Elevated levels and Clinical Association of Serum Cytokines in Untreated HIV-1 Infection

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Research

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Abstract

Background

Human immunodeficiency virus type 1 (HIV-1) infection disturbs the balance of CD4⁺ T cells and monocytes in the immune system. In the early stage of infection, the virus stimulates the activation and proliferation of immune cells, induces the release of cytokines, destroys CD4⁺ T cells, and accelerates HIV-1 replication and AIDS progression. It is essential to explore cytokine changes after HIV-1 infection and further understand the underlying mechanism of HIV infection.

Methods

In this study, we enrolled 38 HIV-infected subjects and 30 healthy subjects. We measured and compared CD4⁺ T cell counts and the serum cytokine levels in different groups.

Results

Our results showed significantly higher serum levels of IL-1 β , IL-2, IL-4, IL-7, IL-10, IL-17, IFN- γ , and TNF- α in HIV-infected patients. Higher levels of IL-6 and IL-17 were observed in the < 200/mL CD4⁺ T cell count group, and higher levels of IL-2 were observed in the CCR5-tropic HIV strain group.

Conclusion

In conclusion, we found that HIV infection-induced activation of the immune system and cytokines could predict the severity of HIV disease and regulate HIV infection and replication differently depending on the type of virus strain.

Introduction

Human immunodeficiency virus type 1 (HIV-1) infection is characterized by the immune cell destruction and immune system hyperactivation, which ultimately causes death in AIDS patients [1]. At the acute stage of infection, a rapid replication of HIV-1 virus and a simultaneous decrease of CD4⁺ T cells accompany with the release of various cytokines [2]. Cytokines as the effectors and modifiers of inflammation produced by innate and adaptive immune cells are responsible for intercellular immune regulation and communication [3, 4]. According to their lineages, different immune cells, including macrophages, Th1, Th2, and Th17 cells, can secrete IL-1 β , IL-2, IL-4, IL-10, IL-17, IFNs, TNF- α , and GM-CSF [5].

The continuous population depletion of helper CD4⁺ T cells is one major characteristics of HIV-1 infection. Thus, the imbalance of cytokines is a common immune state in HIV-1 patients. The dysregulation in cytokine production may act as an important role in the T-cell dysfunction, but also impairs the HIV-specific response in the innate immune system [1]. In previous studies, IL-2, IL-4, IL-7, and IL-10 were found to be critical in the normal proliferation, regulation, function, and homeostasis of T cells and B cells [6, 7]. Secreted by Th1 cells, IL-2 is not only regarded as a T cell growth factor but can induce the differentiation of B cells and NK cells and maintain regulatory T cells (Tregs) [8]. IL-4 and IL-10 secreted by Th2 cells activate B cells to produce IgG and IgE. IL-7 promotes CD4⁺ T cell and CD8⁺ T cell survival while regulating transcription and inducing the reactivation of HIV-1 [9]. IFN- γ secreted by Th1 cells activates NK cells, upregulates antiviral restriction factors, induces CD4⁺ T cell apoptosis, and dampens the effect of antigen-specific CD4⁺ and CD8⁺ T-cell responses [10]. Moreover, HIV-1 infection can trigger inflammasome activation in CD4⁺ T cells, which induces the release of IL-1 β and GM-CSF from blood monocytes [11].

Currently, several studies have shown that cytokines produced by immunocytes affect HIV-1 infection, and recombinant cytokine or agonist treatment has shown promising results. Unfortunately, current HIV remission approaches largely involve single-agent immunotherapy, with very few studies focusing on combinatory approaches and virus tropism. Our study investigates the different cytokine changes in HIV-1 infection and illustrates the association of these cytokines in viral tropism, which aims to further understand the HIV-1 infection mechanism and develop combinatory therapeutic approaches for HIV remission.

Materials And Methods

Patient recruitment and study design

This study was performed to analyze serum cytokine expression in HIV-infected individuals receiving clinical care at the First Affiliated Hospital of Harbin Medical University and Harbin Centers of Disease Control and Prevention from January 2016 to January 2017. HIV-infected pregnant women, age under 18 and HIV-infected individuals with intercurrent diseases were excluded from this study. A total of 24 HIV-infected patients (26 to 72 years old), 4 female and 20 male patients from the First Affiliated Hospital of Harbin Medical University, and 14 HIV-infected patients (21 to 56 years old), 2 female and 12 male patients from Harbin Medical University, were enrolled for cytokine expression analysis during HIV-1 infection. Among these, 14/38 HIV-infected patients were investigated for chemokine coreceptor phenotypes - CXCR4 and CCR5. As a control group, 30 healthy individuals, 9 females and 21 males (23 to 62 years old), were enrolled in this study. Ethics committees at Harbin Medical University approved this study, and all subjects signed a consent form. The study conforms to the PRISMA guidelines.

CD4⁺ T cells counts

Peripheral blood samples of HIV-infected patients were collected and anticoagulated with EDTA, and a BD FACSCalibur platform (BD Biosciences, San Jose, CA) was used to measure CD4⁺ T cells. The serum of the healthy control group was tested for HIV antibody by an ELISA Kit for the detection of anti-HIV (LIVZON DIAGNOSTICS, Zhuhai, China).

Levels of serum cytokines

Blood was centrifuged for the separation of plasma, and all specimens were aliquoted immediately, frozen, and stored at -80°C until all samples were collected. Plasma levels of cytokines IL-2, IFN- γ , IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IL-1 β , TNF- α , IL-7, MCP-1, MIP-1 β , G-CSF, and GM-CSF were analyzed using the Bio-Rad Human Cytokine 17-Plex Assay (Hercules, CA, USA) according to the manufacturer's instructions. Each measurement was taken in duplicate. Standard curves were generated by using the reference cytokine concentrations supplied by the manufacturer.

Statistical analysis

Cytokine levels were normalized in the cultures of each patient according to the control group levels. Comparisons of cytokine dynamics among different groups revealed an asymmetric distribution. Thus, central tendency and dispersion were described using median and interquartile range values. When the differences were significant, analyses of variables between each group were performed using the nonparametric Mann-Whitney U test. Spearman correlation analysis, a variant of the Pearson correlation coefficient, was also used. The R coefficient reflects the direction of the association, with a positive sign indicating a direct correlation and a negative sign indicating an inverse correlation. p values < 0.05 were considered significant.

Results

Overexpression of IL-2, IFN- γ , IL-4, IL-10, IL-17, IL-1 β , TNF- α , GM-CSF, and IL-7 in HIV-infected patients

We compared the serum levels of cytokines between HIV-infected patients and healthy individuals and found that there were higher levels of IL-2 ($p < 0.001$), IFN- γ ($p < 0.001$), IL-4 ($p < 0.001$), IL-10 ($p = 0.001$) and IL-17 ($p = 0.021$) secreted by CD4⁺ T cells in all 38 HIV-infected patients (Fig. 1). Moreover, IL-1 β ($p < 0.001$), TNF- α ($p = 0.003$), and GM-CSF ($p < 0.001$), which are secreted by monocytes, and IL-7, which is secreted by bone marrow stromal cells, were higher in all HIV-infected patients (Figs. 2 and 3).

Correlation analysis of different cytokines in HIV-infected and healthy control groups

A Spearman correlation analysis was applied between serial cytokine production in the groups of HIV-1-infected patients and healthy individuals. In the HIV group, a clear correlation was found between the IL-17 and IL-1 β ($r = 0.539$; $p < 0.001$), IL-10 ($r = 0.535$; $p < 0.001$), GM-CSF ($r = 0.543$; $p < 0.001$), and TNF- α ($r =$

0.558; $p < 0.001$) production level. Strong positive correlations between the IL-2 level and IL-4 level ($r = 0.602$; $p < 0.001$) and IL-7 level ($r = 0.556$; $p < 0.001$) were observed. In the group of HIV-infected patients, positive correlations between the IL-4 level and IL-7 level ($r = 0.593$; $p < 0.001$) and IL-10 level ($r = 0.521$; $p < 0.001$) were also found (Fig. 4). Other than these, we did not find any correlations between cytokines in the healthy control group.

The associations of different cytokines and the relationship between cytokines and CD4⁺ T cells

The CD4⁺ T cell count in peripheral blood is an important indicator of HIV-1 infection progression. A CD4⁺ T cell count less than 200 indicates the terminal stage of AIDS. At this stage, the immune system is severely damaged, and opportunistic infections are easily acquired [8]. Thus, we divided the HIV-infected patients into two groups with CD4⁺ T cell counts $> 200/\text{mL}$ ($n = 25$) and $< 200/\text{mL}$ ($n = 13$) to compare the serum cytokine concentrations. IL-6 ($p = 0.027$) and IL-17 ($p = 0.044$) concentrations increased at the terminal stage of AIDS, as shown in Fig. 5.

Overexpression of IL-2 in the CCR5-positive HIV group

HIV-1 enters into host cells through the combination with the envelope glycoprotein (Env) to the host cell receptor CD4 and the subsequent combination with the chemokine coreceptors - CCR5 or CXCR4 [12]. In this study, 14 patients infected with HIV through blood transmission were tested, and their chemokine coreceptor phenotypes were analyzed. We investigated the differences in viral tropism, and our results showed that IL-2 level was higher in the CCR5-tropic HIV-infected group than the CXCR4-tropic HIV-infected group ($p = 0.047$, Fig. 6).

Discussion

HIV infection may disrupt the balance of cytokines produced by Th1, Th2, and Th17 T cells and B cells, and NK cells. Previous studies have shown that the Th1 response dominates in the early stage of HIV infection. IL-2 and IFN- γ produced by Th1 cells stimulate the activation of CD8⁺ cytotoxic T cells and maintain viremia. Then, during chronic HIV infection, the Th2 response begins to dominate, causing the increased production of IL-4 and IL-10. These studies revealed the importance of understanding the production of cytokines secreted by T cells during HIV infection [13]. In this study, the serum cytokines of HIV-infected patients were measured and compared, and we found that the levels of IL-1 β , IL-2, IL-4, IL-7, IL-10, IL-17, IFN- γ and TNF- α , which are produced from different immune cells, were higher in the HIV-infected group, which indicates that these cytokines may act as biomarkers for HIV-1 infection and predictors of different types of immune cells involved in HIV progression.

In mucosa-associated lymphoid tissue (MALT), the presence of a large number of infected CD4⁺ T cells is directly related to the inflammation of mucosal tissue and destroys the integrity of the mucosa, allowing microorganisms to migrate from the intestinal tract to peripheral blood [14]. Th17 cells play an important

role in maintaining mucosal integrity, and our results indicated a higher level of IL-17 in HIV-infected patients, which is consistent with previous studies [15]. Monocytes are target cells of HIV infection, and their function is affected by the virus. IL-1 β and TNF- α are proinflammatory cytokines secreted by monocytes. Their augment may be due to the persistent stimulation of HIV regulatory proteins by HIV antigens, resulting in an increased monocyte release of IL-1 β and TNF- α [16, 17]. IL-7 secreted by bone marrow stromal cells promotes the development and differentiation of T cells, and HIV replication in infected patients is maintained by IL-7 [18]. IL-6 can activate latent viruses and induce them to replicate in vitro [19]. IL-17 plays an important role in activating neutrophils against infection. The above results show that IL-17 and IL-6 participate in the pathogenesis of HIV and can predict the severity of the disease, providing a new indicator for the clinical observation of disease progression. In this study, our results showed that the levels of IL-6 and IL-17 increased in patients with CD4⁺ T cell counts < 200/mL, which indicates that these cytokines may be biomarkers for the terminal stage of AIDS. This study indicated that HIV infection maintains a state of long-term immune activation in the whole body. This chronic activation state causes T cells to generally lose their immune function, leading to progressive degradation of the immune system and the development of AIDS.

HIV-1 entry into cells is mediated by Env consisting of two subunits, gp120 and gp41. The initial interaction occurs between HIV-1 and host cell mediated by combination of gp120 and the cell surface receptor CD4 [3–5]. Subsequently, structural gp120 rearrangements emerge, which causes exposure of the coreceptor binding sites and secondary combination of CCR5 or CXCR4 chemokine receptor [10]. This combination between gp120 and CCR5 or CXCR4 coreceptor lead to next conformational changes in Env accompanied by exposure of the gp41 fusion peptide. CCR5 antagonists are a relatively novel armamentarium of anti-HIV-1 drugs. In recent investigations of the complex regulation of cytokines during HIV infection, HIV strains should also be taken into account. Previous studies have suggested that IL-4 can upregulate the expression of CXCR4 and inhibit the expression of CCR5, while IL-2 can downregulate the expression of CXCR4 and increase the expression of CCR5 [20]. Other studies suggested that the level of IL-2 increased in the group infected with CCR5-tropic HIV strains, which was consistent with our results [21]. These results indicated that cytokines may influence HIV infection and replication differently depending on the type of virus, suggesting the importance of different cytokine adjuvants in the design of effective vaccines for viruses with different tropisms.

In conclusion, our study demonstrated that the levels of the serum cytokines IL-2, IFN- γ , IL-4, IL-10, IL-17, IL-1 β , TNF- α , and IL-7 were elevated in HIV-infected patients, which indicated that HIV infection caused activation of the immune system. Higher levels of IL-6 and IL-17 could predict the severity of HIV disease. The higher level of IL-2 in patients infected with CCR5-tropic HIV strains indicated that cytokines regulated HIV infection and replication differently depending on the type of virus strain.

Abbreviations

HIV-1 = Human immunodeficiency virus type 1

Tregs = Regulatory T cells

Env = Envelope glycoproteins

MALT = Mucosa-associated lymphoid tissue

Declarations

Ethics approval and consent to participate

Approval of this study was obtained from the Ethics Committee of First Affiliated Hospital of Harbin Medical University (Approval no. IRB-AF/SC-04/01.0), Harbin, China. The protocol was revised and approved by the Ethics Committee of Harbin Medical University and all participants signed consent forms.

Human and animal rights

No animals were used in the study. All human research procedures followed were in accordance with the standards set forth in the Declaration of Helsinki principles of 1975, as revised in 2013 (<http://ethics.iit.edu.ecodes/node/3931>) and performed according to the Harbin Medical University concerning human research.

Consent for publication

Written informed consent was taken from all subjects before participation in this study.

Conflict of interest

All authors declare no conflict of interest in this study.

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Availability of data and materials

The authors confirm that the data supporting the results and findings of this study are available within the article.

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Figures

Figure 1

The serum levels of different cytokines secreted by CD4+ T cells in HIV-infected patients. HIV, HIV-infected group (●); Control, healthy control group (■). The horizontal bars indicate the median value. Differences between groups were tested by the Mann-Whitney U test.

Figure 2

The serum levels of different cytokines secreted by monocytes in HIV-infected patients. HIV, HIV-infected group (●); Control, healthy control group (■). The horizontal bars indicate the median value. Differences between groups were tested by the Mann-Whitney U test.

Figure 3

The comparison of the serum levels of different cytokines secreted by non-B cells or non-monocytes between the HIV-infected group and the healthy control group. HIV, HIV-infected group (●); Control, healthy control group (■). The horizontal bars indicate the median value. Differences between groups were tested by the Mann-Whitney U test.

Figure 4

Correlations between the concentrations of cytokines. (A) The correlation between IL-17 and IL-1β, IL-10, GM-CSF or TNF-α. (B) The correlation between IL-2 and IL-4, IL-7 or IL-10. (C) The correlation between IL-4 and IL-7 or IL-10. The solid line means correlated to each other. Correlation analysis was performed with Spearman's rank correlation to determine correlations between variables. The relevance is considered >0.5.

Figure 5

Comparisons of cytokine levels between the group with CD4+ T cell count>200 cells/mL and the group with CD4+ T cell count<200 cells/mL. HIV, CD4+ T cell count>200 cells/mL (●); CD4+ T cell count<200/mL (□). The horizontal bars indicate the median value. Differences between groups were tested by the Mann-Whitney U test.