 reduced the down-modulation of Bcl-2, thus resulting in increased cellular survival (1). For this reason, colleagues and I investigated the in vivo effects of therapy with IL-2 on spontaneous and activation-induced cell death in HIV-positive patients. Our findings suggest that the additional use of IL-2 did not significantly influence apoptosis, but HAART itself reduced the percentages of CD4 cells undergoing spontaneous and activation-induced cell death after 4 weeks of therapy. The kinetics of lymphocyte apoptosis reduction was slower in CD8 lymphocytes than in CD4 cells since a significant reduction in the level of CD8 apoptosis required at least 24 weeks of therapy (9).

IL-2 AND THYMIC FUNCTION

The thymus is essential for T-cell development not only during childhood but also during adulthood. In fact, it is well known that adults also retain some ability to generate T cells (50). The ability to measure the function of the thymus during physiological as well as pathological conditions is therefore an essential tool for the monitoring of immunopathological processes and therapeutic interventions. Computer-assisted tomography detected a correlation between the thymus size and the number of naive T lymphocytes present in the peripheral blood (26, 40). Therefore, the levels of these cells have been considered markers of thymic function. More recently, it was shown that during intrathymic stages, T-cell precursors undergo rearrangement of the αβ T-cell receptor, resulting in the formation of T-cell receptor rearrangement excision circles (TRECs) that remain stable for a few divisions after T-cell migration from the thymus to the peripheral blood. For this reason, the concentration of TRECs in the peripheral blood can be exploited to assess the levels of recent thymic emigrants, and accordingly, it quantitatively estimates thymic function.

In patients with HIV infection, critical questions are whether the virus affects thymic output and whether this impairment could be reversed by current or innovative therapies. Douek et al. (17) showed that a substantial thymic output was also maintained in healthy subjects into late adulthood and that HIV infection diminished TREC values in untreated patients. Zhang et al. (69) suggested that effective antiretroviral therapy was able to increase TREC levels only in those patients whose baseline TREC values were significantly lower than those in controls. Finally, Hatzakis et al. (25) have found that the concentration of TRECs in the peripheral pool of T cells complemented the HIV-1 RNA load and T-cell counts in predicting the rate of HIV disease progression. Those investigators also concluded that recent thymic emigrants possibly have a role in the pathogenesis of HIV disease. The increases in CD4 counts induced by IL-2 treatment will be of clinical importance for patients, depending on the mechanisms by which this cytokine works. In particular, the increase in naive T-cell levels should be caused by increased thymic output rather than peripheral expansion of preexisting CD4 cells or a redistribution of cells from the lymphoid tissue. Since the effects of IL-2 on thymic functions were completely unknown, colleagues and I investigated T-cell regeneration in IL-2-treated, HIV-positive patients by an immunophenotypic assay that monitored $CD4^+$ naive T cells and by analysis of thymic function through the quantification of the excision DNA products of T-cell receptor rearrangement (TRECs) in lymphocytes (P. De Paoli et al., submitted for publication). The IL-2 combination produced a marked increase in the number of $CD4^+$ T cells bearing a naive phenotype ($CD45RA^+$ $CD62L^+$), which was apparent for over 96 weeks after therapy. To assess whether these cells were the product of improved T-cell generation, colleagues and I exploited a competitive-quantitative molecular biology-based assay to quantify TRECs in peripheral blood lymphocytes, finding that the levels of these molecules were unchanged in these patients. A recent paper provided experimental data which suggest that the occurrence of an elevated T-cell division rate during IL-2 therapy could obscure the interpretation of TREC data (28). As far as this aspect is concerned, we have evidenced that an increased rate of T-lymphocyte proliferation could affect TREC content only during the first 2 to 4 weeks of IL-2 administration (De Paoli et al., submitted), while it was no longer observed after 24 to 96 weeks of treatment. We have therefore concluded that improved thymic function does not account for the early rise in CD4 naive cell numbers in HIV-positive patients treated with IL-2 and that alternative mechanisms of T-cell maturation and differentiation are responsible for this event.

IL-2 AND AUTOIMMUNITY

The systemic administration of IL-2 has been reported to induce thyroid dysfunction as well as thyroid autoantibodies in cancer patients undergoing cytokine therapy (4, 21). Although a complete clinical and laboratory evaluation has not yet been performed, there is still no evidence regarding the occurrence

of autoimmune phenomena in IL-2 treated, HIV-positive patients.

CONCLUSIONS

Therapy with IL-2 has been widely used in patients with HIV disease, and its limited toxicity and its effects on CD4 and CD8 counts are well established at this time. IL-2 would be of substantial benefit if it could also improve the clinical conditions of the patients. The most important findings obtained by previous and ongoing trials with IL-2 can be summarized as follows: IL-2 must not be used alone but must always be used in association with standard antiretroviral therapy; IL-2 produces a stable increase in CD4 counts in the peripheral blood and in the lymphoid organs; very potent immunoactivating regimens, including IL-2 and anti-CD3 T-cell stimulation, do not increase the immunological benefits of IL-2 but, rather, produce deleterious effects on cell-mediated immunity; the effects of IL-2 are due to the preferential stimulation of CD4 T-cell proliferation, resulting also in increased CD4/CD8 ratios at the end of therapy; and IL-2 has a positive effect on some immunological functions (memory response, cytokine production), while other functions are unaffected (thymic output, HIV-specific cytolytic activity).

Even though many data on the immunological effects of IL-2 have accrued in the last few years, some questions remain open and some areas require further work: the most effective route of IL-2 administration is not yet clearly defined; clinical or immunological parameters that allow one to establish very rapidly the degree of individual response to IL-2 are still missing; current protocols limit the activation of resting CD4 T cells, reducing the possibility to purge the latent HIV reservoir; and the long-term clinical efficacy of IL-2 administration must be further studied.

Future investigations on the biological and clinical aspects of IL-2 therapy should follow two directions. The first direction must take into consideration a detailed description and optimization of the activity of IL-2 on immune functions. In light of this consideration, one can hypothesize many situations in which increasing immune reactivity could be beneficial. The second one regards the possibility of increasing the specificity of IL-2 stimulation, i.e., by directing the cytokine against specific cellular targets like resting CD4 T cells harboring latent HIV.

The collection of new experimental and clinical data will be relevant for the definition of future appropriate protocols of immunointervention.

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