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LOCAL BALANCE OF SUB-POPULATIONS OF T-HELPERS AT VARIOUS STAGES OF PRIMARY OPEN-ANGLE GLAUCOMA

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Abstract:

Aim of the present study is to analyze the balance of the Th system in tear fluid in patients with POAG. Study involves evaluation of the content of proinflammatory cytokines in tear fluid in patients with POAG, depending on its stages; evaluation of the content of anti-inflammatory cytokines in tear fluid in patients with POAG, depending on its stages and creation of Th local balance schemes depending on the stages of the disease.

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INTRODUCTION:
The damage of the optic nerve in glaucoma is called glaucoma optic neuropathy (GON), which is considered the main manifestation of the disease and is characterized by accelerated loss of retinal ganglion cells (GCS) and their axons. There are several theories of GON development. The main ones are mechanical (retention), vascular (ischemic), metabolic, neurodegenerative, genetic, and infectious [1]. In recent decades, the immunological theory of pathogenesis has been developed.

Undoubtedly, humoral immunity factors are important in the development of GON. High concentrations of TNF-α, as well as some other cytokines (IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IFN-γ) were found in a tear fluid of glaucoma patients by a number of authors [2,3,4,6,8,9,11,12,15]. V.V. Chernykh et al. in their work [6] raise the ability of IL-1β to stimulate the secretion of metalloproteinases and plasminogen, the prevalence of synthesis of which leads to the growth of destructive processes, increased destruction of the matrix of connective tissue, or a reduction in its recovery. S.A. Petrov et al. [5] established the dependence of concentrations (reduced and elevated) (IL-1, IL-2, IL-4, IL-6, IL-8, IL-10, IL-4) on the narrowing of the visual field boundaries. Much attention is paid to the transforming growth factors. There is evidence that the failure of the molecular mechanism controlling the signaling mechanism of TGFβ in both the anterior and posterior regions of the eye causes, or at least, contributes to the development of POAG [3,11,12].

An analysis of the cytokine profile is important, and as a consequence, an evaluation of the balance of T-helpers (Th) to understand the local state of immunity.

CD4+ lymphocytes or Th perform mostly helper functions, but can also perform a killer one [7]. Carrying out their primary helper function, they help, first, B cells to transform into an antibody-producing plasma cell; secondly, CD8+ lymphocytes - into a mature cytotoxic T-cell; thirdly, macrophages carry out delayed hypersensitivity effects. These functions of T-helpers are implemented due to the fact that they, in turn, are divided into two main subpopulations - the 1st and 2nd type, and the additional subpopulation of the 17th type, 3rd type and Treg, performing various helper functions due to the production of different cytokines – interleukins (see Figure 26, 27).

Type 1 T-helpers produce gamma-interferon, IL-2 and TNF-alpha. These cytokines activate macrophages, NK cells, maturation of cytotoxic T-killers, providing thereby a preferential development of the cellular immune response, including in intracellular infection [10].

In contrast, type 2 T-lymphocytes produce IL-4, IL-5, IL-10 and IL-13, which are responsible for the development of a humoral response, including IgE production.

It was found that Thl and Th2 are responsible for the development of various immunopathological reactions in humans. Thus, for example, the function of Thl prevails in the development of multiple sclerosis, insulin-dependent diabetes mellitus, autoimmune thyroiditis, Crohn's disease, and acute rejection of the allograft, habitual fetal miscarriage, etc. In turn, the Th2 function predominates in normal pregnancy, transplant tolerance, idiopathic pulmonary fibrosis, progressive systemic sclerosis, in HIV-infected patients with rapid disease progression, as well as in allergic pathology. Th17 produce IL-17 and are the initiators of neutrophil inflammation. Th3 - the least studied subpopulation, able of producing growth factors and have anti-inflammatory effect [7,14].

Thus, type 1, 2 and 17 Th are the most important subpopulations of T-lymphocytes, which functional balance determines the directivity of the normal immune response and the peculiarities of clinical manifestations in the development of immunopathology. On this basis, T-helpers were called "immune response conductors".

OBJECTIVE OF THE RESEARCH: to analyze the balance of the Th system in tear fluid in patients with POAG.
The **tasks** we solved are:
1. Evaluation of the content of proinflammatory cytokines in tear fluid in patients with POAG, depending on its stages;
2. Evaluation of the content of anti-inflammatory cytokines in tear fluid in patients with POAG, depending on its stages;
3. Creation of Th local balance schemes depending on the stages of the disease;

MATERIAL AND METHODS:
Cytokines (IL-1β, IL-6,IL-□□ $_{SR}$IL-2, IL-17, IFN-□,IL-10, IL-4, TGF-□$_1$, TGF-□$_2$) were examined in serum and lacrimal fluid, using the sandwich version of the solid-phase enzyme-linked immunosorbent

assay, using the specific test systems "R&D Diagnostics Inc." (USA) according to the instructions attached. The results were recorded using the immunoenzymatic analyzer "Multiscan" (Finland). The amount was expressed in pg/ml or ng/ml. The processing of all digital data was carried out using descriptive, parametric and nonparametric statistics with the help of the Statistica 6.0 and SPSS programs, version 16. The following tests were carried out: quantitative trait distribution normality tests using the Shapiro-Wilk W-test, evaluation of the significance of the differences between the two sample means for the Student t-test for a two-sided confidence interval, comparison of two pairwise non-characteristics using the nonparametric Mann-Whitney-Wilcoxon test, evaluation of the relationships between the investigated indices using the Spearman correlation coefficient, checking of qualitative and quantitative traits using the χ^2 test. Sample parameters given hereinafter in the tables have the following designations: arithmetic mean (M), arithmetic mean average error (m), the volume of the analyzed subgroup (n), p – achieved level of significance. The median (Me), the minimum and maximum values, the lower and upper quartiles (Q25, Q75) were calculated. The level of confidence was set at 95%. Thus, null hypotheses were rejected when the achieved significance level P of the statistical criterion used was less than 5%.

Local level of cytokines was studied in 133 patients with POAG of stage I-III.At the age of 59 years - 32

(24%) people, 60-74 years - 101 (76%) people. In the main group, patients were divided by stages. The number of patients with stage I was 39 (29.3%), with stage II - 56 (42.1%), and stage III - 38 (28.6%). Women predominated - 77 (57.9%), the number of men was 56 people (42.1%). The control group consisted of 50 virtually healthy volunteers aged 53.45 ± 2.35 years, represented by 30 (60%) women and 20 (40%) men. Patients with stage IV (terminal stage) were not included in this study.

RESULTS:

The analysis of proinflammatory cytokines in lacrimal fluid in patients with different stages of POAG (Table 1) revealed the highest IL-1β content in stage III of POAG; the lowest level was recorded in patients with stage I POAG (p<0.05), as well as an increase in its local content in comparison with the reference values.

The study of local IL-6 indices in patients with stage III POAG determined its significant increase in comparison with stage I, II and control group (p<0.05).

The assessment of the IL-2 content found its significant increase only in patients with stage II POAG; patients with stage I and III had the level of this cytokine not differing from the control values (Table 1).

Table 1.The content of proinflammatory cytokines in tear fluid in patients with POAG of different stages

No.	Indicators (Me; Q25-Q75) pg/ml	Control group n=50 people	Patients with POAG n=133		
			Stage I	Stage II	Stage III
			n=39	n=56	n=38
1	IL-1β	2.6 (1.6; 3.2)	3.69* (3.20; 5.06) p1-2<0.05	8.64* (1.50; 12.38) p2-3<0.05	13.20* (3.22; 37.19) p1-3<0.001
2	IL-6	2.0 (1.7; 5.4)	2.1 (2.0; 4.80) p1-2>0.05	2.4 (2.0; 5.70) p2-3<0.05	7.40* (6.5; 9.20) p1-3<0.05
3	IL-2	2.2 (2.0; 3.1)	5.18 (1.95; 8.60) p1-2<0.01	16.19** (8.46; 30.50)↑ p2-3<0.001	3.25 (1.2; 6.84) p1-3>0.05
4	SRIL-2	200.0 (132.4; 240.8)	296.34* (154.0; 328.6) p1-2>0.05	309.170* (268.42; 394.12) p2-3<0.05	228.95 (136.77; 289.17) p1-3<0.05
5	IL-17	5.34 (2.10; 8.90)	47.80** (13.20; 158.20) p1-2<0.05	19.60* (7.32; 72.50) p2-3>0.05	16.76* (8.00; 62.14) p1-3<0.05
6	IFNγ	22.83 (10.00; 26.50)	10.25* (4.78; 10.0) p1-2<0,001	54,30* (27.20; 146.0)↑ p2-3>0.05	44.36* (18.64; 82.0)↑ p1-3<0.01

Note: The statistical significance of the differences between the groups: p - with the control group: p<0.05 - *; p<0.01 - **; p<0.001 - ***; p1,2,3 - study groups; n=people

We registered a high level of SRIL-2 in patients with stage I and II POAG, the lowest level was recorded in patients with stage III ($p<0.05$): its level in this group did not differ from the control group.

The IL-17 content was significantly increased in all the examined patients; the greatest value was found in patients with stage 1 POAG in comparison with patients with stages II, III POAG (Table 1).

The study of the parameters of IFNγ revealed its significant increase in patients with stage II and III; the lowest level was recorded in patients with stage I POAG, which was below the control values (Table 2).

The assessment of the level of anti-inflammatory cytokines in patients with POAG, depending on the stage (Table 2), found that the content of IL-10 significantly higher in patients with stage I; the lowest level was recorded in patients with stage II, the value of this cytokine is lower than in the control group ($p<0.05$).

Table 2:
The content of anti-inflammatory cytokines in tear fluid in patients with POAG of different stages

No.	Indicators (Me; Q_{25}-Q_{75})	Control group n=50	Patients with POAG, n=133		
			Stage I n=39	Stage II n=56	Stage III n=38
1	IL-10, pg/ml	14.05 (7.14; 26.3)	34.51* (6.20; 45.63)↑# $p_{1-2}<0.01$	3.90* (6.3; 14.31)↓ $p_{2-3}>0.05$	7.66 (6.40; 30.50) $p_{1-3}<0.05$
2	IL-4, pg/ml	4.90 (2.5; 6.2)	9.90 (3.2; 14.0) $p_{1-2}>0.05$	7.78 (2.7; 14.3) $p_{2-3}<0.05$	18.20* (5.6; 28.4)↑ $p_{1-3}<0.05$
3	TGFβ_1, pg/ml	360.00 (270.0; 425.0)	270.00* (210.00; 360.00) $p_{1-2}>0.05$	285.0* (255.0; 310.0) $p_{2-3}<0.05$	200.0** (150.0; 240.0)↓ $p_{1-3}<0.05$
4	TGFβ_2, pg/ml	168.50 (110.0; 206.4)	182.96* (173.92; 238.30) $p_{1-2}>0.05$	216.00** (193.80; 280.4) $p_{2-3}>0.05$	288.63*** (201.21; 320.60) $p_{1-3}<0.05$

Note: The statistical significance of the differences between the groups: p - with the control group: $p<0.05$ - *; $p<0.01$ - **; $p<0.001$ - ***; $p_{1,2,3}$ - study groups; n=people

Patients with stage III POAG had a lower level of IL-10 defined, but the indicators were higher than in patients with stage II, but lower than with stage I ($p<0.05$) and did not differ from the values of the control group.

The analysis of IL-4 indicators in patients with stage III POAG found its significant increase in comparison with stages I and II and the control group ($p<0.05$).

The assessment of TGFβ_1 indicators established its significant decrease in all the study groups with the lowest value being found in patients with stage III POAG (Table 2). We detected a high level of TGFβ_2 in patients with POAG, especially with stage III ($p<0.001$).

DISCUSSION
Thus, the analysis of the local cytokine profile in stage I POAG established IL-17 and IL-10

hyperproduction, a moderate increase in IL-1β, SRIL-2, TGFβ_2 and deficiency of IFNγ.

Locally, in stage II POAG, an increase in the level of IL-2, its soluble receptor, IFNγ and TGFβ_2 was detected. The content of IL-6 and IL-4 was within normal limits. A decrease in TGFβ_1 was determined.

The local cytokine profile of advanced (stage III) POAG had more differences from the stage II of the disease: hyperproduction of IL-1β, IL-6, IL-4, and TGFβ_1 deficiency were identified. Analysis of the local content of cytokines as a function of the stage of the process made it possible to reveal a number of regularities. In patients with POAG already at an early stage, hyperproduction of IL-17 and IL-10, a moderate increase in IL-1β, TGFβ_2, SRIL-2 against a background of deficiency of IFNγ and a decrease in TGFβ_1 were registered. Calculation of the ratio of IFNγ to IL-4 made it possible to ascertain that in this category of patients the Th2 immune response prevails against the background of Th17

hyperactivation. An increase in the concentration of IL-17 in the lacrimal fluid by more than 27.0 pg/ml is associated with stage I of POAG ($\chi^2 = 8.64$ at 2nd degree of freedom, p<0.05).

In stage II POAG, an increase in the local level of Th1 of marker cytokine - IFNγ, an increase in the content of IL-1β, IL-2 and its soluble receptor were detected. An increase in TGFβ2 and IL-17 is preserved, but the latter is reliably lower than at the stage I of the disease. A lowered level of TGFβ1 (not different from stage I POAG) was detected, a deficiency of IL-10 was determined. An integrated assessment of the local level of cytokines at stage II POAG revealed a Th1 response with suppression of the anti-inflammatory activity of cells of innate immunity.

In stage III POAG, both early and late proinflammatory cytokines IL-1β and IL-6 and a high level (not differing from stage II) of IL-17 and IFNγ were recorded. A relative reduction (up to reference values) of the local concentration of IL-2 and its soluble receptor was revealed, which may indicate

the depletion of their production or the increased formation of the corresponding complexes.

Evaluation of anti-inflammatory mediators in patients with stage III POAG showed a change in the balance towards intensification of production and secretion of the majority of them: the level of IL-4 increased by 4 times; the content of TGFβ2 and the IL-10 concentration increased gradually but significantly compared to the control and stage I POAG (in comparison with the stage II POAG). A distinctive feature of stage III POAG was the local deficiency of TGFβ1. An increase in IFNγ and IL-4 with a prevalence of the latter level characterizes the Th2 immune response in stage III POAG.

The integrated study of the cytokine status at various stages of POAG formed the basis for evaluating the balance of the Th cell subpopulation (based on the data proposed by Iarilin A.A. [7]) and allowed developing schemes for the mutual influence and predominance of a subpopulation of T-helpers in the development of glaucoma neuropathy (see Figure 1, 2, 3).
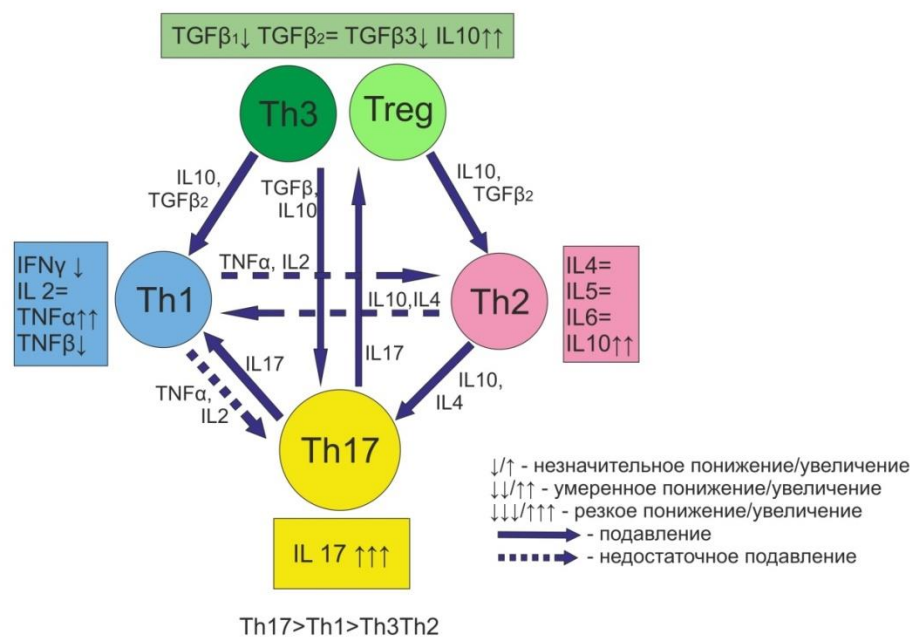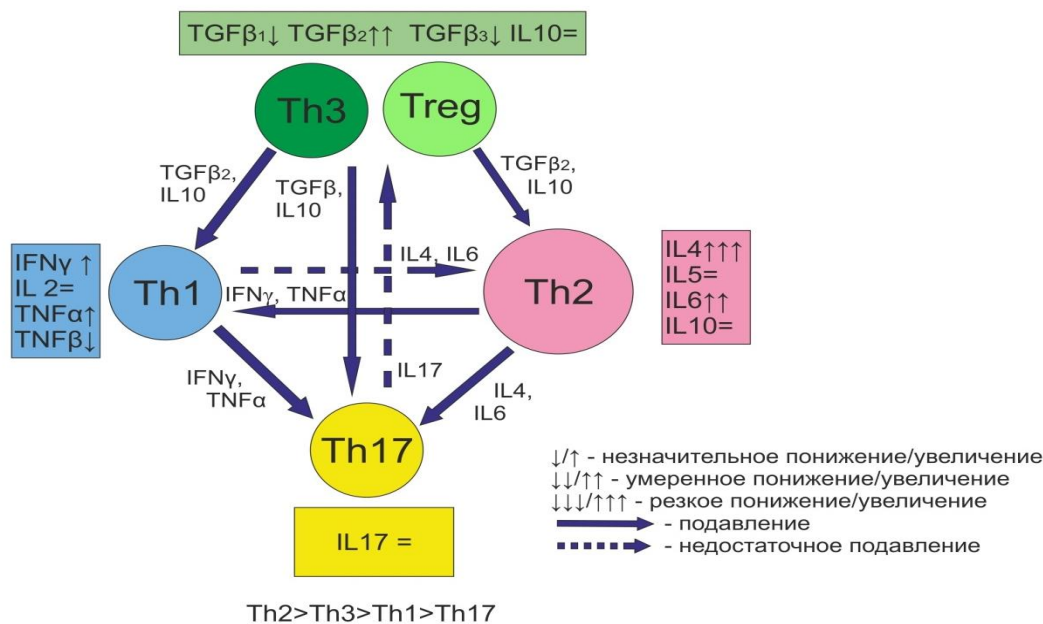

- *Insignificant decrease/increase*
- *Moderate decrease/increase*
- *Sharp decrease/increase*
- *suppression*
- *insignificant suppression*

Fig. 1. The balance of the Th system in stage I POAG (own scheme based on the scheme by Iarilin A.A., 2010)
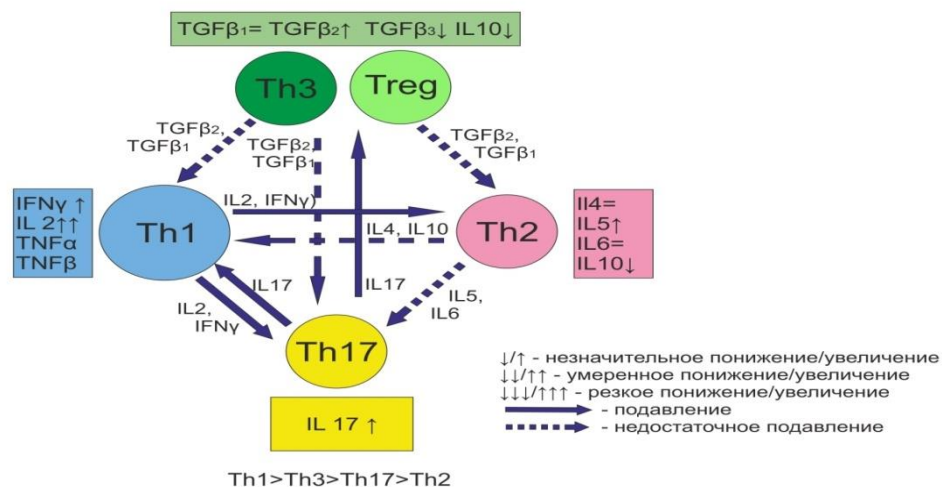
- *Insignificant decrease/increase*
- *Moderate decrease/increase*
- *Sharp decrease/increase*
- *suppression*
- *insignificant suppression*

Fig. 2. The balance of the Th system in stage II POAG (own scheme based on the scheme by Iarilin A.A., 2010)

- *Insignificant decrease/increase*
- *Moderate decrease/increase*
- *Sharp decrease/increase*
- *suppression*
- *insignificant suppression*

Fig. 3. The balance of the Th system in stage III glaucoma (own scheme based on the scheme by Iarilin A.A., 2010)

Thus, by forming an understanding of the Th system in the progression of GON, we can describe the following relationships. The patients with stage I POAG had hyperproduction of IL-17, IL-22 and IL-10, a moderate increase of IL-1β, TGFβ$_2$ against the background of IFNγ deficiency and decrease of TGFβ$_1$ registered. This category of patients have Th17 hyperactivation prevailing. In stage II POAG, an increase in the local level of Th1 marker cytokine - IFNγ, an increase in the content of IL-1β, IL-2 and its soluble receptor was noted. In stage II POAG, Th1 response with suppression of the anti-inflammatory activity of cells of innate immunity was detected. In stage III POAG, both early and late proinflammatory cytokines IL-1β and IL-6, decrease in IL-17 and IL-22 and IFNγ were recorded. A relative reduction (up to reference values) of the local concentration of IL-2 and its soluble receptor was revealed, which may indicate the depletion of their production or the increased formation of the corresponding complexes.

The local profile of cytokines in POAG is characterized by a pronounced proinflammatory profile with switching of types of immune response from Th17 and Th2 in patients with stage I POAG to Th1 in II stage POAG and Th2 in patients with stage III POAG. These data suggest various immune responses in the development of POAG, the activation of early autoimmune processes in the early stage, then cellular immunity in the advanced stage and, at the end of the process, humoral immunity at stage 3. The results obtained, with further study, will become the theoretical basis for diagnosing the progression of GON and targeted immune therapy at various stages of POAG.

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