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The comparison of Th1, Th2, Th9, Th17 and Th22 cytokine profiles in acute and chronic HIV-1 infection

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ABSTRACT

The aim of this study was to compare cytokine expression on both gene and protein levels in acute and chronic phase of HIV type 1 (HIV-1) infection. Thirty four patients were enrolled for cytokine expression analysis on protein level in acute and chronic stage of HIV-1 infection. Using PCR array technology, expression of 84 cytokine genes was measured in 3 patients in acute and 3 patients in chronic stage of HIV-1 infection. Bead-based cytometry was used to quantify levels of Th1/Th2/Th9/Th17/Th22 cytokines. The results showed statistically significant increase of 13 cytokine gene expression (*cd40lg*, *csf2*, *ifna5*, *il12b*, *il1b*, *il20*, *lta*, *osm*, *spp1*, *tgfa*, *tnfsf 11*, *14* and *8*) and downregulation of the *il12a* expression in chronic HIV type 1 infection. Concentrations of IL-10, IL-4 and TNF-α were increased in the acute HIV type 1 infection when compared to control group. During chronic HIV type 1 infection there was an increase of IL-10, TNF-α, IL-2, IL-6, IL-13 and IL-22 levels when compared to control group. Comparison of cytokine expression between two stages of infection showed a significant decrease in IL-9 concentration. This study showed changes in cytokine profiles on both gene and protein levels in different stages of HIV-infection.

1. Introduction

Infection with human immunodeficiency virus type 1 (HIV-1) is characterized by a continuous depletion of helper $CD4^+$ T-cells and hyperactivation of the immune system [1,2]. The course of infection can be classified into several stages. The eclipse phase is a period between 1 and 2 weeks post infection when the virus replicates and spreads from the origin of infection to different tissues and organs [3]. Rapid increase in viremia and simultaneous decrease in the population of $CD4^+$ T-cells (particularly in gut-associated lymphoid tissue, GALT), mark the onset of acute stage of infection [4,5]. According to the Fiebig's score, acute HIV-1 infection can be classified into six stages, based on the serological and molecular assays for detection of p24 antigen, virus-specific antibodies and HIV-1 RNA [6,7]. Upon activation of host-specific cellular immunity, HIV-1 infection progresses to the stage of clinical latency with usually low level viremia for long periods of time (up to 20 years). High level of immune activation is also present during chronic infection, as illustrated by increased expression of activation markers CD38, HLA-DR and Ki67 [8]. In time, constant viral replication and immune activation cause further decrease in $CD4^+$ T-cell counts, immune system loses control over infection leading to opportunistic infections, malignancies and death of untreated individuals [3]. Cytokines are biological response modifiers synthesized by cells of innate and adaptive immunity that are important in intercellular communication and regulation of immune reactions [9–12]. Effector functions of $CD4^+$ T-cells depend on cytokine immunity. Naive $CD4^+$ T-cells differentiate to effector cell after recognition of antigenic peptides bound to MHC Class II molecules expressed on antigen-presenting cells [13]. Depending on cytokine environment, $CD4^+$ T-cells differentiate into Th1, Th2, Th9, Th17, Th22 as well as regulatory (Treg) and follicular (Tfh) cell populations that have different biological functions [14]. The role of cytokines in the pathogenesis of HIV-disease, particularly in the context of Th cytokine profiles, has not been fully elucidated. In acute HIV

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infection, increasing viral replication induces the synthesis of various cytokines [15]. Concentrations of IFN-α and IL-15 increase within the 5 days following the onset of measurable plasma viremia. Subsequently, increased concentrations of TNF-α, MCP-1, IL-6, IL-8, IL-18 and IFN-γ are also detected [16]. Cytokines synthesized in early HIV-1 infection can have a complex effect on the host. They can accelerate the immune response against the virus [17], while simultaneously, cytokine activity amplifies the population of target cells for HIV infection by activating $CD4^+$ T-cells. In chronic HIV infection, elevated concentrations of proinflammatory cytokine TNF-α and its receptor TNF-RII indicate long-term inflammation in infected persons [18,19].

Literature data on the cytokines corresponding to the effector profiles of $CD4^+$ T-cells are scarce. In 1993, Clerici and Shearer introduced a hypothesis that shift from Th1 to Th2 cytokine profile is crucial for the immunopathogenesis of HIV-disease [20] but *in vivo* and *ex vivo* studies by other groups did not support these findings [21] and the issue remains controversial. In untreated HIV-infected individuals, depletion of Th17 cells can be observed in the gut mucosa and is linked to the loss of integrity of gut mucosal barrier [22]. In HIV-infected children, Ndhlovu et al. found a positive correlation between viremia and decrease in Th17 cell count [23]. Th22 cells synthesize IL-22, a cytokine responsible for the maintenance of epithelial barrier integrity. In HIV-1 infection, Th22 cells are depleted leading to the systemic immune activation and loss of mucosal immunity [24].

The aim of this study was to compare the expression of genes coding for cytokines and other biological response modifiers in acute and chronic HIV infection and to analyze the patterns of Th1, Th2, Th9, Th17 and Th22 cytokines in the plasma of HIV-infected persons at the acute and chronic stage of infection.

2. Methods

2.1. Subjects

The study is a retrospective analysis of cytokine expression on both gene and protein levels in HIV-infected individuals receiving clinical care at the Croatian Reference Center for HIV/AIDS of the University Hospital for Infectious Diseases (UHID) in Zagreb, Croatia. HIV-infected pregnant women, minors and HIV-infected individuals with intercurrent disease were excluded from the study. Thirty four patients were enrolled for cytokine expression analysis on protein level in acute and chronic phase of HIV-1 infection. Cytokine gene expression was determined in 6 patients, 3 patients were in acute and 3 patients were in the chronic stage of HIV-infection. As a control group, 22 HIV-negative individuals were enrolled in the study. Ethics committees at the UHID and Faculty of Medicine approved the study and all subjects signed a consent form.

2.2. Study design

Cytokine expression on protein level in acute and chronic stage of HIV-1 infection was determined in 3 time points. The first time point represents plasma samples obtained from HIV-infected individuals with acute infection. Plasma samples from the same patients were also collected after 6 and 12 months, respectively (second and third time point).

2.3. Immune and virological monitoring

HIV-1 RNA quantification and absolute $CD4^+$ T cell count data were extracted from the database of Department of molecular diagnostics and flow cytometry at UHID on dates corresponding the samples used for cytokine expression analysis.

HIV-1 RNA quantification was performed by using COBAS AmpliPrep/COBAS TaqMan HIV-1 Test (Roche Diagnostics, Manheim, Germany) as recommended by the manufacturer.

Absolute $CD4^+$ T cell count was determined using flow cytometer Cytomics FC500 (Beckman Coulter, Brea, California, USA). Periferal blood was stained with fluorochrome-conjugated antibodies CD45-FITC/CD4-RD-1/CD8-ECD/CD3-PC-5 (Beckman Coulter) and quantification reagent Flow-Count Fluorospheres (Beckman Coulter) was used to obtain absolute CD4+T cell count.

2.4. Bead-based cytometry for cytokine levels analysis

To determine the concentrations of 13 cytokines (IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p70, IL-13, IL-17A, IL-22 and TNF-α) in plasma samples Human Th1/Th2/Th9/Th17/Th22 13plex Kit FlowCytomix (eBioscience, San Diego, California, USA) was used according to the manufacturer's instructions. In brief, this test uses beads coated with antibodies specific for each of 13 cytokines to be detected. They are mixed with 25 μl of plasma samples. After incubation, biotin-conjugated second antibody is added and the presence of analyzed cytokine is detected using streptavidin-phycoerithrin that binds to the biotin conjugate and emits fluorescent signal. Detection process is carried out on flow cytometer Cytomix FC500 (Beckman Coulter) and Forward Scatter measurements were collected at 1–8°. Obtained results were processed using FlowCytomixPro software to determine cytokine concentration.

2.5. PCR array technology for cytokine gene expression analysis

Peripheral blood was collected in PAXgene RNA tube (PreAnalytiX, Hombrechtikon, Switzerland) and RNA was isolated using PAXgene RNA Kit (PreAnalytix) according to the manufacturer's instructions. Quality and concentration of isolated RNA were tested and cDNA was synthesized using QIAGEN RT^2 First Strand Kit (QIAGEN, Hilden, Germany) following manufacturer's instructions. Prepared cDNA was added to the RT2 SYBR Green qPCR Master Mix (QIAGEN) and alliquotted on 96-well plate of RT^2 Profiler PCR Array Human Common Cytokines (QIAGEN). Array contains a panel of 96 primer sets of that 84 specific for researched cytokine genes, 5 housekeeping genes and 3 RNA and PCR quality controls. Expression of following genes was determined: group of interferon genes (IFNA1, IFNA2, IFNA4, IFNA5, IFNB1, IFNG), interleukin genes (IL-10, IL-11, IL-12A, IL-12B, IL-13, IL-15, IL-16, IL-17A, IL-17B, IL-17C, IL-18, IL-19, IL-1A, IL-1B, IL-1RN, IL-2, IL-20, IL-21, IL-22, IL-23A, IL-24, IL-25 (IL-17E), IL-27, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, TXLNA (IL-14)), growth factors (CNTF, CSF1 (MCSF), CSF2 (GM-CSF), CSF3 (GCSF), FIGF (VEGFD), LEFTY2 (EBAF), LIF, NODAL, OSM, PDGFA, TGFA, THPO, VEGFA), TGF-β family (BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, GDF2 (BMP9), GDF5 (CDMP-1), GDF9, INHA, INHBA, MSTN (GDF8), TGFB1, TGFB2, TGFB3), TNF family (CD40LG (TNFSF5), CD70 (TNFRSF7), FASLG (TNFSF6), LTA (TNFB), LTB, TNF, TNFRSF11B, TNFSF10 (TRAIL), TNFSF11, TNFSF12, TNFSF13, TNFSF13B, TNFSF14, TNFSF4 (OX40L), TNFSF8) and other cytokines (ADIPOQ, FAM3B, SPP1 (Osteopontin)). PCR reactions were carried out in a ABI 7500 Standard (Applied Biosystems, Foster City, California, USA) under given conditions: 10 min on 95 °C, 40 cycles for 15 s on 95 °C and 1 min on 60 °C. Calculations and analysis of Ct values and dissociation curves for all PCR reaction were made using 7500 System SDS Software v1.4.0 (Applied Biosystems). Data analysis was performed using RT2 Profiler PCR Array Data Analysis v3.5 (http://www.sabiosciences.com/pcrarraydataanalysis.php). Volcano plot was created to visualize and identify significant gene expression changes.