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Original Research Article

IL-4, *IL-10*, *TNF-α* profile and immunological changes in North-Eastern Ukrainian HIV-infected individuals

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ABSTRACT

Background: Cytokines play an important role in controlling the homeostasis of the immune system in HIV infection. The measurement cytokines in patients with HIV may provide additional information to complement prognostic markers and understand disease procession. Aim of the study was to determine *IL-4*, *IL-10* and *TNF-α* profiles in plasma of HIV-infected individuals with different CD4 T-cell levels.
Methods: Quantitative level detection of *IL-4*, *IL-10*, *TNF-α* was carried out by ELISA (test systems "Vektor-Best", RF) in 78 HIV-infected people (group I: 35 persons with level of T-helpers ≥350 cells/μL; group II: 43 persons with level of T-helpers ≤200 cells/μL).
Results: HIV-infection was associated with an increase in plasma levels of *TNF-α* and *IL-10*. Immune imbalance due to changes in concentrations of cytokines is more pronounced in HIV-infected individuals with severe immunosuppression with CD4 T lymphocyte counts ≤200 cells/μL. No significant difference in *IL-4* between surveyed troops and comparison group was found.
Conclusion: Immunological status of the patients with HIV is characterized by inefficiency of cellular compartment with imbalance of relation of immunocompetent cells and increased production of proinflammatory *TNF-α* and anti-inflammatory *IL-10*.

Nowadays human immunodeficiency virus causes a pandemic, in consequence of which increase of HIV infection is a significant medical and social problem [1]. However, numerous researches, devoted to study of pathogenesis mechanisms of illness and peculiarities of disease course, don't find unambiguous solution.

One of the factors influencing virus replication in body is cytokines some of them are able to favour HIV replication increasing expression of regulatory agent genes [2]. Many authors indicate that imbalance of interleukins produced by T-helpers of the 1st and 2nd type form the basis of immune system response to virus antigens. Thus, by progression of disease T1 helper response is suppressed that leads to disorder of stimulation of cellular component. At the same time, there is an increase of levels of anti-inflammatory cytokines, which in their turn cause relatively inefficient antibody resistance [3–5].

TNF-α plays a crucial role in viral genome expression by HIV infection. Increase of serum concentration of *TNF-α* by illness progression is noted by many researchers, the majority of which underline correlation of the following increase with quantity reduction of CD4+ lymphocytes and severity of clinical characteristics of disease [6–8]. Significance of *IL-10* in course of disease consists in its ability both decrease and increase viral replication depending on presence of other cytokines. High levels of *IL-10* are recorded in serum of infected people at late stages of the disease [9,10]. More controversial data about the role of *IL-4* are: despite of separate notes about decrease of cytokine level many researches state about increase of its concentration by infection progression [2,11,12].

Therefore, cytokine balance plays an important role in regulation of homeostasis of immune system and influences the course of HIV infection and the research of cytokine status changes in HIV-infected people will allow to understand better a disease pathogenesis and will give additional information about mechanism of disease course.

Aim of study

The aim of this research was to study changes of cytokine profile in HIV-infected people by indexes of *IL-4*, *IL-10* and *TNF-α* content and the peculiarities of disease course depending on cell-mediated immunity.

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Table 1
Social and demographic characteristics in patients with HIV infection.

Index	Group I ($n = 35$)	Group II ($n = 43$)
Average age, years	31.2 ± 0.86	34.5 ± 1.70
Male, abs./%	23/(65.71 ± 8.14)%	30/(69.77 ± 7.09)%
Parenteral way of transmission, abs./%	25/(71.43 ± 7.85)%	27/(62.79 ± 7.46)%
Sexual way of transmission, abs./%	10/(28.57 ± 7.55)%	16/(37.21 ± 7.46)%
HAART, abs./%	11/(31.43 ± 7.96)%	12/(27.91 ± 6.92)%
Experience of HIV infection, years	3.69 ± 0.31	1.90 ± 0.62[a]

[a] Significant difference of index compared to group I, $p < 0.05$.

Materials and methods

78 HIV-infected people were under supervision, among which there were 53 (67.9%) men and 25 (32.1%) women at the age of (32.61 ± 0.87), who were under hospital treatment in Sumy Regional Clinical Infectious Diseases Hospital named after Z.I. Krasovytskyi (SOKIL), Sumy, Ukraine. The patients were divided into groups depending on level of CD4+ T-lymphocytes. 35 people with level of T-helpers ≥350 cells/μL fell under group I, 43 people with level of T-helpers ≤200 cells/μL – under group II. Comparable by sex and age 30 clinically healthy blood donors made experimental group.

Quantitative level detection of *IL-4, IL-10, TNF-α* was carried out by ELISA (test systems "Vektor-Best", RF) in blood serum according to the instructions of manufacturing plant on the basis of SOKIL Clinical Diagnostic Laboratory. Parameters of cellular compartment of immune system (quantity of CD3+ lymphocytes, quantity of CD4+ subpopulation of T-helpers, quantity of CD8+ subpopulation of T-suppressors in 1 μL of blood, ratio index of CD4+/CD8+ lymphocyte subpopulation) are received on the basis of immunologic laboratory of Regional Centre for Prophylaxis and Fight against HIV infection/AIDS. Moreover, generally accepted and provided by the protocol examinations were carried out. Persons were included into the research only by their written consent.

Results

While studying social and demographic data it was found out that HIV-infected people of investigated groups didn't differ by gender, age characteristics and mode of virus infection (Table 1). The fact, that less illness experience was definitely recorded among the patients of group II, draws attention. Thus, in persons with level of CD4+ T-lymphocytes ≤200 cells/μL the average number of years, which passed after establishment of HIV infection diagnosis to the moment of hospitalization, was 2 times less than in persons from group I ($p < 0.01$).

By structural division of groups according to reason HIV1/2 antibody test (Fig. 1) no reliable differences by patient examination were found: as injecting drug users (code 102); people with sexually transmitted diseases (code 104); people with multiple sexual partners, including men, who have sex with other men (code 105); people who has been deprived of their liberty (code 112); pregnant women (code 109). However, definitely greater number of patients who were screened for clinical indications (code 113) was registered in group II that together with little infection experience can indicate late illness diagnosis in this contingent already at the stage of clinical indications.

Comparison of HIV infection clinical stages (World Health Organization, 2006) of patients of studied groups allowed to discover (Fig. 2) that only in persons with level of T-helpers ≥350 cells/μL stage of carrier without symptoms was noted and III clinical stage was met 2.2 times more often ($p < 0.05$). In group II patients with

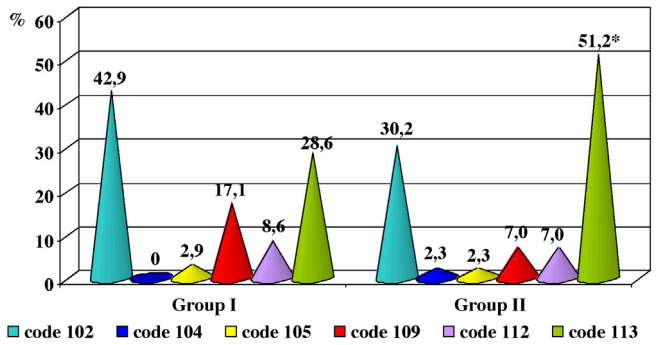

Fig. 1. Groups structure depending on the cause of testing for HIV. *significant difference of index compared to group I, $p < 0.05$.

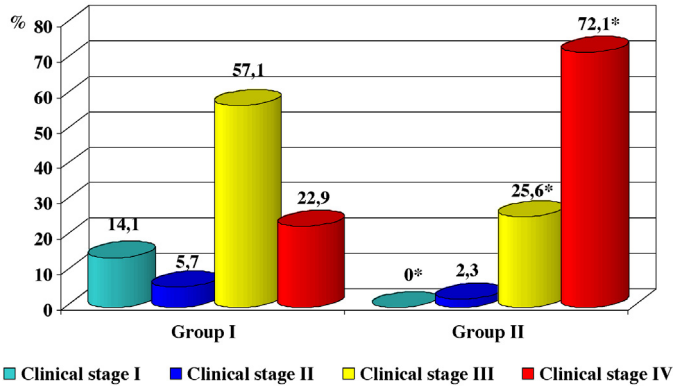

Fig. 2. Groups structure depending on clinical stage of HIV infection. *significant difference of index compared to group I, $p < 0.05$.

final stage of disease dominated (index 3.2 times exceeded the similar one among persons of group I, $p < 0.05$).

At the moment of health encounter general state of patients of group I was estimated as of medium severity in 82.9%, that is 1.7 times more than among the representatives of group II ($p < 0.05$). In patients with level of immunocompetent cells ≤200 cells/μL severe condition was recorded in 30.2% of cases, extremely severe condition – in 18.6%, that 3.5 times exceeds the corresponding indexes of group I and 18.6 relatively ($p < 0.05$).

Study of pathology structure associated with HIV indicated the fact that the most widespread were the infections, caused by Candida fungus and B and C hepatitis viruses (Fig. 3). Only in cohort of persons with level of CD4+ T-lymphocytes ≤200 cells/μL extrapulmonary tuberculosis, cachexia were diagnosed; organic lesion of central nervous system was met definitely more often (2.5 times).

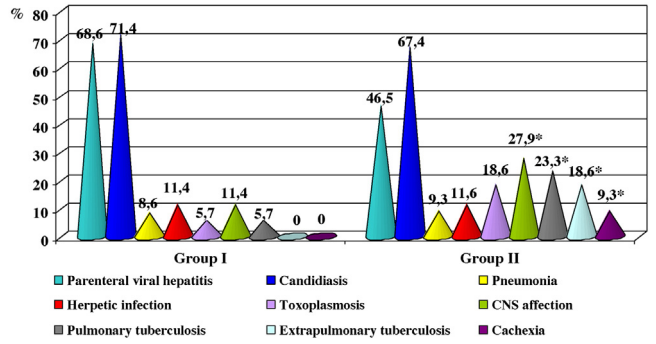

Fig. 3. Clinical manifestations in patients with HIV infection. *significant difference of index compared to group I, $p < 0.05$.

Table 2
Cell immunity indices in patients with HIV infection.

Index	Blood donors ($n=30$)	Group I ($n=35$)	Group II ($n=43$)
CD4+, cells/μL	992.47 ± 29.53	446.35 ± 20.07[a]	115.21 ± 11.72[a,b]
CD3+, cells/μL	1330.77 ± 54.39	1483.08 ± 38.40[a]	835.79 ± 100.65[a,b]
CD8+, cells/μL	828.17 ± 16.01	1042.85 ± 32.01[a]	602.91 ± 95.72[a,b]
CD4+/CD8+	1.21 ± 0.04	0.43 ± 0.01[a]	0.23 ± 0.03[a,b]

[a] Significant difference of index compared to blood donors, $p<0.001$.
[b] Significant difference of index compared to group I, $p<0.001$.

In patients the index of opportunistic infections (number of infections diagnosed with a patient) ranged from 1 to 5. In patients with severe immunodeficiency this index was higher – (3.21 ± 0.22) in group II vs. (1.65 ± 0.32) in I ($p<0.05$).

As it could be seen from the Table 2 in HIV-infected patients by the research of indexes of cellular compartment of immunity significantly decreased indexes of CD4+ T-lymphocytes were recorded in comparison to healthy persons (group I – 2.2; II – 8.6) and ratio decrease of CD4+/CD8+ (group I – 2.8; II – 5.3 times). Average values of pool of CD3+, CD8+ lymphocytes in patients with level of T-helpers ≥350 cells/μL definitely exceeded the values of persons from experimental group and group II. Changes in immune status of patients of group II were more expressed that is proved by presence of definite difference of indexes with patient cohort of group I ($p<0.05$).

As it is showed in Fig. 4 there was increase of level of proinflammatory cytokine *TNF-α* in comparison to control (group I – (0.77 ± 0.08), II – (2.34 ± 0.69), experimental group – (0.51 ± 0.32) pg/ml, $p<0.05$) and anti-inflammatory *IL-10* (group I – (3.99 ± 0.99), II – (20.08 ± 4.44), experimental group – (1.68 ± 0.32) pg/ml, $p<0.001$) in cytokine profile of all HIV-infected. No definitely significant difference in *IL-4* production was found out among examined population.

Significant increase of *TNF-α* and *IL-10* was noted in patients with level of CD4+ T-lymphocytes ≤200 cells/μL in comparison to persons of group I ($p<0.05$) that causes the presence of deep imbalance of immune response at late stages of the disease. Among HIV-infected of group II average level of serum concentration of *TNF-α* 3 times exceeded the corresponding index of group I ($p<0.05$). Great increase of cytokine concentration was noted in persons with severe immunodeficiency by comparison of *IL-10* values (*IL-10* level in group II turned out to be 5 times higher $p<0.05$), that can mediately indicate about more active involvement of *IL-10* in processes of illness progression. Power of correlative relationships in patients of group II between concentration of this cytokine and index of opportunistic infections in comparison to similar

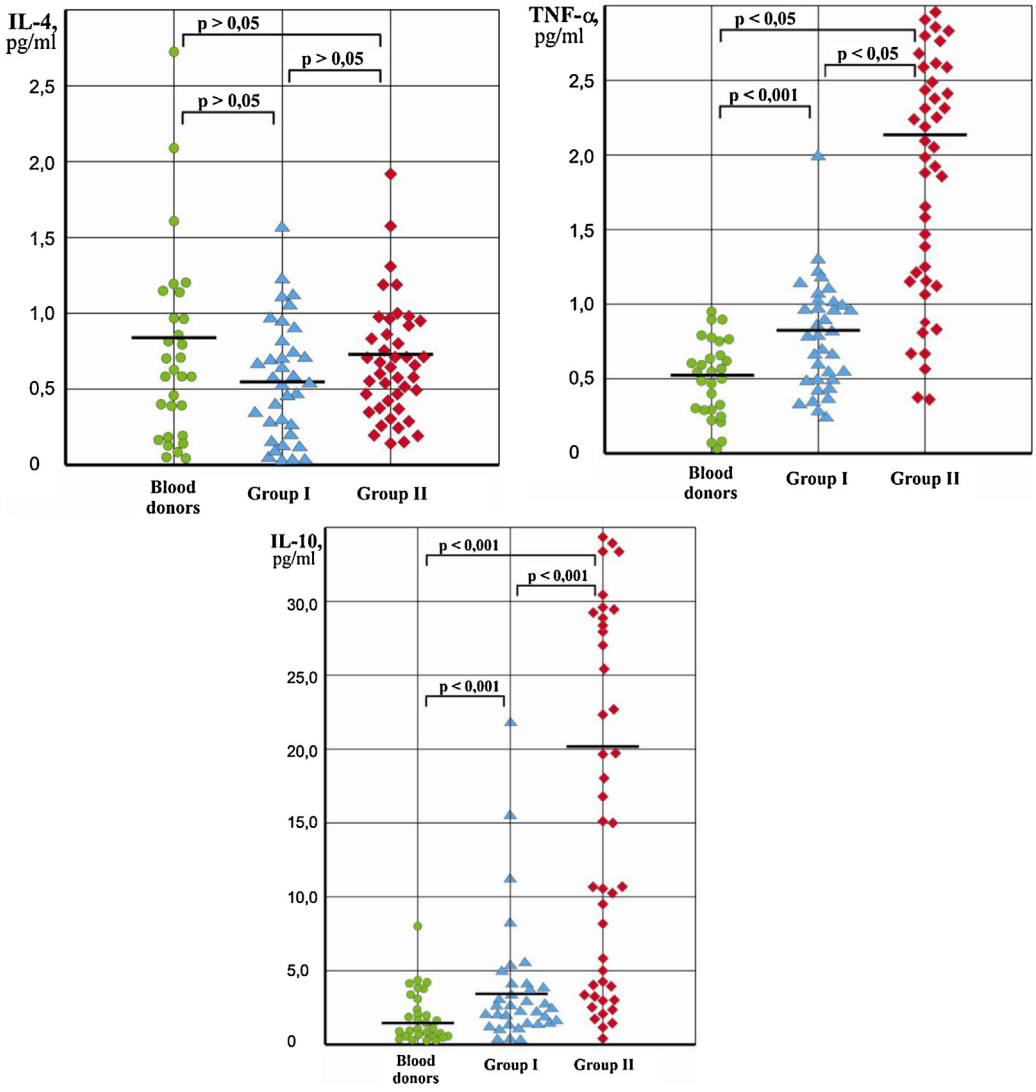

Fig. 4. *IL-4*, *IL-10* and *TNF-α* serum concentration indices in studied groups.

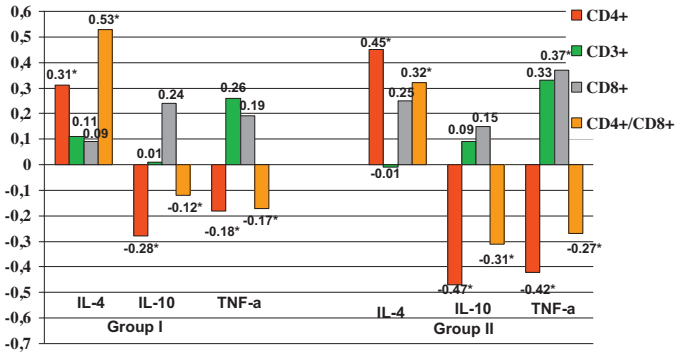

Fig. 5. Cell immunity and cytokine profile indices correlation in studied groups. *reliability correlation, $p < 0.05$.

values *TNF-α* (*IL-10*: $r = 0.23$, $p < 0.05$; *TNF-α*: $r = 0.17$, $p < 0.05$) also speaks in favour of this assumption; with severity of patient's general state (*IL-10*: $r = 0.43$, $p < 0.05$; *TNF-α*: $r = 0.25$, $p < 0.05$).

By research of correlative relationships in groups of HIV-infected patients feedback of various power between quantity of CD4 cells, ratio of CD4+/CD8+ lymphocytes and *IL-10* and *TNF-α* levels was discovered (Fig. 5).

It is necessary to note that in persons with highly expressed immunodeficiency level dependence of immunocompetent cells and serum concentration of cytokines is more significant. Thus, in group with level of T-helpers ≥350 cells/μL, correlative relationship was inverse weak (*IL-10*: $r = -0.28$, $p < 0.05$; *TNF-α*: $r = -0.18$, $p < 0.05$), among the people with level of CD4-lymphocytes ≤200 cells/μL inverse relationship of medium power was recorded (*IL-10*: $r = -0.47$, $p < 0.05$; *TNF-α*: $r = -0.42$, $p < 0.05$).

Discussion

Changes of lymphocyte subpopulation in HIV-infected people were discovered that are characterized by change of number of immunocompetent cells with CD+ phenotype and their ratio, prove modern concept of global immune imbalance by progression of disease [4,6,13]. The obtained results coincide with the data of foreign researches – cytokine balance plays a significant role in HIV-associated immune disfunction, when both anti-inflammatory and proinflammatory cytokine links activate, with high concentration of *IL-10* and *TNF-α* [6,8–10]. Association of high level of circulating *TNF-α* with unfavourable course of infection [2,4] was also proved. It is suggested that *IL-10* has negative consequences by HIV infection because of ability to influence directly on viral replication in T-helpers, monocytes and dendritic cells [9]; to modulate differentially cellular apoptosis of pool of CD4+ and CD8+ lymphocytes [14]; also *IL-10* can renew blockade of T-cellular proliferative response in patients with relatively saved number of T-helpers but this ability is being lost during progression of illness [15].

Conclusions

1. Among the examined HIV-infected with number of CD4+ lymphocytes ≤200 cells/μL late stages of disease, severe course of disease, pulmonary and extrapulmonary tuberculosis, organic lesions of nervous system, cachexia, higher index of opportunistic infections were definitely registered more often.
2. Immune status of HIV-infected persons is characterized by deficiency of cell component of immune system and changes in cytokine status with increased production of proinflammatory *TNF-α* and anti-inflammatory *IL-10*.
3. Immune imbalance that is caused by changes of cell component and cytokine level is more expressed in HIV-infected persons with level of CD4+ lymphocytes ≤200 cells/μL.
4. Cytokine profile interacts with level of immunocompetent cells that is proved by presence of correlative relationships between serum concentration of *IL-10, TNF-α* and T-helpers.
5. Evident changes of *IL-10* level in patients at late stages of illness and correlative relationships with index of opportunistic infections and severity of general state indicate active involving of this cytokine in immunopathogenesis of HIV infection.

Therefore, the notions of levels of *IL-10* and *TNF-α* cytokines can be considered as additional prognostic markers of HIV infection progression and the conducted immunological researches point out the necessity of further study of cytokine chain for the purpose of determination of pathogenic relationships of cell cooperation by deteriorative course of disease and potential of use of interleukins for therapeutic purposes.

Conflict of interest

Conflict of interest is not notified.

Financial disclosure

Study design, data collection, analysis and interpretation of data, writing the manuscript and the decision to submit the manuscript for publication were carried out without the participation of sponsors.

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