Research Article

Cytokine Levels in Plasma Samples of Individuals with HIV Infection

Enrique Vera Tudela[1], Manpreet Kaur Singh[1], Minette Lagman[1], Judy Ly[2], Nishita Patel[3], Cesar Ochoa[3] and Vishwanath Venketaraman[1, 2*]

[1]Department of Biomedical Sciences, Western University of Health Sciences, USA

[2]Department of Basic Medical Sciences, Western University of Health Sciences, USA

[3]Western Diabetes Institute, Western University of Health Sciences, USA

***Corresponding author:** Vishwanath Venketaraman, Department of Basic Medical Sciences, College of Osteopathic Medicine of the Pacific, Western University of Health Sciences, 309 East Second Street, Pomona, CA 91766-1854, USA, Tel: 9097063736; Email: vvenketaraman@westernu.edu

Abstract

CD4+ T helper cells play a vital role in the immune system by secreting cytokines, which regulate the immune response. Cytokines are secreted by T cells when an intracellular infection is detected as in case of HIV infection. HIV is known to infect T cells that have CD4+ receptors present on their surface. These cells, among others in the immune system, secrete cytokines. The levels of cytokines present in the plasma become an indicator of the nature of the immune response. In this study we are measuring levels of plasma cytokines in HIV infected individuals and healthy control subjects using ELISA. Our results indicate that cytokine plasma levels between the two groups are substantially different. In general, we observed that there was an increase in anti-inflammatory cytokines and a decrease in pro-inflammatory cytokines in individuals that were positive for HIV infection, likely due to the role of HIV in suppressing the appropriate immune response in HIV positive individuals.

Introduction

Having claimed more than 36 million lives so far and with approximately 35.3 million people infected with HIV as of October 2013, HIV is a major global health issue [1]. The Human Immunodeficiency Virus (HIV) targets the immune system and weakens the surveillance and defense system of the body against infections, resulting in HIV infected individuals becoming more susceptible to a wide range of infections normally cleared by the immune system of a healthy individual [1].

Cytokine levels are indicative of the nature of the immune response in a particular individual. When immune cells detect intracellular pathogens, the immune response that results is specifically tailored to optimize the clearance of the pathogen [2]. T helper cells, also named CD4+ T helper cells (Th), are immune cells with an important role in directing the response necessary to accomplish this task. When the response calls for the clearance of an intracellular pathogen, a Th1 response is induced [2]. On the contrary, when removal of an extracellular pathogen is necessary, the resultant Th2 response is induced [2]. In contrast to the Th1 response, the Th2 response mediates antibody production [3]. Cytokines generally can be divided into 2 major categories, the pro-inflammatory cytokines, which stimulates the immune system, or the anti-inflammatory cytokines which suppresses the immune system [2]. Not only are CD4+ T helper (Th) lymphocytes crucial regulators of the immune system, but they also play a major role in inflammatory disease progression. One of the most important factors in directing CD4+ T helper cells towards the appropriate response are cytokines produced by cells of the innate immune system [4]. Antigen presenting cells (APC) are innate cells that activate T helper cells, causing them to differentiate into effector cells specialized in cytokine secretion and function. Th1 activation also requires interleukin 12 (IL-12) [5]. IL-12 is secreted by a class of APC called dendritic cells (DC). IL-12 induces native T-cells into the Th1 (CD4+) subset. Th1 cells then go onto produce cytokines IL-2 and interferon gamma (*IFN*-γ). *IFN*-γ acts by activating macrophages and is essential for eliminating intracellular pathogens [6]. Interleukin 2 (IL-2) is crucial for growth, proliferation and differentiation of T-cells, acting as a growth factor for T-cells [3].

Studies conducted earlier have revealed that HIV-infected individuals have a weaker immune system; this is due to the inability of CD4+ T cells to proliferate, due to the decrease in the levels of IL-12 [7]. A consequence of this decrease in IL-12 results in a decrease in IL-2 and *IFN*-γ, leading to immunosuppressive effects and allowing room for opportunistic infections, which is a trademark of HIV progression. This shows how CD4+ T cells play a central role in disease progression [8]. CD4 T cell counts are important biomarkers for AIDS progression in HIV patients [9]. A range of 500-1000 cells/mm^3 is considered a normal range for healthy individuals, while a CD4 count of 200 cells/mm^3 is diagnostic for AIDS [10].

Given HIV's role in dampening the immune response towards intracellular pathogens, pro-inflammatory cytokines should be decreased in HIV infected individuals, while the opposite effect would be true of anti-inflammatory cytokines. Levels of anti-inflammatory cytokines interleukin 10 (IL-10), for example, should be relatively decreased in healthy individuals with intracellular infections, allowing the infection to be better cleared by the appropriate Th1 response. Likewise, Transforming Growth Factor Beta (TGF-β), a cytokine with an important role in augmenting a regulatory T cell response (T-regs) [11] that inhibits the immune system, should be decreased in healthy individuals with intracellular infection. Another cytokine, interleukin 6 (IL-6), has been found to serve as a growth factor for the HIV [12]. Because HIV infection weakens the cell mediated immune response and directs it to allow for its own survival, we would expect levels

of IL-10 and TGF-β to be elevated in HIV-infected subjects. IL-6, serving its role as a growth factor for HIV, should also be elevated in HIV infection, as compared to healthy individuals.

In our controlled study, we aim to shed light on various components of the innate and adaptive immune system, and subsets within adaptive immunity such as the Th1, Th2, and regulatory signaling in the context of HIV infection. In light of previous findings, we hypothesize that IFN-γ, IL-12, and IL-2 will be decreased in the plasma of HIV infected subjects, while IL-6, IL-10, and TGF-β will be elevated in such subjects.

Methods and Materials

Study Participants

A total of 17 individuals were recruited to participate in this study. The age of study subjects ranged between 18-65. Of the 17 individuals, ten individuals were healthy and seven were positive for HIV infection. All of the study participants were volunteers that were local to Pomona or the nearby areas and were recruited using email, flyers at the Patient Care Center at Western University of Health Sciences in Pomona, California. The purpose of the study and what was required from each participant was explained to each individual before obtaining their consent to be subjects in our study. Background information for the HIV infected individual, such as time since seroconversion and CD4 T helper cell counts was obtained.

Collection of blood and separation of plasma sample

On day one, all of the study participants had about 40CCs of blood collected using Vanutainer Safety-Lok blood collection set. Plasma was separated from the whole blood by density gradient centrifugation using ficoll hypaque, followed by allocation into tubes and storage at -80 degrees. This plasma was then tested for cytokine levels using Enzyme-linked Immunosorbent Assay (ELISA).

Plasma cytokine measurements

Cytokine levels were measured using a Ready-Set-Go! ELISA kit from ebioscience. A total of six cytokines were measured in the plasma of all subjects. The ebioscience protocol outlined in each kit was followed. All the standard safety precautions were taken at all times.

Statistical Analysis

Graph Pad Prism 6 was used to perform statistical analysis on our data. To test for a statistically significant result, we analyzed the cytokine plasma concentrations using the Mann-Whitney test of unpaired t test data.
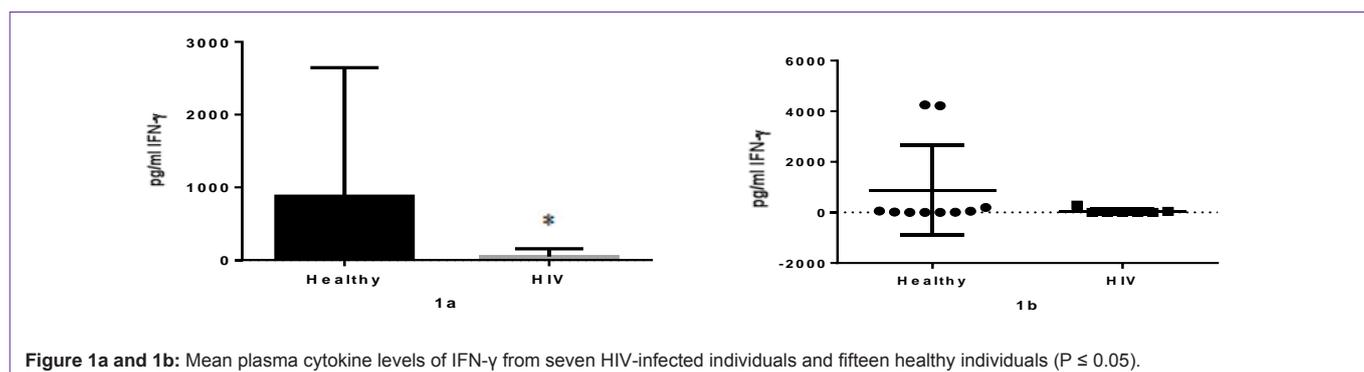
Results

HIV positive participants in this study had CD4 T cell counts below the normal range of 500-1000 cells/mm^3. The HIV positive participants consisted of two females and five males between the ages of between 28 and 53 (Table I). Overall, we did not observe a significant correlation among age and sex and CD4 counts. Likewise, duration of HIV treatment did not appear to affect the results of this study. There was a statistically significant difference in the plasma cytokine concentration between HIV positive and healthy individuals, as assayed with ELISA. Furthermore, those cytokines that strengthen the immune response to HIV infection such as IL-12 (Figures 2a and 2b), IL-2 (Figures 3a and 3b), and *IFN*-γ (Figures 1a and 1b), were significantly lower in HIV positive participants. On the other hand, cytokines that encourage HIV replication and proliferation, IL-6 (Figures 4a and 4b), IL-10 (Figures 5a and 5b), and TGF-β (Figures 6a and 6b), were significantly increased in participants positive for HIV infection. Overall, homeostasis between pro-inflammatory and anti-inflammatory cytokines was not observed. Additionally, there was a wide variation between the participants in plasma CD4 count, possibly in result to previous treatment or lifestyle habits of the participants.
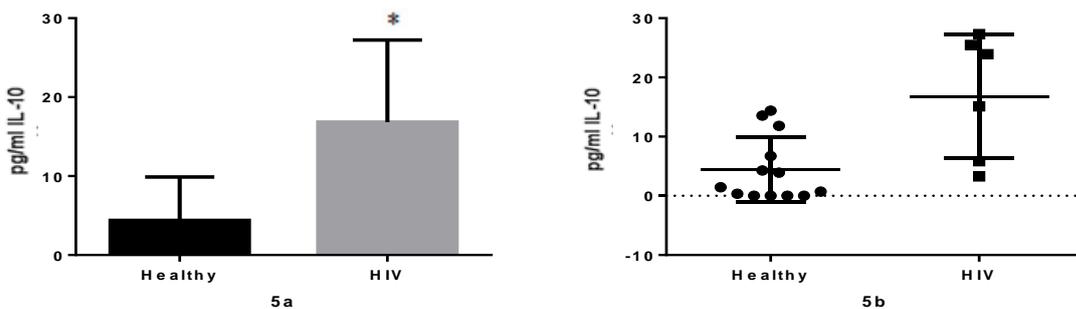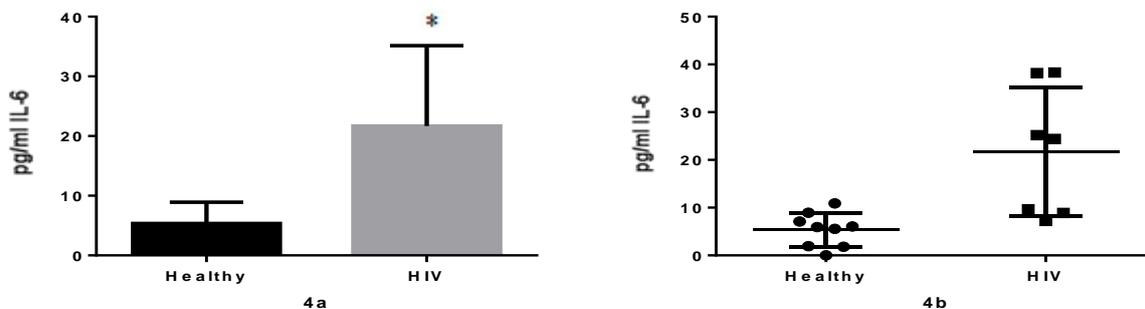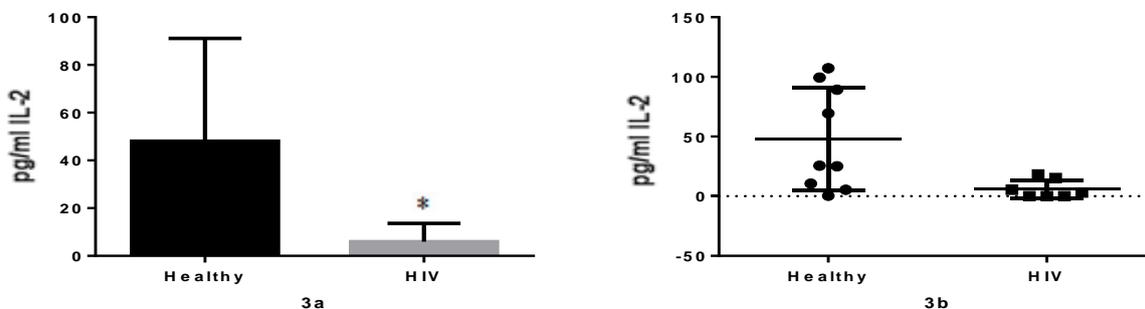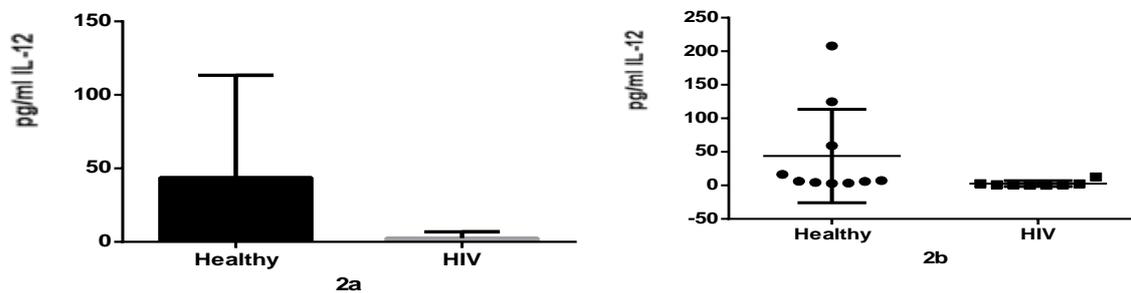
Discussion

In cases of HIV infection, levels of plasma cytokines of affected individuals are understandably altered from the normal cytokine profile found in those without HIV infection. Those cytokines known to stimulate the Th1 subset would expectedly be lower than found in healthy subjects. In contrast, those known to stimulate the Th2 subset

Table 1: Participant demographics collected from HIV positive participants

Patient #	Gender	Age	Year diagnosed	Race/ Ethnicity 1=Hispanic 2=Black 3=White	Language 1=Spanish 2=English	Baseline CD4+ Count
1	F	52	2007	2	2	235
2	M	34	2000	3	2	89
3	M	34	2000	3	2	296
4	F	53	2001	Unknown		167
5	M	28	2010	3	2	436
6	M	28	2011	3	2	509
7	M	45	2003	3	2	456

Figure 1a and 1b: Mean plasma cytokine levels of IFN-γ from seven HIV-infected individuals and fifteen healthy individuals (P ≤ 0.05).

Figure 2a and 2b: Mean plasma cytokine levels of IL-12 in eight HIV-positive individuals and fourteen healthy individuals (P ≤ 0.05).

Figure 3a and 3b: Mean plasma cytokine levels of IL-2 in seven individuals with HIV infection and ten individuals not infected with HIV (P ≤ 0.05).

Figures 4a and 4b: Mean plasma levels of IL-6 in seven HIV infected participants and thirteen healthy participants (P ≤ 0.05).

Figures 5a and 5b: Mean plasma levels of IL-10 in six subjects with HIV infection and fifteen without HIV infection is illustrated (P ≤ 0.05).
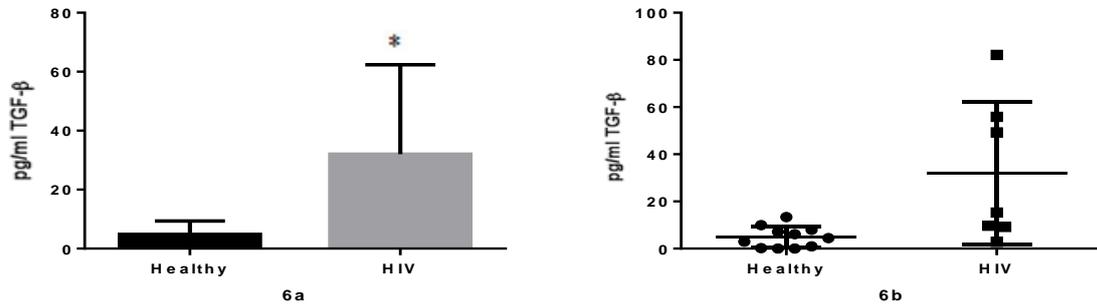
Figure 6a and 6b: Mean plasma cytokine levels of TGF-β in seven HIV positive individuals and nine healthy subjects (P ≤ 0.05). Variants in the number of participants are a result of an ongoing recruitment process for future studies. All samples were assayed for levels of plasma cytokines using ELISA kit from ebioscience.

would also be altered to misdirect the immune system in cases of HIV infection. Likewise, cytokines with an important role in suppressing the immune system as a whole, such as those elevated in individuals with autoimmune disease, would possibly also be elevated in HIV infection. With the goal to document these differences, we carried out this study to measure the cytokine levels in subjects afflicted with HIV, comparing the cytokine levels with those found in subjects without HIV. In our study, six distinct cytokines were measured and compared using ELISA. Based on our data, it is clear that there is a statistically significant difference among those with and without HIV infection.

Given the nature of CD4 cell counts as an important biomarker for HIV progression, our results showed CD4 counts were significantly lower in HIV participants versus healthy participants, as expected. This may contribute to the altered cytokine profile of the participants.

With its role in activating the Th1 subset, we anticipated IL-12 to be decreased in those with HIV infection. Our assay revealed that this was in fact the case. The results showed markedly decreased plasma levels of IL-12 in subjects with HIV infection (Figures 2a and 2b). Similarly, IL-2, a major growth factor for T cells, was also expected to be decreased in HIV infected participants. The data collected confirmed these expectations, as the plasma levels of IL-2 were markedly decreased (Figures 3a and 3b). Another Th1 subset cytokine essential for eliminating intracellular pathogens, IFN-γ, was thought to be decreased with HIV infection, and our results confirmed these assumptions (Figures 1a and 1b).

In contrast to the previously mentioned cytokines, we expected another group of cytokines with important roles in managing the immune response to be elevated in subjects with HIV. Specifically, we predicted IL-10, an immunosuppressive cytokine with roles in inhibiting the Th1 response, to be increased in patients with HIV infection. Per our data, the plasma levels of this cytokine in HIV subjects did show a significant increase (Figures 5a and 5b). With a similar role in suppressing the immune response, TGF-β was also considerably increased in those with HIV (Figures 6a and 6b). Finally, our analysis also shows that IL-6, a proinflammatory cytokine serving as a growth factor for the HIV virus, was evidently elevated in our participants with HIV (Figures 4a and 4b).

After carefully examining our data, we conclude the following: Those proinflammatory cytokines, IL-2, IL-12, and IFN-γ, with their role in stimulating the Th1 response, are significantly decreased in

patients with HIV. In contrast, IL-6, also a proinflammatory cytokine with other roles in the immune system, is elevated in patients with HIV. Our data also show that those cytokines that enhance HIV replication, IL-10 and TGF-β, were noticeably increased.

A recent study carried out by our lab has shown that HIV infection leads to excessive production of pro-inflammatory cytokines, in turn decreasing the level of glutathione (GSH) in these subjects [13]. It was also previously reported by our lab that GSH, a tripeptide antioxidant, activates the function of T-lymphocytes [14]. GSH is a major intracellular hydrosoluble antioxidant agent, responsible for maintaining important functions in biochemical processes and cell control. More specifically, it is involved in the control of the immune response and antioxidant defenses [15]. In vitro studies have also shown that a decrease in GSH levels not only promotes HIV expression but also impairs T-cell function, which helps explain the link between GSH depletion and HIV disease progression [16]. For a future study, we will supplement study participants with GSH to test the hypothesis that GSH will normalize the cytokine levels in these patients, thereby improving their immune responses and decreasing their chance of opportunistic infections.

Conflict of interest

The people providing the funds for this study had no input in any aspect of this study. There was no conflict of interest for any of the authors.

Acknowledgments

References

1. World Health Organization. Global HIV/AIDS response. 2013.

2. Dinarello CA . Proinflammatory cytokines. Chest. 2000; 118: 503-508.

3. Morris D, Guerra C, Khurasany M, Guilford F, Saviola B, Huang Y, et al. Glutathione supplementation improves macrophage functions in HIV. J Interferon Cytokine Res. 2013; 33: 270-279.

4. Elenkov IJ, Chrousos GP . Stress Hormones, Th1/Th2 patterns, Pro/Anti-inflammatory Cytokines and Susceptibility to Disease. Trends Endocrinol Metab. 1999; 10: 359-368.

5. Park H, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, et al. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. Nat Immunol. 2005; 6: 1133-1141.

6. Fan J, Bass HZ, Fahey JL . Elevated IFN-gamma and decreased IL-2 gene expression are associated with HIV infection. J Immunol. 1993; 151: 5031-5040.

7. Noble A, Thomas MJ, Kemeny DM . Early Th1/Th2 cell polarization in the absence of IL-4 and IL-12: T cell receptor signaling regulates the response to cytokines in CD4 and CD8 T cells. Eur J Immunol. 2001; 31: 2227-2235.

8. Li Y, Ling W, Xu H, Wang M, Wu C . The activation and dynamics of cytokine expression by CD4+ T cells and AIDS progression in HIV-1-infected Chinese individuals. Microb Pathog. 2012; 53: 189-197.

9. Mellors JW, Muñoz A, Giorgi JV, Margolick JB, Tassoni CJ, Gupta P, et al. Plasma viral load and CD4+ lymphocytes as prognostic markers of HIV-1 infection. Ann Intern Med. 1997; 126: 946-954.

10. Ray K, Gupta SM, Bala M, Muralidhar S, Kumar J . CD4/CD8 lymphocyte counts in healthy, HIV-positive individuals & AIDS patients. Indian J Med Res. 2006; 124: 319-330.

11. Guerra C, Morris D, Sipin A, Kung S, Franklin M, Gray D, et al. Glutathione and adaptive immune responses against Mycobacterium tuberculosis infection in healthy and HIV infected individuals. PLoS One. 2011; 6: e28378.

12. Miles SA, Rezai AR, Salazar-González JF, Vander Meyden M, Stevens RH, Logan DM, et al. AIDS Kaposi sarcoma-derived cells produce and respond to interleukin 6. Proc Natl Acad Sci U S A. 1990; 87: 4068-4072.

13. Morris D, Guerra C, Donohue C, Oh H, Khurasany M, Venketaraman V. Unveiling the mechanisms for decreased glutathione in individuals with HIV infection. Clin Dev Immunol. 2012; 734125.

14. Morris D, Gonzalez B, Khurasany M, Kassissa C, Luong J, Sarah Kasko, et al. Characterization of Dendritic Cell and Regulatory T Cell Functions against Mycobacterium tuberculosis Infection. BioMed Research International. 2013.

15. Borges-Santos MD, Moreto F, Pereira PC, Ming-Yu Y, Burini RC . Plasma glutathione of HIV⁺ patients responded positively and differently to dietary supplementation with cysteine or glutamine. Nutrition. 2012; 28: 753-756.

16. Herzenberg LA, De Rosa SC, Dubs JG, Roederer M, Anderson MT, Ela SW, et al. Glutathione deficiency is associated with impaired survival in HIV disease. Proc Natl Acad Sci U S A. 1997; 94: 1967-1972.

Citation: Tudela EV, Singh MK, Lagman M, Ly J, Venketaraman V et al. Cytokine Levels in Plasma Samples of Individuals with HIV Infection. Austin J Clin Immunol. 2014;1(1): 1003.