Journal of AIDS and HIV Research Vol. 3(1), pp. 6-10, January 2011
Available online http:// academicjournals.org/JAHR
ISSN 2141-2359 ©2011 Academic Journals

Full Length Research Paper

Upregulation of interleukin-27 expression is correlated with higher CD4+ T cell counts in treatment of naive human immunodeficiency virus-infected Chinese

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Accepted 6 January, 2011

Human immunodeficiency virus (HIV) infection is a major global health problem and causes a huge number of deaths each year. Interleukin-27 (IL-27), which is composed of Epstein Barr virus-induced gene 3 (EBI3) protein and p28 protein, inhibits HIV replication *in vitro*. However, the status of IL27 and its relationship with CD4+ T cell counts *in vivo* in treatment-naïve HIV infected individuals has not yet investigated. We recruited 108 healthy and 120 HIV-infected but treatment-naive Chinese individuals to participate this study in the last two years. We determined the IL-27 titers in all the participants by using enzyme-linked immunosorbent assay (ELISA), and measured the CD4+ T cell counts in HIV-infected individuals by using fluorescence activated cell sorting (FACS) assays. We showed that IL-27 titers were significantly elevated in HIV-infected individuals as compared to HIV negative healthy controls (612 ± 355 pg/ml vs 413 ± 230 pg/ml; *P* < 0.001). We also showed a significant positive correlation between plasma IL-27 titers and $CD4^+$ T cell counts (*r* = 0.206, *P* = 0.024) in HIV-infected individuals. These findings suggest that the elevated plasma IL-27 levels may play important role in slowing down the CD4+ T cell declining in HIV-infected individuals and the process of AIDS disease.

Key words: Human immunodeficiency virus (HIV) infection, interleukin-27, $CD4^+$ T cell counts.

INTRODUCTION

Human immunodeficiency virus (HIV), a member of the genus Lentivirus, causes progressive impairment of the immune system which leads to acquired immunodeficiency syndrome (AIDS). Interleukin-27 (IL-27) is a heterodimeric cytokine composed of EBI3 protein and p28 protein and belongs to the IL-6/IL-12 family of type I cytokines (Pflanz et al., 2002). IL-27 is mainly secreted by antigen-presenting cells in response to inflammatory stimuli (Pflanz et al., 2002). IL-27 not only induces naïve $CD4^+$ T cell proliferation, but also promotes polarization of naïve $CD4^+$ T cells into Th1 phenotype and IFN-γ production, which are mediated through its functional signal-transducing receptor composed of gp130 and orphan cytokine receptor WSX-1/TCCR (Pflanz et al., 2002; Pflanz et al., 2004).

Shimizu et al. (2006) have demonstrated that IL-27 also possess antiangiogenic and antitumor activities via $CD8^+$ T cells. IL-27 inhibits X4 and R5 HIV replication in peripheral blood mononuclear cells (PBMC), $CD4^+$ T cells and MDMs through the induction of type I interferon and activation of multiple interferon-inducible genes (Fakruddin et al., 2007; Greenwell-Wild et al., 2009; Imamichi et al., 2008). HBV infected patients showed significantly elevated IL-27 levels and serum IL-27 levels correlate with HBV e antigen (Zhu et al., 2009). However, the status of IL-27 levels in HIV-infected individuals has not yet been reported.

In this study, we assessed the correlation of HIV infection and IL-27 titer in a Chinese population as well

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as the relationship of plasma IL-27 levels and $CD4^+$ T cell counts in HIV-infected treatment naïve individuals.

MATERIALS AND METHODS

Study participants

A Chinese population was recruited from an ongoing HIV/AIDS surveillance study in Shenzhen, an immigrant city in Guangdong province, from September 2007 to July 2009. The study participants were recruited by voluntary-based HIV/AIDS screening program. Written consent was obtained from each participant following an interview by experienced research staff. Blood samples were tested for HIV, HBV and HCV infections. Confirmed HIV positive participants were referred to on-going HIV prevention, treatment, and care programs. HBV and HCV infected participants were referred to local hospitals for treatments. All infections were recorded following the requirements of reportable infectious diseases.

The study protocol was approved by Shenzhen Center for Disease Control and Prevention (Shenzhen CDC) and the Chinese University of Hong Kong.

HIV, HBV and HCV measures

Whole blood specimens were collected from all subjects into EDTA-containing tubes (BD Biosciences, San Jose, CA). Plasma was stored at -80°C until analysis. Plasma was screened for HIV antibody according to standard procedures of Shenzhen CDC laboratory. In brief, samples were tested using ABBOTT PRISM HIV O Plus (Detect anti-HIV-1 group O and M, anti-HIV-2, Abbott Laboratories, IL) and ELISA (Beijing Wantai Biological Pharmacy Enterprise CO., LTD, Beijing) for HIV infection screening, positive samples were then confirmed by HIV-1/-2 western blot assay (HIV Blot 2.2 WB; Genelabs Diagnostics, Singapore).

HBsAg positivity was determined by ELISA kit (Beijing Wantai Biological Pharmacy Enterprise CO., LTD, Beijing) according to manufacturer's protocol. HCV antibody was tested by ELISA kit (Beijing Wantai Biological Pharmacy Enterprise CO., LTD, Beijing) according to manufacture's instructions.

CD4 counting

$CD4^+$ T cell count of HIV-positive individuals was assessed by three-color fluorescence kit for CD4/CD8/CD3 (BD Biosciences, San Jose, CA) counting according to the manufacturer's instructions. Fresh whole blood was analyzed on FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) within 8 h. Multiset software was used for CD4/CD8/CD3 analysis.

Detection of IL-27 in plasma

IL-27 titer in plasma was measured with LEGEND MAX™ Human IL-27 ELISA Kit (BioLegend Inc., San Diego, CA) according to manufacturer's instructions. Standard curve was generated by using serial diluted recombinant human IL-27 with known concentrations. The minimum detectable concentration of the ELISA kit is 11 pg/ml.

Statistical analysis

Data were analyzed with SPSS version 13.0 (SPSS Inc., Chicago, IL, USA). Independent-samples *T* test was used for comparison of plasma IL-27 between HIV-infected group and healthy control group. Pearson's correlation coefficient was used to evaluate the correlation between IL-27 titers and $CD4^+$ T cell counts in HIV positive group. P value < 0.05 was considered as statistically significant.

RESULTS

Age and gender of participants

After HBsAg positive and HCV positive individuals were excluded, 228 antiretroviral therapy-naïve Chinese were tested for IL-27 concentration. Among them, 108 participants were HIV negative and 120 participants were HIV positive. The average ages were 27.6+5.7 years and 29.7+6.2 years for healthy control group and HIV positive group, respectively. There were 84 males and 24 females in the control group and 89 males and 31 females in the HIV-infected group. There were no statistical differences of ages and gender between the two groups.

Upregulation of IL-27 levels in HIV-infected persons

IL-27 levels were among 27-945 pg/ml in the control group and 131-1892 pg/ml in HIV-positive group. Compared to healthy controls, IL-27 level was significantly higher in HIV-infected individuals (612 ± 355 vs. 413 ± 230 pg/ml; $P < 0.001$; Figure 1).

Correlation of plasma IL-27 titers and $CD4^+$ T cell counts

$CD4^+$ T cell count in HIV positive individuals was determined by flow cytometer and analyzed with plasma IL-27 titer by Pearson's correlation coefficient. As illustrated in Figure 2, a significant positive correlation between $CD4^+$ T cell counts and plasma IL-27 titers was observed ($r = 0.206$, $P = 0.024$; Pearson's Correlation).

DISCUSSION

HIV infection is a global challenge for public health. However, how human body responds to HIV infection and the consequences of host response to disease progression are not well understood. Recently, it was reported that IL-27 inhibited HIV replication in CD4+ T cells and macrophage and stimulated the proliferation of CD4+ T cell and macrophages (Fakruddin et al., 2007) *in vitro*. It is elusive if HIV triggers IL-27 response in human bodies *in vivo*. In this study, we recruited treatment-naive HIV infected individuals to address this question. In the past two decades, China has experienced a remarkable increase in HIV infection along with other sexual

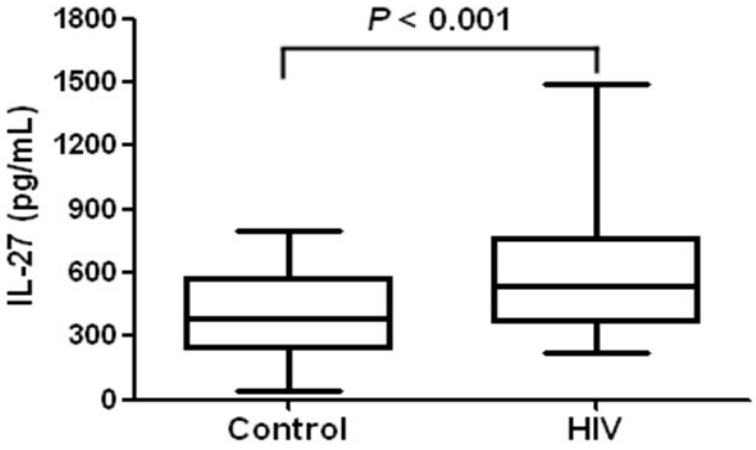

Figure 1. IL-27 titer in HIV-infected persons and healthy controls. Plasma IL-27 concentration was measured in 120 HIV-infected individuals and 108 healthy controls by ELISA. Box-plot illustrated the medians with 25 and 75%, error bars indicate 5 and 95% percentiles.

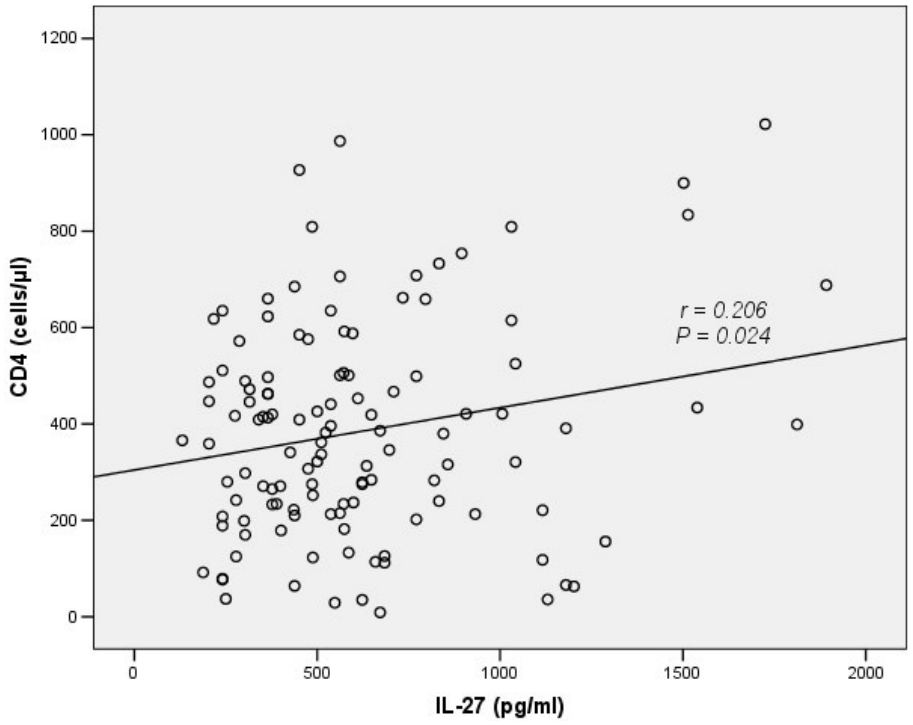

Figure 2. Correlation between plasma IL-27 titers and $CD4^+$ T cell counts in HIV-positive individuals. IL-27 titers (pg/ml) of 120 HIV-positive Chinese were plotted with $CD4^+$ T cell counts (cells/µl). Pearson's coefficient and *P* value of the correlation were shown (r = 0.206, P = 0.024).

transmitted diseases (STDs) due to the changes of social values, sexual behaviors, and also the population shift by migration (Yang et al., 2005). In 2007, the estimated number of people living with HIV/AIDS in China was 700,000; approximately half of them were infected through sexual transmission (Ruan et al., 2009).

As HIV, HBV and HCV share routes of transmission; HIV/HBV and HIV/HCV coinfections are common. Both HIV/HBV and HIV/HCV coinfections significantly elevate liver-related mortality (Thio et al., 2002; Rockstroh et al., 2005). HBV infection enhances IL-27 expression, and HCV coinfection with HIV suppresses IL-27 expression

(Zhu et al., 2009; Guzzo et al., 2010). To minimize the influence of HBV and HCV coinfection on IL-27 expression, we excluded those HBV and HCV positive participants in this study. The HIV-infected group and healthy control group were similar in age and gender. HIV infection changes the cytokine profile both in T cells infected with HIV *in vitro* and HIV-infected individuals *in vivo*. IL-1, IL-4, IL-6, IL-8, IL-10, IFN-α, IFN-β, M-CSF, TNF-α, TNF-β, SDF-1, MIP-1α, MIP-1β and RANTES are elevated by HIV infection both *in vitro* and/or *in vivo*; whereas IL-2, IL-12, IL-13, IFN-γ, and GM-CSF are downregulated (Kedzierska and Crowe, 2001; Cocchi et al, 1995). IL-32, a pro-inflammatory cytokine produced by T-lymphocytes, NK cells and blood monocytes, is also significantly elevated in serum of HIV patients. As IL-32 suppresses HIV replication *in vitro*, it is possible that the upregulation of IL-32 in serum after HIV infection may inhibit HIV replication *in vivo* (Rasool et al., 2008).

In a small scale study**,** Guzzo et al. (2010) reported serum IL-27 was modulated in HIV infection, but noted that this was clearest in patients on antiretroviral therapy with low plasma HIV RNA. They suggested that high viral loads may reduce IL-27 levels. In our study, we showed that plasma IL-27 levels were markedly elevated in therapy-naïve HIV infected individuals compared to healthy controls, suggesting that more studies on larger populations should be carried out in the future. IL-27 is secreted by antigen-presenting cells and induces naïve $CD4^+$ T cell proliferation (Pflanz et al., 2002). In the study, a positive correlation was witnessed between IL-27 titers and $CD4^+$ T cell counts among untreated individuals, suggesting IL-27 may indeed prompt expansion of naïve $CD4^+$ T cell *in vivo*. In HIV infection circumstance, immune system counterattack HIV by ensuring its integrity to slow down disease progression.

In this process, IL-27 may play a central role by directly inhibits HIV replication and meanwhile drives rapid clonal expansion of naïve $CD4^+$ T cell to neutralize or refine the damage caused by HIV replication. It is also possible that viral control is poor in individuals with low levels of plasma IL-27 since IL-27 plays a crucial role in combating HIV infection. IL-27 inhibits HIV replication in PBMCs, $CD4^+$ T cells and MDMs *in vitro*. Fakruddin (2007) and colleagues described that IL-27, released by PBMCs and MDMs upon human papilloma virus-like particles treatment, inhibited HIV replication. IL-27 hampered the replication of both X4 and R5 HIV without affecting cell surface CD4, CXCR4 and CCR5 expression. The inhibitory effect of IL-27 on HIV replication was dose-dependent, and the underlying mechanism was activation of genes encoding antiviral proteins (Fakruddin et al., 2007). DNA microarray experiments revealed that IL-27 activated multiple IFN-inducible genes (IFIG) in MDMs similar to IFN-α induction (Imamichi et al., 2008). Further study on macrophages and $CD4^+$ T cells uncovered that IL-27 induced the expression of type I interferons which drove the intracellular antiretroviral cytidine deaminases expression to counterattack HIV (Greenwell-Wild et al., 2009).

In our study, for the first time, we showed that IL-27 was significantly increased in HIV-infected Chinese and further demonstrated that plasma IL-27 titer is positively correlated with $CD4^+$ T cell count, indicating that IL-27 was indeed responds to HIV infection and may be an important factor for inhibition of HIV replication and slow down the disease progression in human beings.

ACKNOWLEDGEMENTS

This study was partially supported by the Supporting Program of the “Eleventh Five-year Plan” for Sci and Tech Research of China (2008ZX10001-004), National Nature Science Foundation of China (30901224), and a special grant from the Medical Key Laboratory of Shenzhen; and Research Grant for Control of Infectious Diseases (RFCID, Ref. No.09080482), Food and Health Bureau and General research grant (GRF, CUHK4428/06M), the government of Hong Kong Special Administrative Region, China.

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