Original Research

Dynamic observation of IL-33 and its receptors in HIV patients who received HAART

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Abstract: The purpose of this study was to observe the changes in serum IL-33 and its soluble receptors, serum sST2 level and ST2L expression in PBMCs of HIV-infected patients receiving highly-active antiretroviral therapy (HAART) for 12 monthes. Fifty-five chronic HIV-1-infected adults were recruited for this study before initiation of HAART. 30 age and gender matched healthy adults were recruited as control. Blood was obtained from each patient at baseline (0 month) and 12 months after initiation of highly active antiretroviral therapy(HAART), and each healthy person. The concentration of serum IL-33 and sST2 were detected by ELISA. plasma HIV RNA was detected by real-time fluorescent quantitative PCR. peripheral blood $CD3^+/CD4^+$ cell count, the ratio of CD4+ST2L+ positive peripheral blood mononuclear cells (PBMCs) were determined by flow cytometry. In HIV-infected patients, serum IL-33 and sST2 levels are higher and percentage of ST2L in PBMCs is lower than normal control significantly. During HAART, serum IL-33 and sST2 levels were decreased, whereas CD4+ST2L+ level was increased in PBMCs gradually. Serum IL-33 and sST2 levels positively correlated with plasma HIV RNA levels, but negatively correlated with the peripheral blood $CD3^+/CD4^+$ cell count. CD4+ST2L+ receptor in PBMC are positively correlated with the peripheral blood $CD3^+/CD4^+$ cell count, but negatively correlated with the plasma HIV viral loading. Serum IL-33 and sST2 levels, and CD4+ST2L+ expression in PBMCs are closely associated with HIV-1 infection and immune reconstitution in patients received HAART.

Key words: Interleukin-33; sST2 receptor; ST2L receptor; Human immunodeficiency virus; Highly active antiretroviral therapy.

Introduction

Progressive depletion of $CD4^+$ T cells and subsequent profound immune suppression is the main immune pathogenesis in hosts infected with the HIV-1 virus. Ultimately, the host is incapable of defending against AIDS-induced opportunistic infections and malignancies (1,2). Therefore, the key to successful HIV/ AIDS therapy is reconstruction of the immune system. Although the emergence of highly active antiretroviral therapy (HAART) has leaded to dramatic progression in the recovery of HIV/AIDS patients, the development of resistance and immune reconstruction inflammation syndrome (IRIS) limit its application (3,4). However, the roles played by HAART in rebuilding immune function in HIV/AIDS patients have demonstrated that the immune system in HIV/AIDS patients could be enhanced. Therefore, understanding immune reconstitution in HIV/AIDS patients under HAART could provide insights on the mechanisms of HIV-1 infection and provide new clues for developing therapeutic strategies for HIV/AIDS patients.

Interleukin 33 (IL-33) is a recently discovered member of the IL-1 family that is related to infections and inflammation. IL-33 can activate mast cells, lymphocytes, and eosinophils to produce Th2 cytokines in response to various infections and inflammatory immune responses. IL-33 is produced mainly by stromal cells, but inflammatory stimuli can induce its expression in epithelial and endothelial cells (5). IL-33 exerts biological effects through interactions with the ST2 receptor (IL-1RL1) and IL-1 receptor accessory protein (IL-1RAcP). However, ST2 and IL-1RAcP are widely expressed on the surface of innate immune cells and Th2 lymphocytes (6). IL-33 / ST2 pathway is involved in T cell-mediated immune response and the pathogenesis of many viral diseases.

In this study, the changes in serum IL-33 and its receptor sST2 levels in HIV-infected patients under HAART, as well as their associations with serum HIV virus loading, peripheral blood $CD3^+/CD4^+$ cell count, percentage of CD4+ and ST2L expression on the surface of PBMC were investigated, to explore IL-33/ ST2 axis how to play the roles in HIV-1 pathagenesis and anti-viral immune responses.

Materials and Methods

Subject

55 HIV / AIDS outpatients, of which 35 males and 20 females, with an average age of 32.29 ± 10.6 years were recruited before initiation of HAART for this study from June 2012 to December 2012 at Second Xiangya Hospital, Central South University. HIV-1 infection was confirmed in all patients by enzyme-linked immunosorbent assay (ELISA) and Western blot assay for HIV-1 antibodies. The inclusion criteria are as follows: 1) the patients that have never used anti-HIV medications before this study, 2) patients with no obvious AIDS manifestations, 3) the patients with no current tumors, 4) female patients that were not pregnant, 5) patients with normal heart, lung, liver and

kidney functions . The blood of 30 age and gender matched healthy subjects (20 males and 10 females with an average age of 35.45 ± 12.06 years) was collected as normal control during the same period. All subjects signed and returned the "informed consent" forms. This study has been approved by the medical ethics committee of Second Xiangya Hospital, Central South University.

Antiretroviral treatment regimen

55 HIV / AIDS patients were treated with first-line anti-HIV medications provided by the national AIDS program. The regimen included: two nucleoside reverse transcriptase inhibitors (NRTIs) and one non-nucleoside reverse transcriptase inhibitor (NNRTI). 27 patients chose zidovudine (AZT) + lamivudine (3TC) + Efabirenz (EFV) regimen, 21 patients chose AZT + 3TC + Nevirapine (NVP) regimen, 6 patients chose Tenofovir Disoprox (TDF) + 3TC + EFV regimen, and 1 patient selected Stavudine (D4T) + 3TC + EFV regimen. Blood was collected from all patients before (0 month) and 12 months after HAART initiation.

Peripheral blood CD3+CD4+ subset count and plasma HIV RNA quantification

2 mL of blood was collected from all patients before (0 months) and 12 months after HARRT initiation and from healthy controls in tubes containing EDTA. CD3-PreCP and CD4-PE fluorescent monoclonal antibody (BD company) were added to 100 µl of whole blood from each patient and healthy control. After mixing the solution, reactions were incubated at room temperature in the dark, followed by incubation with hemolysin. After centrifugation, the pellets were washed with 1xPBS for 2 times and then subjected to flow cytometry assay using BD FACS Calibur™ cytometer (BD company). The total number of $CD3^+/CD4^+$ lymphocytes were counted. The plasma was stored at -80°C until further use. plasma HIV nucleicacid was amplified by fluorescence quantitative detection kit (FQ-PCR HIV RNA kit, Zhunzi SZ20020041) purchased from Shenzhen PG Biotechnology Company using Gene Amp 7300 Sequence Detection System from US PE Company. This kit can detect HIV at a linear range of 50 copies / ml ~ 1,000, 000 copies / ml.

Flow cytometry assay of peripheral blood mononuclear cell surface CD4 and ST2L expression

10 mL of blood was collected from all HIV patients at 0 and 12 months after initiation of HARRT and from 30 healthy persons used EDTA tudes. Within 2 hrs, peripheral blood mononuclear cells (PBMC) were isolated using the Ficoll method. PBMCs were subjected to flow cytometry of CD4 and ST2L expression using CD4-PE (Biolegend company) and ST2L (ST2L-FITC antibody, MD company) antibodies, respectively. Briefly, the isolated peripheral blood mononuclear cells were washed twice with PBS and then the cell number was adjusted to 106 cell/ mL. After resuspending the pellet in 1 ml of PBS containing 5% human serum and incubated on ice for 10 minutes, cells were pelleted by centrifugation at 800 rpm for 5 minutes. 200 µl of PBS containing 0.5% BSA and saturated amounts of fluorescently labeled antibody (5 µl CD4-PE and 10 µl ST2L-FITC antibody)

were added and incubated in the dark at room temperature for 30 minutes. After washing with PBS to remove excess unbound antibodies, 400 µl of 1% paraformaldehyde were applied before flow cytometry assay.

Measurements of serum IL-33 and sST2 concentrations

The serum concentration of IL-33 and sST2 was detected using a Human IL-33 ELISA kit (eBioscience company) and Human IL-1 R4 / ST2 ELISA kit (RayBiotech companies).

Statistical analysis

Data were presented as mean ± standard deviation (SD), and analyzed using SPSS19.0, Graphpad Prism 5 statistical software, and Excel software. Differences in Parametric data were analyzed using t test, while non-parametric data were analyzed using the Wilcoxon rank sum test. A P<0.05 was considered statistically significant.

Results

The changes of peripheral blood $CD3^+/CD4^+$ cell count and plasma HIV RNA levels

The mean peripheral blood HIV-1 RNA copies in 55 HIV-1 infected patients were 3.72 ± 1.21 and 1.81 ± 0.59 (Log10 copies / ml) for 0 and 12 months after HAART initiation, respectively. The mean peripheral blood $CD3^+CD4^+$ cell count was 232.73 ± 93.29 and 367.25 ± 107.41 (cells / µl) for 0 and 12 months, respectively. The mean plasma HIV-1 RNA level significantly decreased (P<0.001), whereas the mean $CD3^+/CD4^+$ cell count significantly increased (P<0.001) during HAART. The HIV-1 RNA levels were negatively correlated with $CD3^+/CD4^+$ cell count (P<0.001).

Changes in serum IL-33 and sST2 levels, positive ST2L and CD4 ratio in PMBCs

HIV infection significantly increased serum IL-33 and sST2 levels than normal control. However, HAART significantly decreased their levels. In contrast, HIV infection decreased the percentage of ST2L and $CD4^+$ of PBMC, HAART significantly increased CD4+ST2L+ expression in PBMCs, and the number of peripheral blood $CD3^+/CD4^+$ count with significance (Table 1).

Correlations of serum IL-33 and sST2 with plasma HIV RNA copies and peripheral blood $CD3^+/CD4^+$ cell count

Correlation analysis showed that serum IL-33 levels at 0 month (Fig. 1A) and 12 month of HAART initiation (Fig. 1B) negatively correlated with the percentage blood of $CD3^+/CD4^+$ cell counts at 0 month (R=-0.861, P<0.001) and 12 month of HAART initiation (R=-0.838, P<0.001). In contrast, serum IL-33 levels at 0 month were positively correlated with plasma HIV RNA copies (R=0.658, P<0.001) (Fig. 1C). The serum sST2 levels at 0 month (Fig. 1D) and 12 months (Fig. 1E) of HAART initiation negatively correlated with the peripheral blood $CD3^+/CD4^+$ cell counts at 0 month (R=-0.553, P<0.001) and 12 months of HAART initiation (R=-0.789, P<0.001). Also, the serum sST2 levels at 0 month were positively correlated with the plasma

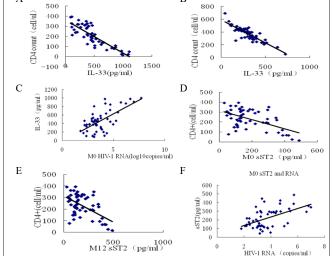**Table 1.** Comparison of IL-33, sST2, ST2L, and CD4+ T levels.

	Healthy controls (n=30)	HAART 0 Month (n=55)	HAART 12Month (n=55)
Serum IL-33 (pg/ml)	173.307±152.319	459.048±308.029**	294.071±198.080Δ*
Serum sST2 (pg/ml)	109.783±56.741	192.394±125.363 **	123.138±69.138Δ√
CD3CD4 count(cell/μl)	987.22±362.04	232.11±73.50**	367.25±107.41Δ*
ST2L+ (%) of PBMC	50.136±17.702	31.604±10.391**	41.138±24.077Δ*
CD4+(%) of PBMC	46.575±5.902	24.814±7.362**	34.559±6.454Δ*

* HAART 12 month vs. Healthy control, $p<0.001$
** HAART 0 month vs. Healthy control, $p<0.001$
Δ HAART 12 month vs. HAART 0 month, $p<0.001$
√ HAART 12 month vs. HAART 0 month, $p<0.001$

Figure 1. Correlation analysis. Serum IL-33 levels at month 0 (A) and month 12 of HAART initiation (B) correlated with peripheral blood $CD3^+CD4^+$ cell count. (C) Serum IL- 33 levels at month 0 positively correlated with plasma HIV RNA level. (D) The serum sST2 levels at month 0 and (E) month 12 of HAART initiation significantly correlated with peripheral blood $CD3^+CD4^+$ cells count. (F) Serum sST2 levels positively correlated with the serum HIV RNA level at month 0 of HAART initiation.

HIV RNA copies (R=0.532, P<0.001) (Fig. 1F).

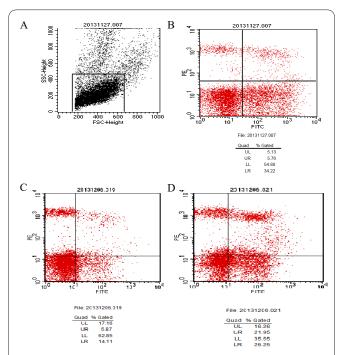**The percentage of ST2L and CD4+ on the surface of PMBCs**

Flow cytometry showed that the percentage of positive CD4 and ST2L in PMBCs expression was significantly decreased in HIV patients at 0 month of HAART (20.03% and %, respectively) compared to healthy controls (50.93% and 66.82%, respectively). 12 month after initiation of treatment, the percentage of positive CD4 and ST2L of PBMCs expression (38.21% and 48.2%, respectively) was significantly increased compared to 0 month (Fig. 2, p < 0.01).

Correlation analysis showed that the percentage of PBMC surface CD4/ST2L expression positively correlated with peripheral blood $CD3^+/CD4^+$ cell counts in healthy controls (Fig. 3A), HIV patients before (Fig. 3B) HAART, and HIV patients 12 months (Fig. 3C) after initiation of HAART. The percentage of PBMC surface CD4/ST2L expression negatively correlated with plasma HIV RNA copies in patients before HAART (R= -0.378, P= 0.04)(Fig. 3D).
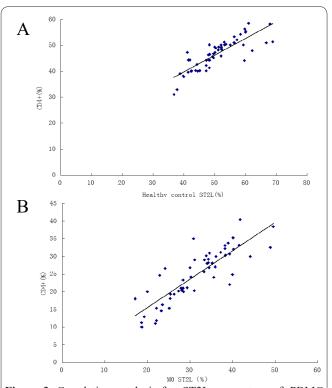
Discussion

This study found elevated serum IL-33 and sST2 levels, decreased ST2L expression on the surface of peripheral blood mononuclear cells in the patients with HIV-1 than healthy control, and their correlations with plsma HIV RNA copies and peripheral blood $CD3^+CD4^+$ T cell count. HAART can partially correct the changes in IL-33, sST2, and ST2L expression. This study suggested that IL-33, sST2, and ST2L correlate with disease progression of HIV infection and may be involved in anti-viral immune reconstitution of HIV/AIDS patients.

Recent studies have demonstrated that blood IL-

Figure 2. Flow cytometry for CD4 (PE staining) and ST2L (FITC staining) expression of peripheral blood mononuclear cells(PBMC): (A) Delineating lymphocytes with FSC and SSC and gating. (B) Healthy controls. The cells expressing CD4 (left upper UL+ right upper UR quadrant), cells expressing ST2L (right upper UR + right lower LR quadrant), and cells expressing both CD4 and ST2L (upper right RU quadrant) (C) Patients at Month 0 of HAART. The cells expressing CD4 (left upper UL+ right upper UR quadrant), cells expressing ST2L (right upper UR+ right lower LR quadrant), and cells expressing both CD4 and ST2L (upper right UR quadrant). (D) Patients at 12 month of HAART. The cells expressing CD4 (left upper UL + right upper UR quadrant), cells expressing ST2L (right upper UR + right lower LR quadrant), and cells expressing both CD4 and ST2L (upper right UR quadrant).

Figure 3. Correlation analysis for ST2L percentage of PBMC and peripheral blood CD3/CD4 count: (A) Healthy control. (B) Patients at Month 0 of HAART. (C) Patients at Month 0 of HAART. Totally, ST2L percentage of PBMC showed positively correlated with CD3/CD4 cell count in healthy controls and patients at Month 0 and Month 12 of HAART. ST2L percentage and HIV RNA level: (D) Positive relation between ST2L percentage of PBMC and plasma HIV RNA level in HIV patients before HAART.

33 and its cell surface receptor ST2L play an important role in a variety of diseases. For instance, IL-33 expression was significantly increased in the blood of patients with anaphylactic shock. IL-33 plays a role in the enhancement of degranulation in IgE sensitized mast cells (7). IL-33 is also linked to cardiovascular disease due to the finding that soluble ST2 is significantly increased in patients with myocardial ischemia (8) and is a predictive factor of disease severity (9). The involvement of IL-33 in infectious diseases is a center of focus in recent research. Becerra et al study found increased serum sST2 level in patients infected with the dengue virus and its level increased more in relapse patients, suggesting that sST2 is related to the severity of diseases (10). Wang et al study (11) found that peripheral blood IL-33 and sST2 levels were significantly higher in chronic hepatitis B patients than in healthy controls. Adefovir therapy significantly decreased IL-33 and sST2 levels, suggesting that immune responses mediated by IL-33 are involved in the process of HBV infection. Elevated IL-33 level was also observed in patients with chronic hepatitis C (HCV) infection (12). IL-33 levels positively correlated with serum ALT and AST levels. Serum IL-33 levels were therefore suggested to be associated with the degree of inflammation and severity of liver damage in hepatitis C (12). Only one study investigated serum IL-33 and sST2 levels, but it had a small sample size with HIV infection compared to healthy controls (13). The changes in IL-33 and sST2 levels and their associations with immune reconstruction in HIV/AIDS patients under

HAART have not been reported.

This study found that serum IL-33 and sST2 levels were significantly increased in HIV- infected patients compared with healthy controls. However, HAART for 12 months significantly lowered IL-33 and sST2 levels. In HIV-infected patients, IL-33 and sST2 levels negatively correlated with peripheral blood $CD3^+CD4^+$ cell counts. $CD3^+/CD4^+$ cell count is the most important immunological parameter in HIV / AIDS(14,15). Therefore, the elevated IL-33 and sST2 levels may reflect the degree of immune suppression. In addition, serum IL- 33 and sST2 level positively correlated with HIV viral loading. Therefore, changes in IL-33 and sST2 may also reflect the extent of infection and antiretroviral therapy effect(16,17). Although IL-33 and sST2 levels decreased after anti-viral therapy, this study found no significant correlations between both of them. However, Miyagaki et al study (13) reported that IL-33 level was significantly lower, but sST2 expression was higher than that of healthy controls. This inconsistency may be associated with the small sample size and low HIV-1 RNA copies in the study. Meanwhile, HIV-1 RNA copy number in 20 of the 26 cases was less than 50 copies / ml and the result might be deviation.

In conclusion, serum IL-33 and sST2 levels, and CD4+ST2L+ percentage of PBMCs are closely associated with HIV-1 infection and immune reconstitution in HIV-1 patients who received HAART.

Acknowledgments

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