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Source / Izvornik: **Microbial Pathogenesis, 2016, 97, 125 - 30**

Journal article, Accepted version
Rad u časopisu, Završna verzija rukopisa prihvaćena za objavljivanje (postprint)

https://doi.org/10.1016/j.micpath.2016.06.008

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:105:186418

Gorenec L., Židovec Lepej S., Grgić I., Planinić A., Iščić Beš J., Vince A., Begovac J. (2016) ***The comparison of Th1, Th2, Th9, Th17 and Th22 cytokine profiles in acute and chronic HIV-1 infection.*** **Microbial Pathogenesis, 97. pp. 125-30. ISSN 0882-4010**

http://www.elsevier.com/locate/issn/08824010

http://www.sciencedirect.com/science/journal/08824010

http://dx.doi.org/10.1016/j.micpath.2016.06.008

TITLE: 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.

KEYWORDS: HIV-1; biological response modifiers; Th1/Th2 cytokine shift; acute HIV-1 infection; chronic HIV-1 infection

HIGHLIGHTS:

- HIV-1 infection causes cytokine profile changes on both gene and protein levels
- In chronic HIV-1 infection 13 cytokine-encoding genes were overexpressed
- Levels of IL-9 were significantly reduced with the progression of HIV-1 infection
- The progression of HIV-1 infection is not associated with Th1 to Th2 shift

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] and [2]. The course of infection can be classified into several stages. The eclipse phase is a period between 1-2 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] and [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] and [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], [10], [11] and [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 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] and [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 synthesised 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.

2.6. Statistical analysis

Statistical analysis was performed using SAS v9.3 software (SAS Institute, Cary, North Carolina, USA). Variables with non-normal distributions were described with medians and interquartile ranges. Independant groups were compared with the Fisher`s exact test. Within the same group of examinees Wilcoxon signed rank test was used. Relative changes in gene expression were calculated using $\Delta\Delta C_t$ method and the differences between gene expression in acute and chronic HIV-1 infection were compared with the Student`s t-test. Statistical significance was set at $P < 0.05$

3. RESULTS:

3.1. Comparison of cytokine gene expression in acute and chronic stage of HIV-1 infection

Cytokine gene expression was compared in six HIV-infected individuals, three of them were in the acute and three in chronic stage of HIV-1 infection. Of 84 analyzed genes, 29 genes were overexpressed in chronic stage when compared to the acute stage of HIV-1 infection. Statistically significant gene expression increase between two stages of infection was noted in 13 genes (Table 1). During chronic stage of HIV-1 infection 4 genes were downregulated and for one of them, *il12a*, this decrease in expression was statistically significant (Table 2).

Table 1. Overexpressed genes in chronic compared to the acute stage of HIV-1 infection

Gene symbol	Fold regulation	*P*-value
BMP3	3.893	0.271
BMP4	2.949	0.131
BMP6	2.054	0.609
CD40LG	2.472	**0.031**
CSF2	3.067	**0.015**
FAM3B	3.138	0.481
IFNA5	3.502	**0.026**
IL12B	2.123	**0.003**
IL17A	2.025	0.158
IL1A	3.253	0.419
IL1B	2.869	**0.049**
IL20	4.833	**0.017**
IL24	2.156	0.157
IL4	3.312	0.256
IL8	3.601	0.073
INHBA	2.077	0.225
LTA	2.249	**0.005**
LTB	2.733	0.069
OSM	2.141	**0.025**
PDGFA	2.499	0.372
SPP1	47.099	**0.045**
TGFA	2.857	**0.045**
TGFB2	5.405	0.146
TNF	2.651	0.225
TNFSF11	5.030	**0.009**
TNFSF13	2.843	0.165
TNFSF14	2.263	**0.042**
TNFSF4	2.255	0.236
TNFSF8	2.386	**0.047**

Gene symbols in bold represent genes with statistically significant increase in expression in chronic compared to the acute stage of HIV-1 infection. Expression of 84 cytokine genes was

measured in 3 patients in acute and 3 patients in the chronic phase of HIV-1 infection. Gene symbols: **BMP3** (Bone morphogenic protein 3), **BMP4** (Bone morphogenic protein 4), **BMP6** (Bone morphogenic protein 6), **CD40LG** (CD40 ligand), **CSF2** (Colony stimulating factor 2), **FAM3B** (family with sequence similarity 3, member B), **IFNA5** (Interferon alpha 5), **IL12B** (Interleukin 12, subunit β), **IL17A** (Interleukin 17, subunit α), **IL1A** (Interleukin 1, subunit α), **IL1B** (Interleukin 1, subunit β), **IL20** (Interleukin 20), **IL24** (Interleukin 24), **IL4** (Interleukin 4), **IL8** (Interleukin 8), **INHBA** (Inhibin beta A), **LTA** (Lymphotoxin α), **LTB** (Lymphotoxin β), **OSM** (Oncostatin M), **PDGFA** (Platelet-derived growth factor α polypeptide), **SPP1** (Secreted phosphoprotein 1), **TGFA** (Transforming growth factor α), **TGFB2** (Transforming growth factor β2), **TNF** (Tumor necrosis factor), **TNFSF11** (Tumor necrosis factor superfamily, member 11), **TNFSF13** (Tumor necrosis factor superfamily, member 13), **TNFSF14** (Tumor necrosis factor superfamily, member 14), **TNFSF4** (Tumor necrosis factor superfamily, member 4), **TNFSF8** (Tumor necrosis factor superfamily, member 8).

Table 2. Downregulated genes in chronic compared to the acute stage of HIV-1 infection

Gene symbol	Fold change	*P*-value
CD70	-3.134	0.079
IL12A	-2.259	**0.021**
IL21	-4.611	0.147
THPO	-3.713	0.131

Gene symbol in bold represent gene with statistically significant decrease in expression in chronic compared to the acute stage of HIV-1 infection. Expression of 84 cytokine genes was measured in 3 patients in acute and 3 patients in the chronic phase of HIV-1 infection. Gene symbols: **CD70** (Cluster of differentiation 70), **IL12A** (Interleukin 12, subunit α), **IL21** (Interleukin 21), **THPO** (Thrombopoietin).

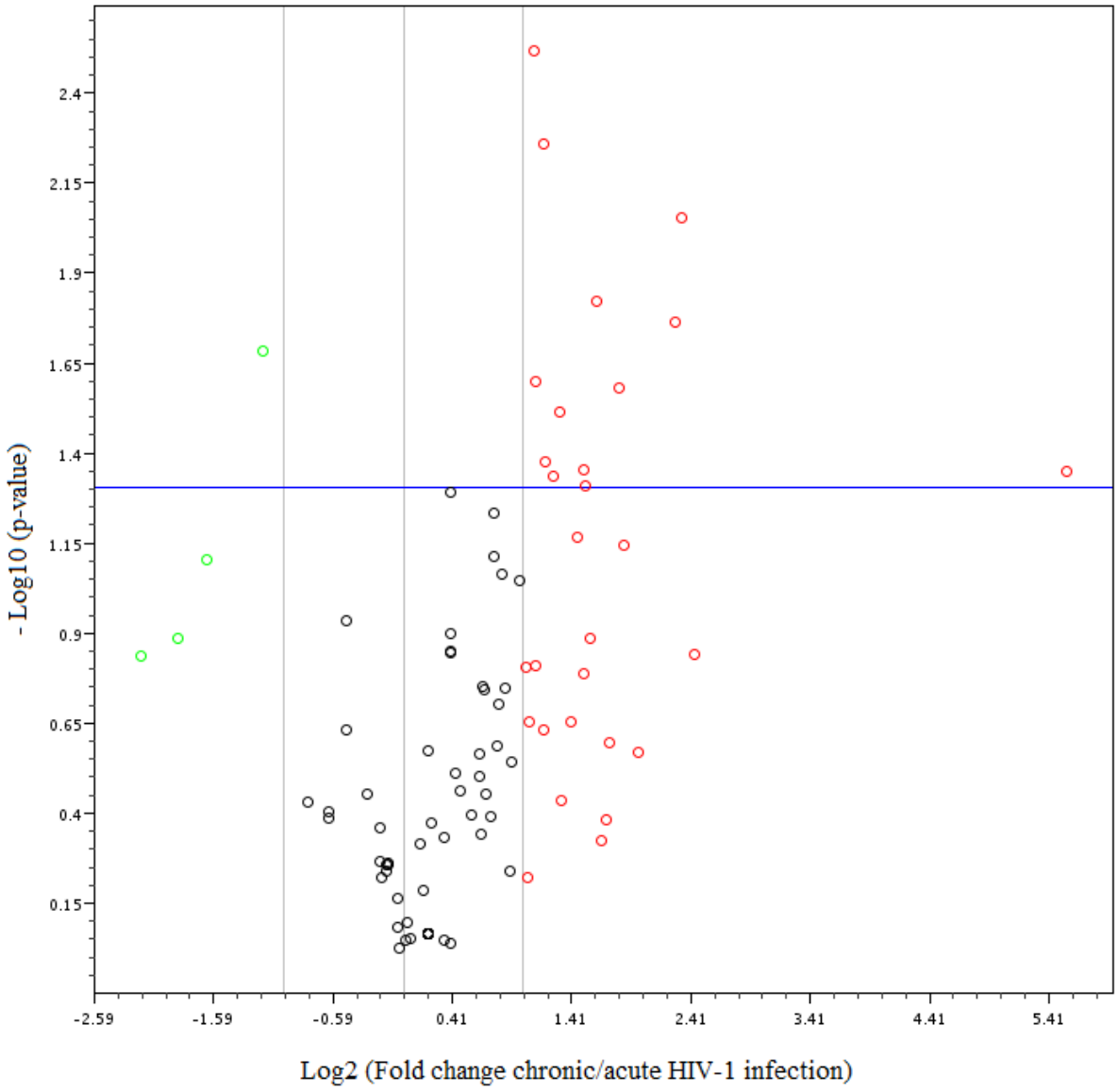

FIG. 1. Volcano plot for 84 analyzed genes. On X-axis is the log2 value of the fold change between chronic and acute HIV-1 infection and Y-axis represents –log10 p-value. Red coloured circles symbolize overexpressed and green coloured circles represent underexpressed genes. Horizontal blue line shows threshold where $P = 0.05$.

3.2. Th1, Th2, Th9, Th17 and Th22 cytokine levels in acute and chronic HIV-1 infection

Concentration measurements of 13 cytokines in plasma samples obtained from 34 HIV-1 positive individuals were conducted in 3 time points. First sample was taken during acute stage (T1) of HIV-1 infection. The median plasma viremia was 224 500 HIV-1 RNA copies/ml (IQR 74 808-883 000 HIV-1 RNA copies/ml). The median of CD4+ T cell count was 396 cells/µL (IQR 269-466 cells/µL). Six months after first time point concentrations of cytokines were again measured. In this second time point (T2) viremia decreased with median of 52 HIV-1 RNA copies/ml (IQR 0-30 214 HIV-1 RNA copies/ml). The median of CD4+ T

cell count was 569 cells/μL (IQR 395-700 cells/μL). In third time point (T3), 12 months after first, the median plasma viremia was 31 HIV-1 RNA copies/ml (IQR 0-541 HIV-1 RNA copies/ml). The median of CD4+ T cell count was 593 cells/μL (IQR 457-734 cells/μL). Concentration measurements of 13 cytokines were also performed in 22 HIV-negative individuals. All the data for both HIV-infected patients as well as control group are presented in Table 3.

Table 3. Concentrations of 13 cytokines measured during acute and chronic HIV-1 infection in 34 individuals and 22 controls, HIV-negative group

	Acute infection (T1)	**Chronic infection (T2)**	**Chronic infection (T3)**	**HIV-negative group**
Cytokine	**Median cytokine conc (IQR; pg/mL)**	**Median cytokine conc (IQR; pg/mL)**	**Median cytokine conc (IQR; pg/mL)**	**Median cytokine conc (IQR; pg/mL)**
IL-12(p70)	3.7 (0.3-11.1)	3.9 (0.1-11.1)	3.4 (1.4-15.1)	0.3 (0.0-5.49)
IFN-γ	16.7 (0.0-34.2)	0.0 (0.0-21.8)	9.2 (0.0-31.3)	1.2 (0.0-107.0)
IL-17A	30.5 (0.0-100.8)	18.2 (4.4-74.2)	20.3 (1.7-131.1)	13.1 (0.0-123.4)
IL-2	34.1 (0.0-113.6)	32.0 (10.2-179.6)	47.9 (29.7-255.6)	3.0 (0.0-118.5)
IL-10	12.1 (3.8-29.5)	11.2 (4.9-23.5)	12.1 (5.5-23.8)	0.5 (0.0-10.6)
IL-9	29.6 (0.0-220.6)	0.6 (0.0-39.1)	5.9 (0.0-42.0)	0.3 (0.0-46.3)
IL-22	127.6 (0.0-215)	141.9 (0.0-223.7)	156.5 (35.8-263.5)	0.0 (0.0-72.5)
IL-6	1.9 (0.0-5.1)	0.7 (0.0-3.1)	1.1 (0.0-150.4)	0.0 (0.0-0.7)
IL-13	67.1 (2.4-115)	98.5 (28.6-121.7)	42.5 (22.2-136.9)	0.0 (0.0-119.7)
IL-4	77.6 (0.0-252.4)	63.6 (0.0-164.8)	45.8 (0.0-150.4)	0.3 (0.0-82.3)
IL-5	18.2 (7.9-37.8)	21.1 (3.9-32.9)	19.8 (12.3-30.5)	20.7 (11.1-86.0)

IL-1β	38.0 (12.7-54.4)	32.4 (13.4-79)	39.3 (21.8-78.8)	17.6 (0.0-74.9)
TNF-α	5.4 (0.0-36.2)	8.1 (0.0-16.2)	11.3 (1.7-23.4)	0.0 (0.0-5.3)

T1- acute HIV-1 infection; T2- 6 months post acute infection; T3- 12 months post acute infection

Our results show statistically significant increase in IL-10, IL-4 and TNF-α concentrations in acute stage of HIV-1 infection when compared to results obtained from healthy controls (P = 0.008; P = 0.019; P = 0.04, respectively). When we measured cytokine concentrations in samples gathered six months after acute infection, levels of IL-10 and TNF-α were still significantly higher than those in control group and we also noticed incerase in IL-22 concentration (P = 0.01; P = 0.037; P = 0.02, respectively). The same three cytokines, IL-10, TNF-α and IL-22 maintained significantly increased during chronic stage in samples collected 12 months after acute HIV-1 infection (P = 0.003; P = 0.007 and P = 0.014, respectively). Besides them, in the same time period IL-2, IL-6 and IL-13 concentrations were also elevated when compared to the control group (P = 0.028; P = 0.046 and P = 0.037, respectively). To see is there a change in cytokine concentrations with the progression of HIV-1 infection we compared their levels among HIV-1 infected patients. The only cytokine with statistically significant change was IL-9 with its levels decreasing from acute to chronic stage of HIV-1 infection (P = 0.04). When comparing cytokine levels in the same group of 34 HIV-1 infected individuals, but only in those with undetectable viremia at the third time point, 12 months after acute HIV-1 infection (N=16, 42.1%), concentrations of IL-2, IL-10, IL-22, IL-4 and TNF- α were significantly increased compared to controls (P = 0.024; P = 0.014; P = 0.026; P = 0.044 and P = 0.018, respectively).

4. DISCUSSION:

The results of this study showed increased transcriptional activity of 13 cytokine genes and downregulation of *il12a* gene in chronic versus acute HIV-1 infection. Increased serum concentrations of selected cytokines (IL-10, IL-4, TNF-α, IL-2, IL-6, IL-13 and IL-22) were found in acute and/or chronic stage of infection compared to controls. Norris et al showed increased concentrations of IL-10, TNF-α and IFN-γ in acute HIV infection compared to controls as well as a correlation between the concentration of the three cytokines and plasma viremia prior to seroconversion [25]. The results of our study also showed increased concentrations of IL-10 and TNF-α in acute HIV infection compared to controls but failed to demonstrate a correlation of cytokine concentrations in the serum and viremia. The difference in the patient selection strategies and timeline of sampling (newly diagnosed HIV-infected plasma donors and patients with symptomatic primary HIV-1 infection vs. patients with acute/recent HIV-infection) could have contributed to the observed difference in the results.

Stacey et al analysed the changes in the cytokine profiles during the „eclipse“ phase of acute HIV-1 infection by analysing samples collected in 2-5 day periods [26]. Initial increase in IFN- α, IL-15, TNF- α, IP-10 and MCP-1 concentrations was followed by a rise in IL-6, IL-8, IL-10, IL-18 and IFN-γ whereas the increase in IL-4, IL-5, IL-12 and IL-22 concentrations was observed later. Contrary to these results indicating a „cytokine storm“ during the acute HIV-1 infection, the results of our study indicate increase in a limited number of cytokines (IL-10, IL-4 and TNF-α) in acute infection. However, it should be noted that a direct comparison between these results is not possible due to the differences in the sampling period in the two studies (eclipse phase vs. acute/recent stage of infection). Stylianou et al showed increasing levels of IL-10 and TNF- α level in HIV-infected patients, regardless of the stage of infection but with highest concentrations in patients with the most advanced disease [27]. Our study also demonstrated increased concentrations of IL-10 and TNF- α in HIV-infected patients in both acute and chronic stage of infection compared to controls. However, increased concentrations of these cytokines in advanced disease were not observed. The concept of a shift from Th1 to Th2 cytokine profiles as one of the key factors in the pathogenesis of HIV disease has been a controversial issue for many years. Several studies on this issue using diverse methodological approaches showed conflicting results [20], [21], [28] and [29]. According to our results, selected Th2 cytokines are already increased in acute HIV infection as well as during subsequent chronic stages of infection. Furthermore, the results of this study confirm the absence of Th1 to Th2 cytokine profile shift. IL-22 is important for the maintenance of the intestinal mucosa integrity. It has been previously shown that chronic HIV infection leads to the exhaustion of Th22 cell subset [30]. However, our study showed increased concentrations of IL-22 in chronic HIV-1 infection and this issue requires further investigations. The results of our study showed increased concentrations of IL-13 in the samples collected one year after acute HIV infection compared with controls. These results are in concordance with a study showing increased concentrations of IL-13 and IFN-γ levels following a decline in the viral load in HIV-infected persons [31]. IL-6 is a cytokine with various biological functions including B-cell stimulation, monocyte differentiation and induction of IL-4 producing cells. In vitro data in mitogen-stimulated CD4+ T-cells from HIV-infected individuals showed increased synthesis of IL-6 [32] and [33]. The results of our study also demonstrated increased levels of this cytokine in chronic HIV-1 infection which is consistent with literature data. Literature data on the role of IL-9 in HIV infection are scarce. Guzman-Fulgenzio et al showed that HIV/HCV coinfected persons with increased concentrations of IL-9 and IL-6 were less likely to achieve sustained virological response during double (PEG IFN-α and ribavirin) treatment of chronic hepatitis C [34]. Our study provides the first available evidence on a decrease in the serum concentrations of IL-9 between acute and chronic stage HIV-1 infection. The results of our study showed consistently increased concentrations of IL-12 in HIV-infected individuals compared with controls, irrespective of the stage of infection. Interestingly, we observed a different effect of HIV-infection on the levels gene expression for the two IL-12 subunits (*il12a* was downregulated whereas *il12b* was upregulated). Since similar observations have not been previously reported, these results require further investigation.

In conclusion, this study showed important changes in the expression of Th1, Th2, Th9, Th17 and Th22 cytokines in acute and chronic stage of HIV-infection. Increased cytokine synthesis during the chronic stage of infection, even in treated patients with undetectable viremia, suggests persistent hyperactivation of the immune system in HIV infection.

Acknowledgments

This research was in part funded by the Croatian science foundation project titled „Molecular, epidemiological and clinical features of HIV infection in Croatia“ (principal investigator Prof. Josip Begovac) and the Croatian Ministry of Science project titled „Immune reconstitution and resistance to antiretroviral drugs in HIV-patients from Croatia“ (principal investigator Snjezana Zidovec Lepej, PhD, Scientific Advisor)

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