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Changes in the levels of some acute-phase proteins in human immunodeficiency virus-1 infected patients, following interleukin-2 treatment

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Summary

Intermittent interleukin (IL)-2 administration to human immunodeficiency virus (HIV)-1 infected patients is well documented and generally used, but there is limited information about the changes of acute-phase protein (APP) levels in response to this treatment. Fifteen patients undergoing highly active anti-retroviral therapy (HAART) treatment, with undetectable viral load, but low CD4$^+$ cell count (<300/μl), have been treated with 3·6 M IU Proleukine® administered twice daily by subcutaneous injection over 5 days. C-reactive protein (CRP), D-dimer, C3, C9, C1-inh and alpha-2HS glycoprotein levels were measured immediately before IL-2 administration, as well as on day 5 and 2–3 weeks thereafter. After IL-2 administration, both mean D-dimer and CRP levels increased significantly ($P < 0{\cdot}001$), but returned ($P < 0{\cdot}001$) to baseline within the subsequent 2–3 weeks. Alpha-2HS glycoprotein decreased immediately after IL-2 administration. No significant differences were detected in the levels of C3, C9 and C1-inh. A significant, positive correlation ($r = 0{\cdot}5178$, $P = 0{\cdot}0008$) was ascertained between the changes of CRP level, measured immediately before as well as 5 days after IL-2 administration, and changes in CD4 T cell counts measured 2–3 weeks before and after treatment, respectively. IL-2 administration induces rapid elevation of two major APPs (CRP, D-dimer). The positive correlation observed between the changes of CRP levels and CD4$^+$ cell counts after IL-2 administration may indicate that the abrupt, but transitory overproduction of CRP might contribute to the CD4$^+$ cell count-increasing effect of the drug and/ or may be associated with serious side effects.

Keywords: acute-phase protein, HIV infection, interleukin-2

Introduction

Interleukin (IL)-2 is a cytokine secreted by activated T cells *in vivo*. IL-2 can regulate the proliferation, differentiation and survival of lymphocytes [1]. Exogenous IL-2 has been used as immunotherapy in oncology and in human immunodeficiency virus (HIV)-infected patients. In clinical trials, high-dose recombinant IL-2 is used to stop the decline of CD4$^+$ T cell count in HIV-infected patients [2–6]. According to the latest data, the increase of CD4$^+$ cell count accomplished by combined treatment with IL-2 and highly active anti-retroviral therapy (HAART) does not confer clinical benefit, but may induce grade 4 clinical side effects in patients with advanced, chronic HIV infection [7,8].

Because the causes of these side effects and the exact mechanism whereby treatment with IL-2 increases CD4$^+$ T cell count are both unknown, studies into these questions seem to be warranted.

One of the pathological effects of IL-2 administration could be its action on other cytokines, including the proinflammatory cytokines IL-6, tumour necrosis factor (TNF)-α and IL-1β [9–11]. These cytokines are responsible for enhanced (or diminished) synthesis of acute-phase proteins (APPs) during the acute phase of inflammation and in chronic diseases [12,13].

Acute-phase proteins are a special type of proteins, the serum levels of which change significantly during inflammation, sudden tissue destruction, in acute and chronic

infections – this is known as the 'acute-phase reaction'. The serum concentrations of positive APPs [e.g. C-reactive protein (CRP), fibrinogen] rise, whereas those of negative APPs (e.g. albumin, alpha-2 HS glycoprotein) decrease during the acute-phase reaction [14,15]. Certain complement proteins [e.g. C3, C9, C1-inhibitor (C1-inh)] are regarded as positive APPs. In HIV-1 infected patients undergoing HAART, the concentrations of positive APPs are higher than in uninfected healthy controls, whereas there are no differences in the serum concentrations of negative APPs [16].

The most important APP is CRP. Lower levels of CRP have been shown to predict longer survival in HIV-infected individuals [17,18]. Moreover, as permanent HIV infection is not a highly inflammatory disease, the level of CRP was found to be relatively low (median <4 mg/l) [18]. A significant increase in CRP level may induce cardiovascular complications [19]. Furthermore, CRP levels have been observed to correlate inversely with $CD4^+$ lymphocyte counts and directly with HIV RNA levels [18,20].

When measuring fibrinogen levels, its degradation product D-dimer is often used as a surrogate. There are conflicting literature data on the changes of fibrinogen or D-dimer levels in HIV-infected patients. Wolf *et al.* [21] found decreased D-dimer levels in HIV^+ patients after the initiation of protease inhibitor (PI) or non-nucleoside reverse transcriptase inhibitors (NNRTI) treatment. Madden *et al.* [22], by contrast, reported that treatment with a PI was associated with elevated fibrinogen levels, whereas NNRTI administration did not result in any increase. An elevated D-dimer concentration identifies HIV-infected patients at a high risk of thrombotic thrombocytopenic purpura, cardiovascular events and death [23–25].

Several APPs (such as C3, C9, C1-inh) belong to the complement system. Measurement of their serum concentrations may provide additional information. For example, Senaldi *et al.* [26] showed that HIV infection is correlated *in vivo* with high levels of complement activation, as shown by both a decrease of serum concentration of complement proteins and an increase of activation fragments in the blood. In addition, complement and anti-HIV antibodies increase HIV replication through a mechanism of enhanced entry [27,28].

Although no correlation between increase of alpha-2 HS glycoprotein and HIV infection was observed [29], measuring this negative APP seems to be reasonable due to its increased sensitivity [30]. Until recently, no data have been available on the effect of IL-2 treatment on APPs. Porter *et al.* [31] were the first to report, in a paper published online in August 2009, the significant increase of CRP and D-dimer levels in two cohorts of HIV patients. Here, we present our investigation into the changes of six important APPs (CRP, D-dimer, C3, C9, C1-inh, alpha-2 HS glycoprotein) following IL-2 treatment of HIV-infected patients.

Methods

Patients and IL-2 treatment

Fifteen patients undergoing HAART, with undetectable viral load and low $CD4^+$ T cell count (fewer than 300/μl blood), were studied. The relevant data of study subjects are summarized in Tables 1 and 2. Patients treated with HAART received recombinant human IL-2 (Proleukine®) 3·6 million international units by subcutaneous injection, twice daily, for 5 days (which is considered one treatment cycle). Six patients underwent several cycles of IL-2 treatment; three of these patients received at least three cycles. The protocol of this study was approved by the local ethics board and all subjects contributed written informed consent.

Clinical specimens

Blood samples were drawn at three different times: immediately before IL-2 administration (day 0), on day 5 at the end of the cycle and 2–3 weeks thereafter. Blood samples for D-dimer assay were collected in citrate-containing tubes. Blood samples for CRP and complement measurements were allowed to clot and the serum was tested within 2 h or was stored frozen at −20°C until assayed. Blood samples [one tube, anti-coagulated with K_3-ethylenediamine tetraacetic acid (EDTA)] were collected 2–3 weeks before and after IL-2 treatment for viral load determination and $CD4^+$ T cell analysis.

Measurement of acute-phase proteins

The CRP and D-dimer concentrations were measured by immunoturbidimetry on an Olympus system. Serum concentrations of C1-inh, C9, C3 and alpha2-HS glycoprotein were measured by the radial immunodiffusion method [32], using anti-human C1-inh, C9 (Quidel, San Diego, CA, USA), C3 and alpha2-HS glycoprotein (Dako, Glostrup, Denmark). Pooled human serum of healthy blood donors was used to establish the reference levels of C1-inh, C3 and alpha2-HS glycoprotein. C9 concentrations were expressed as the percentages of those measured in the above-mentioned standard human serum.

Measurement $CD4^+$ T cell counts and HIV viral load

Determination of $CD4^+$ T cell subsets was performed as described previously [33]. We performed the haematological test with Sysmex XE-2100 (Sysmex Corporation, Kobe, Japan) and immunophenotyping with fluorescence activated cell sorter (FACS) Canto II (BD Immunocytometry Systems, San Jose, CA, USA). Plasma HIV RNA concentrations were determined using Cobas TaqMan® polymerase chain reaction (Roche Magyarország Kft, Diagnosztika Divizió, Budaörs, Hungary).

Table 1. Characteristics of patients and design of the study.

Patients' ID	Gender	Age	Other infection	Date of examined IL-2 administration	Number of IL-2 administration before the examined IL-2 administration	Absolute CD4+ T cell numbers (cell/μl) 2–3 weeks before the examined IL-2 administration
VG	Male	27	HCV	19 September 2008	–	161
				19 November 2008	1	180
PT	Male	36	–	01 July 2008	–	258
VZ	Male	39	CMV	03 September 2008	–	138
MA	Male	30	–	30 April 2008	–	157
SK	Female	56	*Mycobacterium avium*	13 February 2008	–	74
				30 April 2008	1	100
ML	Male	43	HBV	25 January 2008	–	78
				21 March 2008	1	98
				13 June 2008	2	148
PR	Female	39	–	19 February 2008	2	136
BP	Male	42	*Cryptosporidium*	02 January 2008	3	106
				07 March 2008	4	114
				30 May 2008	5	124
				29 August 2008	6	138
				21 November 2008	7	185
ON	Male	35	–	04 September 2008	5	286
KA	Male	41	–	30 November 2007	4	111
				04 April 2008	5	105
				12 June 2008	6	161
FA	Female	37	–	31 March 2008	4	119
BM	Male	39	–	05 August 2008	6	299
SS	Male	40	*Pneumocystis*	11 February 2009	–	210
KP	Male	39	*Mycobacterium tuberculosis*	20 October 2008	–	135
				27 December 2008	1	158
KI	Male	63	*Mycobacterium tuberculosis*	19 December 2008	–	161

CMV, cytomegalovirus; HBV, hepatitis B virus; HCV, hepatitis C virus; IL, interleukin.

Statistical analysis

Statistical analysis was performed with Prism for Windows version 5 (GraphPad Software, San Diego, CA, USA) statistical software. We used the Friedman test for repeated measures analysis of variance (using Dunn's paired *post-hoc* test), Wilcoxon's matched-pairs test to compare paired values into the same group and Spearman's rho to calculate correlations.

Results

The effect of IL-2 therapy on absolute CD4+ T cell numbers

Absolute numbers of CD4+ T cells were determined 2–3 weeks before, and after the IL-2 treatment cycle. On average, the number of CD4+ T cells increased significantly ($P = 0{\cdot}001$) from a baseline count of 74-286-90-563 (Fig. 1a). In patients who received more cycles of IL-2 treatment, the elevation of CD4+ T cell numbers persisted for a longer period, during which intense fluctuations (patients KA, ML) or steady but small increases (patients BP, SK, KP, VG) were ascertained (Fig. 1b).

Changes of APPs during IL-2 administration

D-dimer. On day 0, the average D-dimer level of IL-2 treated patients was below 0·5 μg/ml and exceeded 1 μg/ml in just a single patient. There was a significant ($P < 0{\cdot}001$) increase after the conclusion of the IL-2 treatment cycle (by day 5). In four cases D-dimer concentration increased more than 10-fold, and reached a level of 3·44–3·97 μg/ml on day 5. There were no differences between these patients and the remaining patients either in the number of IL-2 cycles or the baseline CD4+ cell counts.

A significant ($P < 0{\cdot}001$) decrease was observed 2–3 weeks after treatment, and except for three cases D-dimer concentrations returned to baseline level (Fig. 2).

CRP. Mean baseline CRP level was 2·5 μg/ml (range: 1–10 μg/ml), except in two patients infected with

Table 2. The total time of follow-up for patients.

Patients' ID	Date of identification of HIV infection	Date of first HAART	Date of first virological success HAART	Date of IL-2 administration
VG	July 1990	September 1997	October 1997	September 2008 November 2008
PT	February 2005	May 2007	September 2007	July 2008
VZ	October 2007	October 2007	July 2008	September 2008
MA	October 2007	October 2007	January 2008	April 2008
SK	October 2007	October 2007	February 2008	February 2008 April 2008
ML	February 2007	February 2007	August 2007	January 2008 March 2008 June 2008
PR	February 2006	June 2006	January 2007	July 2007 September 2007 February 2008
BP	October 2006	November 2006	December 2006	June 2007 October 2007 January 2008 March 2008 May 2008 August 2008 November 2008
ON	December 2002	December 2002	January 2003	February 2003 May 2003 July 2003 November 2003 February 2004 September 2008
KA	June 2006	June 2006	July 2006	August 2006 November 2006 March 2007 June 2007 November 2007 April 2008 June 2008
FA	February 2004	February 2004	April 2004	June 2004 July 2004 October 2004 December 2004 March 2008
BM	February 2001	February 2001	April 2001	October 2003 December 2003 February 2004 April 2004 June 2004 August 2004 December 2004 December 2005 August 2007 August 2008
SS	March 2008	March 2008	October 2008	February 2009
KP	February 1998	February 1998	October 1998	October 2008 December 2008
KI	February 2003	February 2003	August 2003	December 2008

HAART, highly active anti-retroviral therapy; HIV, human immunodeficiency virus; IL, interleukin.

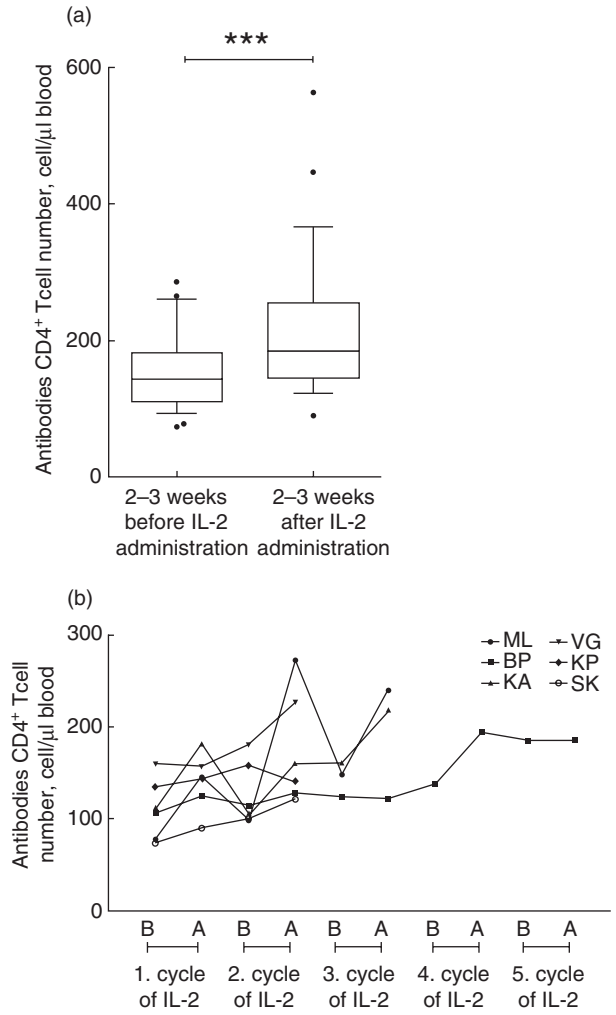

Fig. 1. (a) Changes of absolute CD4+ T cell numbers 2–3 weeks before and after treatment. ***$P < 0{\cdot}001$. (b) Changes of absolute CD4+ T cell numbers 2–3 weeks before (B) and after (A) treatment, in patients who received more interleukin-2 cycles.

Mycobacterium avium (78·5 μg/ml) or *Cryptosporidium* (28·4 μg/ml). After IL-2 administration, a significant elevation of CRP level, by two orders of magnitude, was seen on day 5, although some interindividual differences occurred. A significant decrease ($P < 0{\cdot}001$) of CRP level was observed 2–3 weeks after IL-2 administration (Fig. 3). In the two patients infected with *M. avium* or *Cryptosporidium* a decline of CRP level was observed following a limited increase (mean change of CRP was 27 μg/ml in the *M. avium*-infected and 43 μg/ml in the *Cryptosporidium*-infected patients). In the three patients who had received at least three cycles of IL-2 treatment, the pattern of CRP changes was identical during all cycles (Fig. 3b).

C9, C3, C1-inh. No significant changes in the serum levels of these complement-associated APPs were observed (Fig. 4a–c).

Alpha-2-HS. The concentration of this negative APP changed inversely to that of CRP and the D-dimer; it decreased significantly after IL-2 administration and returned to baseline level 2–3 weeks after treatment (Fig. 4d).

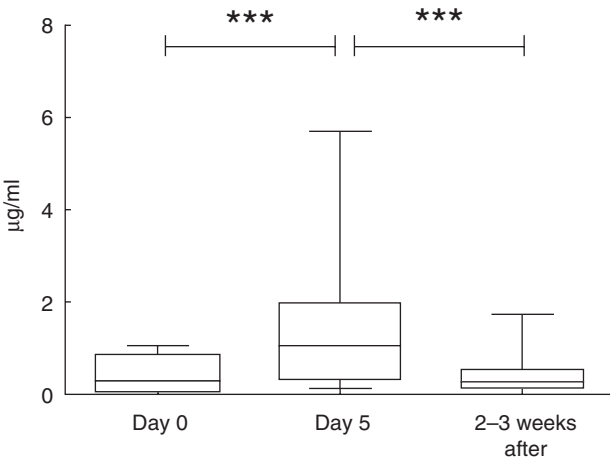

Fig. 2. Changes of D-dimer levels following interleukin-2 treatment. D-dimer levels increased sharply from day 0 to day 5 and decreased after the discontinuation treatment. Presented values are means. ***$P < 0{\cdot}001$.

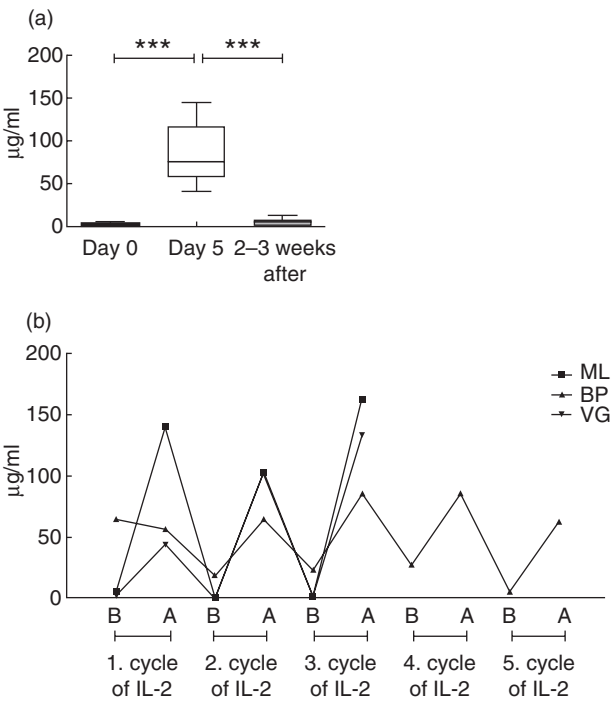

Fig. 3. (a) Changes of C-reactive protein (CRP) level during interleukin-2 treatment of human immunodeficiency virus-infected patients (with the exception of patients co-infected by Pneumocystis and Cryptosporidium). Presented values are means. ***$P < 0{\cdot}001$. (b) Changes of CRP concentration immediately before (B) and after (A) treatment, in patients who received at least three cycles.

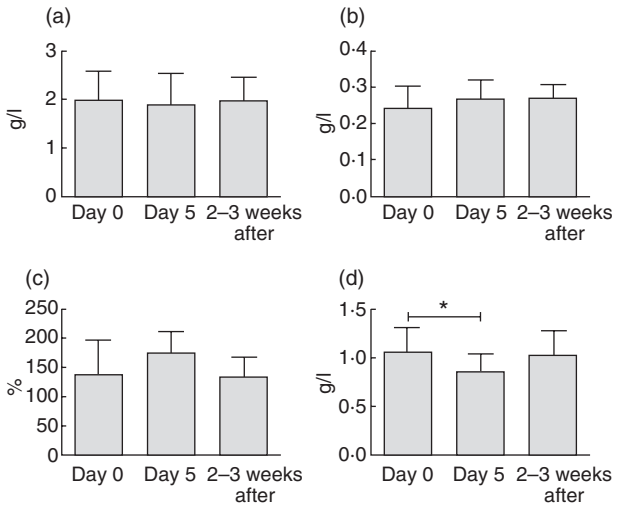

Fig. 4. Changes in the levels of (a) C3, (b) C1-inh, (c) C9 and (d) alpha-2HS glycoprotein during follow-up. Presented values are means. $^*P < 0{\cdot}05$.

Correlation between the changes of CRP level and of absolute CD4$^+$ T cell count

Changes of absolute CD4$^+$ T cell numbers determined 2–3 weeks before and after IL-2 treatment and changes of CRP concentrations between day 0 and day 5 were calculated. A significant, positive correlation was found between the changes of absolute CD4$^+$ T cell counts and CRP levels ($r = 0{\cdot}5178$, $P = 0{\cdot}0080$; Fig. 5), whereas no significant correlation was detected with the changes of other APPs (data not shown).

Discussion

The HIV-infected individuals displaying poor immune reconstitution to ongoing long-term HAART, despite full HIV viraemia suppression, might be ideal candidates to adjunct IL-2, and several controlled studies have been conducted to investigate IL-2 in these cohorts, but only one examined the changes of APPs (CRP and D-dimer) in these patients.

Our findings are in complete agreement with the very recently published findings from Porter *et al.* [31]. However, we have made some novel observations in addition. First, a significant, positive correlation was found between the extent of the elevation in CRP level during the 5 days of IL-2 administration, on one hand, and the changes of CD4$^+$ T cell count occurring after IL-2 treatment on the other hand. Second, investigating the effect of IL-2 on the serum levels of other APPs, we have observed interesting differences. While the levels of a negative APP (alpha2-HS) decreased after IL-2 treatment (similar to the changes of CRP and D-dimer levels), no significant differences occurred in the serum levels of other three APPs (C3, C9 and C1-inh) belonging to the complement system. The failure of IL-2 to increase C1-inh concentration is an apparently important observation, as the administration of C1-inhibitor is known to attenuate the vascular leakage syndrome induced by high doses of IL-2 [34,35].

These findings may have some relevance for the mechanism of action and the effectiveness of IL-2 treatment in patients with HIV infection or cancer. In line with the recent results from Porter *et al.* [31], our present findings obtained in a large patient population indicate that the levels of the most important APP – CRP – are elevated and reach a concentration about 200 times higher than the baseline value. Elevated serum CRP levels can cause adverse effects. Volanakis [36] reported that high CRP concentration occurring directly after treatment with high doses of IL-2 can lead to the fixation of C1 and C4 complexed to cell surfaces. Complement fragments bound to their cellular receptors may effect the function of these cells and may possibly contribute to the side effects of the IL-2 therapy. The latest data [8] suggest a causal relationship between IL-2 therapy and cardiovascular events. As proposed by Kuller *et al.* [24], it is possible that these adverse events are associated with the temporary elevation of CRP and other serum proteins. Elevated D-dimer levels predict cardiovascular complications. Interestingly, Baars *et al.* [13] studied neoplastic patients and their results indicated that IL-2 activates blood coagulation and fibrinolysis, even in doses which do not cause serious side effects.

Conversely, our novel observation on the positive correlation between the extent of IL-2-induced elevation and the changes of CD4$^+$ cell count (if this proves reproducible in a larger cohort of patients) may indicate that the transient, but abrupt, increase in CRP levels may contribute to the IL-2-induced increase of CD4$^+$ cell count.

Our patient's therapy was successful virologically; the plasma HIV RNA level was under detection limit. No signifi-

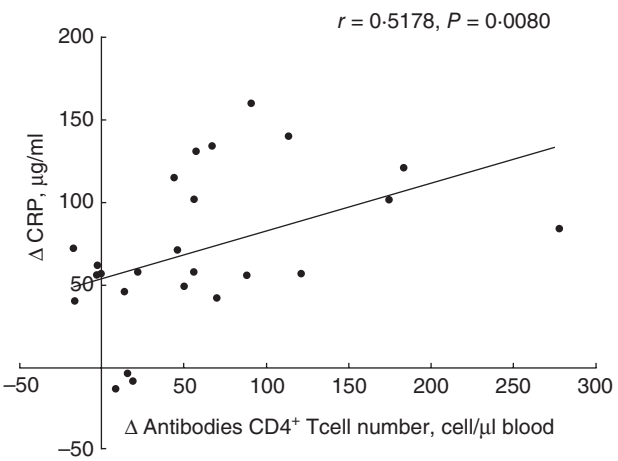

Fig. 5. Correlation between the differences in serum C-reactive protein levels and absolute CD4$^+$ T cell numbers measured 2–3 weeks before and after interleukin-2 treatment.

cant viral load changes have been observed following IL-2 treatment, in line with more recent studies [37–39].

To conclude, in accord with the recent findings from Porter *et al.* [31], our results suggest that the administration of high doses of IL-2 to HIV-infected patients induces an acute-phase reaction of rapid onset. These processes can underlie both the mechanism of the IL-2-induced increase of $CD4^+$ cell count and the cardiovascular side effects of IL-2 treatment.

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Disclosure

None.

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