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Original Article

No Evidence of the Th1 to Th2 Cytokine Shift during the Course of HIV Infection

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ABSTRACT

Background and Objectives: Infection with human immunodeficiency virus (HIV) results in dysregulation of the cytokine profile. A switch from a T helper 1 (Th1) to a Th2 cytokine has been proposed as an important factor in progression of HIV infection to AIDS. The aim of the present study was to assess the level of Th1 and Th2 cytokines in HIV infected individuals in order to identify the switch from Th1 to Th2 cytokines.

Materials and Methods: This study was carried out on 140 HIV infected patients (21 treatment naïve and 119 under treatment) and 35 matched healthy controls refereed to Iranian Research Center for HIV/AIDS, Tehran, Iran. The serum samples were checked with enzyme-linked immunosorbent assay (ELISA) for interleukin (IL)-2, IL-4, IL-10 and interferon (IFN)-gamma. The Chi-square and t^2-tests were used with the SPSS 16 package program for statistical analysis

Results: A total of 140 HIV positive patients with mean age 36.9±9.2 years and 35 matched controls were enrolled in the study. IL-2 level was relatively higher and IL-10, IL-4 and IFN-gamma levels were relatively lower in the treatment naïve group than the under treatment group. Except for IL-2, all of the other cytokines exhibited a negative correlation with the CD4 cell counts and IFN-gamma levels showed the strongest negative correlation.

Conclusion: Our observations did not demonstrate switching of the type 1 to type 2 T helper cells cytokine profile in HIV infected patients and suggested more complex changes in Th1 to Th2 cytokine patterns in HIV infection.

Keywords: T helper 1 (Th1), T helper 2 (Th2), Cytokine, Human immunodeficiency virus (HIV)

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Introduction

Cytokines serve as the immune response molecules which have various physiological functions and regulate the immunologic, inflammatory and reparative host responses. The cytokines include the interleukins (ILs), interferon (IFN) as well as tumor necrosis factor (TNF), which all shows different functions in vivo. Cytokines provide complicated networks, which regulate the immune responsiveness. The T helper (Th) 1/Th2 balance represents one of these networks (1). This balance determines the differentiation of T cells into Th1 cells which stimulate the cellular immunity or Th2 cells which activate the humoral immunity (2).

Human immunodeficiency virus (HIV) is the etiological agent of AIDS in humans. The infection is usually accompanied by changes in the synthesis and secretion of several immunologically important cytokines, such as IL-15, TNF-α, IFN–gamma, IL-4, IL-12, IL-10 and IL-18 (3). The production of many of these cytokines revealed an aberration in HIV infected individuals. These cytokine disturbances play significant role in progression of HIV infection to AIDS (4–6).

The HIV infection is characterized by a progressive destruction of CD4 T cells, which leads to severe immunodeficiency and disease progression (7, 8). This depletion of CD4 T cells in the circulation of HIV infected individuals is a hallmark of AIDS (9).

Clerici and Shearer originally proposed a model wherein a Th1 → Th2 shift in cytokine expression occurs in HIV infected patients progressing to AIDS (1, 10). This imbalance between Th1 and Th2 responses favors humoral immune responses over cell mediated immunity. It was proposed that an activation of the Th1 associated cytokines (cellular immune response) have protective effects against HIV infection, whereas a shift towards an augmented humoral Th2 response may be adverse and lead to the progression of HIV infection to AIDS. The cytokines responsible for the Th1/Th2 balance, such as IL-2 (11), IL-6 (12), IL-10 (13), and IFN-gamma (14), have been studied in various surveys. However, conflicting data has questioned the validity the Th1 → Th2 hypothesis (15-17). To date some groups have shown evidence for supporting the Th1 → Th2 hypothesis, while others have shown the opposite (1, 10, 15-17).

In the pre-HAART (Highly Active Antiretroviral Therapy) era, the levels of several cytokines increased, along with the progression of immunodeficiency but, after HAART initiation, the boosted cytokines were decreased by antiretroviral treatment (14, 18- 21).

The aim of the present study was to assess the level of Th1 and Th2 cytokines (IL-2, IL-4, IL-10 and IFN-gamma) in treatment naïve and undergoing treatment HIV infected individuals in order to identify the switch from Th1 to Th2 cytokines in these patients.

Materials and Methods

In this cross sectional study, 140 HIV positive patients referred to Iranian Research Center for HIV/AIDS in Tehran, Iran and 35 matched (age and sex) HIV-seronegative healthy subjects were enrolled from September 2009 to September 2010. Twenty one HIV infected patients were treatment naive and 119 cases were under HAART.

Informed consent was taken from all patients. A questionnaire that gathered epidemiological and clinical data was completed by clinicians. The project was approved by Ethical Committee of Pasteur Institute of Iran.

HIV-antibody was determined by ELISA (MP Biomedicals, Illkirch, France); with

positive tests confirmed by Western blot assay (Diaplus, San Francisco, USA). All assay protocols, cut-offs, and result interpretations were carried out according to the manufacturers' instructions.

CD4 count was determined by flowcytometry and defined as cells/mm^3. All cases were tested for HBsAg, anti-HBs and hepatitis C antibody (anti-HCV) by ELISA. The commercial enzyme immunoassay kits used were as follows: HBsAg and anti-HBs (Hepanosticka Biomerieux, Boxtel, The Netherlands) and anti-HCV (Biorad, Segrate, Italy). Recombinant immunoblot assay (RIBA Innogenetics, Ghent, Belgium) was employed to confirm anti-HCV reactivity.

All subjects were tested for IL-2, IL-4, IL-10 and IFN-gamma by ELISA (Ani Biotech Oy, Helsinki, Finland).

Statistical Analysis

The Chi-square and t^2-tests were used with the SPSS 16 package program for statistical analysis (Chicago, IL, USA). Multiple comparisons were carried out using the Kruskal-Wallis non-parametric analysis of variance (ANOVA) test. The Spearman rank test was used for correlation. The significance level was set at $P<0.05$. Data are presented as mean ± SD or, when indicated, as an absolute number and percentage.

Results

A total of 140 HIV positive patients (21 treatment naïve and 119 under treatment) with mean age 36.9±8.8 (range: 18-67) years and 35 matched healthy controls were enrolled in the study. Seventy percent of patients were male and 30% were female. The mean CD4 count of patients was 277.2±176.8 (17-800) cells/mm^3. The possible routes of HIV transmission were intravenous drug use (52.9%), heterosexual contact (30%), infected blood and blood products transfusion (2.9%), tattooing (1.4%), IDU and tattooing (0.7%), heterosexual contact and intravenous drug use (5.7%), heterosexual contact and infected blood (0.7%) and in 5.7% the route of HIV acquisition was not identified. Co-infection with HCV and HBV occurred in 52.9% and 5% of patients, respectively. HBV/HCV co-infection was observed in 2.9% of cases. Anti-HBs was positive in 35.7% of cases.

First we determined the levels of Th1 and Th2 cytokines (IL-2, IL-4, IL-10 and IFN-gamma) in cases and controls.

To examine the relationship between the serum cytokine levels and HIV infection, the patients were classified into three groups on the basis of their CD4 counts [low (<200); moderate (200–350) and high CD4 cell counts (>350)].

We showed low serum levels of IL-2, IL-4, IL-10 and IFN-gamma in HIV infected individuals compared with HIV negative subjects.

IL-2 levels was relatively higher in the treatment naive group (mean; 5.7±8.7 pg/ml) than the under treatment group (mean value; 4.7±9.9 pg/ml). Whereas IL-10, IL-4 and IFN-gamma levels was relatively lower in the treatment naive group (mean; 5.8±8.02 pg/ml, 3.9±2.6 pg/ml, 3.8±1.5 pg/ml) than the under treatment group (mean; 6.14±10.08, 4.05 ±2.9, 3.9 ±2.8 pg/ml).

Subsequently, the correlation between the serum cytokine levels and CD4 counts was analyzed using Spearman's rank test. Except for IL-2, all of the other cytokines exhibited a negative correlation with the CD4 cell counts. Among the cytokines we examined, IFN-gamma levels showed the strongest negative correlation (Spearman's $r = -0.18$).

Additionally, we employed the Kruskal–Wallis test for each of the HIV infected groups to examine whether cytokines levels differed with CD4 cell count. IL-4, IL-10 and

IFN-gamma peaked within the low CD4 cell count group (<200), whereas a boost in IL-2 was seen in high CD4 cell counts (>350).

We investigated whether HCV co-infection might influence cytokines levels and observed a decrease in IL-2, IL-4 and IFN-gamma in patients co-infected with HCV compared to those monoinfected with HIV, whereas IL-10 was higher in HCV/HIV co-infected patients. A similar pattern was also seen in HIV/HBV co-infected patients.

Discussion

The levels of Th1 and Th2 cytokines (IL-2, IL-4, IL-10 and IFN-gamma) were assessed in HIV infected individuals in order to identify the switch from Th1 to Th2 cytokines in these patients. Our survey showed that IL-4, IL-10 and IFN-gamma levels increased with disease progression, but IL-2 levels decreased with advanced disease. In treatment naïve patients IL-2 was relatively higher and IL-4, IL-10 and IFN-gamma was relatively lower than under treatment patients. So our study demonstrated a decrease in IL-2 levels but not the Th1 to the Th2 shift in the advanced stages of HIV infection. These data also demonstrate that IL-4, IL-10 and IFN-gamma levels were increased and remained high in under treatment patients.

Cytokines are immune system soluble mediators that regulate inflammation, allergy, development and hematopoiesis and contribute in regulation of the immune system (22). Most Cytokines have a wide spectrum of biological effects in several different cell types and mediate their effects by binding to specific high affinity receptors on target cells. HIV infection results dysregulation of the cytokine profiles (23). Two subsets of the human CD4 T helper lymphocytes (Th1, Th2) were initially identified (17). During the course of HIV infection secretion of Th1 cytokines, such as IL-2 and IFN-gamma, is generally decreased, whereas production of Th2 cytokines such as IL-4 and IL-10 is increased. Such abnormal cytokine production plays an important role in the pathogenesis of HIV progression by impairing cellular immunity (23).

In 1993, Clerici *et al.* first reported that an immune dysregulation, such as reduction in antigen induced T cell proliferation and defects in intracellular signaling, which develops with the disease progression in HIV infected individuals, was caused by a shift in the cytokine profile from Th1 to Th2 T cell subsets (24). This cytokine shift may create conditions unfavorable to clearing the virus (25).

Many studies have been conducted on the importance of cytokines in the pathogenesis of HIV infection because it affects the immune system. There are conflicting data from these studies regarding the validity of the Th1 → Th2 hypothesis. Some groups have shown evidence for supporting this hypothesis (10, 26), while others have shown the opposite (1, 10, 15-17).

Graziosi *et al.* (16) demonstrated a constitutive cytokine expression in the HIV infected individuals at different stages of the disease and concluded that the switch from Th1 to Th2 did not occur during the progression of the disease. Westby *et al.* (27) also could not identify the subset shift along with disease progression.

A decreased percentage of cells expressing IL-2 and IFN-gamma in conjunction with an increased proportion of IL-4 and IL-10-producing cells among the CD4 T cells in HIV infected individuals was seen and demonstrated a Th1 to Th2 cytokine shift in the course of HIV infection on a single cell level (28). Becker has also proposed that a Th1 → Th2 shift in cytokine occurs in HIV infected individuals (25).

Our survey showed variability in the cytokine production pattern among HIV infected individuals. We did not find the Th1 to the Th2 shift in the advanced stages of HIV infection. Our results are in agreement with Fakoya (15), Graziosi (16), Tanaka (17) and Westby (27) studies but in contrast with Klein (28), Becker (25) and some other studies (10, 26). These conflicting data may be related to various factors such as the size of the study groups and the demographic and epidemiologic factors.

Conclusion

Our observations did not suggest switching of the type 1 to type 2 T helper cells cytokine profile in HIV infected patients and suggested more complex changes in Th1 to Th2 cytokine patterns in HIV infection. However, further studies including longitudinal studies as well as a larger population sample are necessary to confirm our findings.

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