IL-21 level in Chinese HIV infected individuals and its dynamics undergoing HAART

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Abstract: **Objective** To investigate the dynamics of interleukin-21 (IL-21) cytokine in the Chinese HIV patients undergoing highly active antiretroviral therapy (HAAPT). **Methods** A total of 25 adults with chronic HIV infections, responding to combined highly active antiretroviral therapy (HAART) guideline criteria were enrolled for a 1-year follow-up. After signing an informed consent, 20 mL blood was collected from each patient at the base line, 6 month and 12 month, respectively. CD4 and CD8 cell count was quantified by flux cytometry, serum HIV RNA quantified by real time PCR and IL-21 concentrations by ELISA. **Results** IL-21 levels increased gradually during the follow-up but did not reach the healthy levels. IL-21 correlated positively with the CD4 cells but not with CD8 T cells. HIV RNA correlated negatively with CD4 cell count but did not show any relationship with the CD8 cells. **Conclusion** IL-21 has potential role in the immunopathogenesis of HIV, and might be an important factor in immune construction during HAART.

Key words: human immunodeficiency virus; highly active antiretroviral therapy; interleukin-21; CD4 cell; CD8 cell; HIV RNA

中国 HIV 感染者的 IL-21 水平及其在高效抗反转录病毒治疗中的动态变化

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[**摘要**] **目的**:观察在接受抗 HIV 治疗的中国 HIV 感染者队列中血白细胞介素 21(IL-21)的动态变化。**方法**:将符合高效抗反转录病毒治疗(HAART)指南标准的 25 例慢性 HIV 成年感染者纳入研究,在启动 HAART 的 0,6,12 个月时各抽取感染者的 20 mL 血液。运用流式细胞仪进行 $CD4^+$ T 细胞和 $CD8^+$ T 细胞计数,以 RT-PCR 检测 HIV 的 RNA 水平,ELISA 法测定 IL-21 的水平。**结果**:中国 HIV 感染者的 IL-21 水平低于正常人,在接受 HAART 治疗过程中逐渐升高,但未达到正常人水平。IL-21 的水平和 $CD4^+$ T 细胞数呈正相

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关,但与 CD8⁺T 细胞数无关;HIV RNA 的水平与 CD4⁺T 细胞数呈负相关,但与 CD8⁺T 细胞数无关。**结论:** IL-21 与 HIV 免疫致病机制有一定关系,并在抗反转录病毒治疗的免疫重建中起重要作用。

[**关键词**] 人类免疫缺陷病毒; 高效抗反转录病毒治疗; 白介素 21; CD4 细胞; CD8 细胞; HIV RNA

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Interleukin-21 (IL-21) is a relatively newly discovered immune-enhancing cytokine that plays an essential role in controlling chronic viral infections. It is produced mainly by $CD4^+$ T cells, which are also the main targets of HIV-1 and are often depleted in HIV-infected individuals. IL-21 stimulates secretion of interferon-γ (IFN-γ) by NK and T cells, and induces their proliferation and cytotoxic activity [1-3]. In general, IL-21 appears to primarily modulate the function of mature lymphocytes. For CD8 T cells, IL-21 has little effect on in vitro T cell proliferation, but can synergize with IL-15 or IL-7[4-5]. IL-21 has been reported to have biphasic effects, for example, at low doses it promotes, but a high dose inhibits NK cell proliferation [6]. $CD4^+$ T cells stimulated via their T cell receipter (TCR) are main producers of IL-21, and TCR stimulation increases the expression of IL-21R in $CD4^+$ T cell, giving IL-21 an autocrine role in $CD4^+$ T cell responses[7-8]. In addition to B cells, $CD8^+$ T cells are the primary responders to IL-21. $CD8^+$ T cells also increase their expression of the IL-21R in response to TCR stimulation, showing that IL-21 primarily affects activated $CD8^+$ T cells. Alone, IL-21 does not induce significant proliferation of $CD8^+$ T cells, but in response to antigen-independent stimulation IL-21 costimulates proliferation and expansion together with IL-7 or IL-15 of both naive and activated $CD8^+$ T cells. Taken together, IL-21 co-stimulates antigen-dependent and -independent proliferation, expansion, survival, and cytotoxicity of $CD8^+$ T cells. Furthermore, IL-21 maintains $CD8^+$ T cell expression of CD28 and increases their IFN-γ and IL-2 production, creating a more robust and independent $CD8^+$ T cell response[9-10]. In HIV-infected subjects, the functions of NK cells such as cytotoxicity and production of IFN-γ are augmented with cytokines, e.g. IFN-α and common γ-chain family cytokines[11-13]. For all these reasons we were interested in investigating the production of this cytokine in HIV-infected individuals. In order to address this issue, we measured IL-21 in the sera of 25 adult HIV-infected patients before initiating highly active antiretroviral therapy (HAART) and during a 1-year follow-up after HAART initiation, and compared the sera with that from age-matched HIV-seronegative healthy subjects.

1 MATERIAL AND METHODS

1.1 Study population

This study was conducted from December 2009 to November 2010 and included 25 adults with chronic HIV infection (Tab. 1) and 10 age-matched healthy donors. The patients were included based on the HIV-1/2 diagnosis confirmation, the absence of highly active antiretroviral therapy (HAART) initiation, the absence of obvious opportunistic diseases infection and the absence of pregnancy and childbearing experience for adult women. Patients did not receive any medications known to affect immune functions. Twenty milliliter of blood were collected from the HIV patients and 10 mL from the healthy donors. All the patients had one or more AIDS-defining conditions and were not receiving HAART at the time of enrollment. All participants signed the informed consent approved by the Institutional Review Board of Central South University. This study was approved by the Second Xiangya Hospital Ethics Committees of Central South University.

1.2 IL-21 concentrations

The concentration of IL-21 was determined in plasma from healthy donors and HIV infected individuals using human interleukin ELISA kit test provided by 4A Biotech Co. Ltd. following the manufacturer's instructions. The lowest test sensitivity was 7 pg/mL.

1.3 Quantification of $CD4^+$ and $CD8^+$ T lymphocytes

Blood circulating absolute CD3, CD4 and CD8 cell counts were determined at month 0, 6 and 12 and were expressed as cells/μL. Cells were labeled with fluorescently conjugated anti-CD3, CD4, CD8 and analyzed on a FACS Calibur instrument (Becton Dickinson, USA). Quantification of viral RNA was performed using the QIAamp viral RNA mini kit (Qiagen, USA) according to the manufacturer's protocol.

Tab. 1 Summary of patient characteristics at the onset of HAART

Indexes	Data
Number of cases	25
Age/years	37.7(21-59)
Gender	
Male	23(92%)
Females	2 (8%)
HIV WHO stage	
I-II	20 (80%)
III-IV	5 (20%)
Duration of infection /years	3-5
Antecedents of OIs	No
$CD4^+$ count /(cells/μL)	195(65-328)
$CD8^+$ count /(cells/μL)	558 (397-700)
≥200 cells/μL/No. (%)	13 (52%)
50-200 cells/μL/No. (%)	12 (48%)
Mean IL-21 /(pg/mL)	70.25
HIV RNA / $\log_{10}$ copies/mL	4.01(2.08-9.85)
HAART regimen	
AZT + 3TC + NVP (A3N)	22(88%)
AZT + 3TC + EFV (A3E)	2(8%)
d4T + 3TC + EFV (34N)	1(4%)

1.4 Statistical analysis

Data were carried out with SPSS 18.0 software (SSPS Inc, Chicago, IL) and expressed as mean ± standard deviation ($\bar{x} \pm s$). Comparisons between variables were performed using one-way analysis of variance or paired samples t test. Association between continuous variables was tested using a non-parametric Spearman rank correlation test. $P < 0.05$ was considered as statistical significance.

2 RESULTS

After HAART initiation, all the parameters except the RNA load increased significatively during the whole time of the follow-up (Tab. 2). The CD4 cell count increased above 500 cells/μL in patients whose baseline CD4 cell count was above 200 cells/μL but was below 500 cells/μL in patients whose baseline CD4 count was under 200 cells/μL. The viral load decreased drastically within the first 6 month and was below the lowest detectable level of our machine (400 copies/mL). The CD4 cell count positively correlated with the IL-21 (Fig. 1). The levels of IL-21 increased gradually during the follow-up but did not reach the health controls levels. The increase of the IL-21 positively correlated with the CD4 cell count during all the follow-up (Fig. 2). The relationship between the increase of CD4 count and the type of HAART was not found. The Tab. 2 summarizes the evolution of biological patterns after HAART initiation.

Tab. 2 Evolution of the different immune parameters after HAART initiation

Times	CD4 count/(cells/μL)	CD8 count/(cells/μL)	HIV RNA/$\log_{10}$(copies/mL)	IL-21/(pg/mL)
Month 0	211 ± 103.09	1055.9 ± 83.80	4.21 ± 1.7	72.56 ± 37.43
Month 6	355.47 ± 105.93	847.47 ± 67.68	2.69 ± 1.84	134.9 ± 55.86
Month 12	530.76 ± 117.02	583 ± 115.09	1.68 ± 1.66	180.52 ± 54.15
P	≤0.001	≤0.001	≤0.008	≤0.002

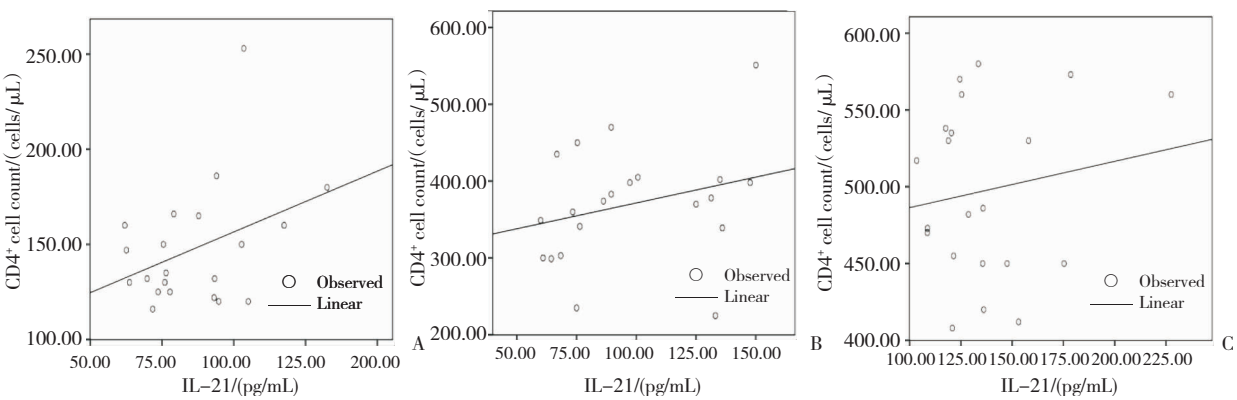

Fig. 1 Relationships between IL-21 with CD4 count during HAART. A: Relationship between IL-21 and CD4 at month 0 ($r = 0.085$); B: Positive correlation between IL-21 and CD4 cells at month 6 ($r = 0.028$); C: Positive correlation between CD4 cells and IL-21 at month 12 ($r = 0.019$).

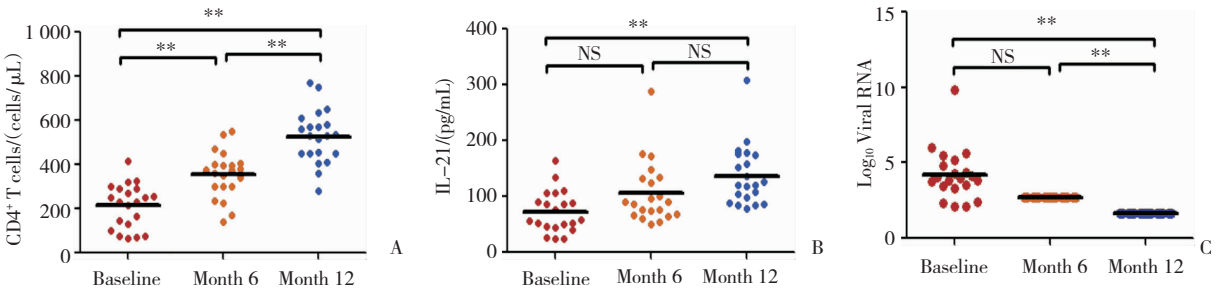

Fig. 2 Dynamic of CD4 count, IL-21 level and HIV RNA level undergoing HAART. Compared with each other, * * $P<0.01$; NS: no significance.

3 DISCUSSION

Infection with HIV-1 induces a progressive deterioration of the immune system that ultimately leads to acquired immune deficiency syndrome (AIDS). γ-chain(γC) cytokines in general have proven potential function for immunotherapy. IL-21 is a relatively newly discovered immune-enhancing γC cytokine that plays an essential role in controlling chronic viral infections. In our study, patients did show neither opportunistic infection nor IRIS development during all the follow up. Therefore, the variation of the immune paterns is supposed to be exclusively due to HIV infection. At baseline, patients were generally characterized by a low CD4, high CD8 cell count, a low IL-21 levels and associated with a high viral load. However, we could distinguish these patients 2 into subgroups: those with CD4 count less than 200 cells/μL, high CD8 count above low IL-21 levels, and those with CD4 count below 200 cells/μL, relatively low CD8 count and high IL-21 levels. The low levels of IL-21 observed in these patients are related to the decrease of the CD4 cell count since the later are the main IL-21 producer cells. Innello et al. have also observed that the IL-21 production is compromised early in the course of the infection and that its serum concentrations correlate with the $CD4^+$ T cell counts in the infected persons [14]. Furthermore, it has been shown that the HIV infection of human $CD4^+$ T cells inhibits the cytokine production and that highly active antiretroviral therapy only partially restores its production[3]. Other researches[15-16] have observed greater circulating IL-21-producing $CD4^+$ T cells in HIV-infected individuals compared with uninfected volunteers. Moreover, the HIV-specific IL-21-producing $CD4^+$ T cells were detected in blood during untreated acute and chronic HIV infection, and that elevated frequencies of these cells correlated with relative viral control. These cells had an effector memory or end effector phenotype and expressed CXCR5. However, Low or aviremic long-term nonprogressors, showed absent or low HIV-specific IL-21 $CD4^+$ T cells. It is also observed that HIV-specific $CD8^+$ T cells exhibited high levels of IL-21R, indicating sensitivity to IL-21. Thus, it suggested that IL-21-producing $CD4^+$ T cells are induced in viremic HIV infection and likely contribute to viral control by affecting $CD8^+$ T cell maintenance[15]. After HAART initiation, we observed a progress increase of the CD4 and the IL-21 levels associated with a progressive decrease of the viral load. Our results showed a negative relationship between the viral load and the CD4 T cells and a positive relationship between the IL-21 and the CD4 cells. The hallmark of HIV infection is the rapid loss of the CD4 T cells leading to generalized immune dysfunction, luckily, the HAART initiation partially restores these adverse symptoms. The increase of the CD4 cells is consecutive to the decrease of the viral load, and the increase of the CD4 cells induces the increase of the IL21 which in return enhances the function of the CD8 T cells and the NK cells. The function of these cells contributes to the production of the granulysin and perforin, two biological factors which play key roles in the control of the viral infection. Investigating the control of the viral infection in elite controllers, Williams et al.[16] found that IL-21 production during HIV-1 infection is closely associated with enhanced CD8 T cell function, allowing improved viral control and that IL-21-producing HIV-1-specific CD4 T cells were the best indicator of functional CD8 T cells. As it appears here, the decrease of the IL-21-CD4 producer cells has great impact on the viral control. HAART not only partially restore the number of total CD4 but also restore partially their cytotoxicity which in return lead to a long control of the viral infection. As a consequence

viral infection, it has been noticed a functional defects in cytotoxic $CD8^+$ T cell responses in chronic human viral infections but the mechanisms involved are not well understood. In mice for exemple, it has been shown that CD4 cell-mediated IL-21 production is necessary for the maintenance of $CD8^+$ T cell function and control of persistent viral infections. Investigation the potential role of IL-21 in a chronic human viral infection, Chevalier et al. [17] found that HIV-specific triggering of IL-21 by $CD4^+$ T cells was significantly enriched in elite controlers, while isolated loss of IL-21-secreting $CD4^+$ T cells was characteristic for subjects with persistent viremia and progressive disease. IL-21 responses were mediated by recognition of discrete epitopes largely in the Gag protein, and expansion of IL-21$^+$ $CD4^+$ T cells in acute infection resulted in lower viral set points. Moreover, IL-21 production by $CD4^+$ T cells of HIV controllers enhanced perforin production by HIV-1-specific $CD8^+$ T cells from chronic progressors even in late stages of disease, and HIV-1-specific effector $CD8^+$ T cells showed an enhanced ability to efficiently inhibit viral replication in vitro after IL-21 binding. One critical element of $CD8^+$ T-cell effector function and differentiation is the T-box transcription factor T-bet. Hersperger et al. assessed T-bet expression, together with the effector proteins perforin, granzyme A, granzyme B, and granulysin, in HIV-specific $CD8^+$ T cells from elite controllers (EC), chronically-infected progressors, and HAART-suppressed individuals. When compared to the other cohort groups, HIV-specific $CD8^+$ T cells among EC demonstrated a superior ability to express perforin and granzyme B but with no detectable difference in the levels of granzyme A or granulysin. Notably, they observed higher levels of T-bet in HIV-specific $CD8^+$ T cells from EC, with an ensuing positive correlation between T-bet and levels of both perforin and granzyme B. Moreover, HIV-specific $CD8^+$ T cells in EC upregulated T-bet to a greater extent than progressors after in vitro expansion with concomitant upregulation of perforin and granzyme B. Collectively, these results suggest that T-bet may be playing an important role in driving effector function and that IL-21 may be involved in the expression of this transcriptor[18]. Others studies have also shown that beside the CD8 T cells, IL-21 could induce an increase expression of the perforin in NK 56 dim and in association with IL-15, it induce significative perforin expression on NK56 bright[19]. Investigating how exogenous IL-21 enhances NK cell responses in HIV-infected persons, Iannello et al. [20] showed that the cytokine receptors are expressed equally on all NK cell subsets defined by expression of CD16 and CD56. In these cells, IL-21 activates STAT-3, MAPK, and Akt to enhance NK cell functions; increases expression of antiapoptotic proteins Bcl-2 and Bcl-X(L) and enhances viability of NK cells but has no effect on their proliferation. The cytokine also enhances HIV-specific ADCC, secretory, and cytotoxic functions, as well as viability of NK cells from HIV-infected persons; it exerts its biological effects on NK cells with minimal stimulation of HIV-1 replication; and the cytokine-activated NK cells inhibit viral replication in cocultured, HIV-infected, autologous $CD4^+$ T cells in a perforin- and LFA-1-dependent manner. Researches on γC cytokines and AIDS have been conducted for years abroad, but still rare data are reported in this field in Chinese HIV infected individuals. Due to differences in ethnicity, countries or regions, and differences in epidemic virus subtypes might have somewhat similary or different exhibition [21-22]. Thus, it is of scientific and practical values that analysis of relations betweem with γC cytokines and HIV infection in China to search better way for control HIV-AIDS.

This was the first study conducted on the role of IL-21 in viral control in chinese HIV infected individuals. In summary our data show Il-21 is highly depleted during HIV infection but HAART initiation restores partially the levels of IL-21. Though the increase of the IL-21 concentration showed no correlation with the viral load or the CD8 cell count, these 3 patterns seem to be closely linked. Investigation on large cohort studies might reveal more significative results. Cytokine immunotherapy is being evaluated as adjunct treatment in infectious diseases. Combination treatment with antiretroviral therapy (ART) and IL-21 may induce sustained increases of circulating CD4 and NKT and their effector functions in HIV infected individuals.

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