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Immunologic status and sensitivity of peripheral blood T cells to neurotransmitters in patients with optic neuritis

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Background: *Optic neuritis (ON) is an optic nerve inflammation that can result in impairment of visual function. Studying expression patterns of immune cell subpopulations and genes is of importance for revealing the role and influence of each of them in the early ongoing ON pathological process.*

Purpose: *To examine the immunologic status and sensitivity of peripheral blood T cells to adrenaline and acetylcholine neurotransmitters in patients with ON and its sequelae.*

Material and Methods: *The study cohort included 45 patients with idiopathic ON who underwent an examination: group 1, 27 patients with primary acute ON; group 2, 9 patients with partial optic atrophy (POA) following ON; and group 3, 9 patients with loss of posterior pole structure following ON. Group 4 (controls) was composed of 27 healthy volunteers. The specific reactivity of lymphocytes to adrenaline and acetylcholine was assessed using our complex methodology (in conjunction with a parallel sampling method) for assessing the individual's sensitivity to medicaments (biological regulators) which has been developed at Immunology laboratory, Filatov Institute of Eye Diseases and Tissue Therapy. The methodology involves obtaining lymphocytes from an individual, culturing lymphocytes with examined drugs immunohistochemically, and use of a peroxidase anti-peroxidase method with monoclonal antibodies*

Results: *Patients with acute idiopathic ON showed increased cell immunity activity, with increased absolute numbers of CD3, CD4, and CD8 cells compared to the control group. The CD4:CD8 ratio for acute ON was not significantly different from the norm. We noted increased levels of activity of humoral immunity components (increased absolute numbers of B lymphocytes and increased IgA and IgM levels) in acute ON compared to the control group. A reduction in and normalization of absolute numbers of CD3 and CD4 T cells compared to acute ON were characteristic features of cell-mediated immunity in patients with POA following ON and those with loss of posterior pole structure following ON. The percentage of CD8 cells in patients with POA following ON was lower than in controls. It is these characteristic proportions of immunocompetent cell ratios that mirrored in increased CD4:CD8 ratios in ON groups, which is associated with a reduced level of cytotoxic T cells. Patients in groups 1, 2 and 3 had increased absolute numbers of peripheral blood T cells sensitive to adrenaline, being 3.4-fold, 2.4-fold, and 1.7-fold more than controls, respectively. In addition, patients in groups 1 and 2 had increased absolute numbers of peripheral blood T cells sensitive to acetylcholine, being 2.8-fold and 2.6-fold more than controls, respectively. We found significant direct correlations of the level of the T cells sensitive to adrenaline and acetylcholine with the leukocyte cell count and CD3, CD8, CD19 and CD16 lymphocyte subset counts in patients with ON and its sequelae.*

Conclusion: *The levels of some cell-mediated immunity components (CD3, CD4, and CD8) and humoral immunity components (B lymphocytes, IgA and IgM) were increased in acute ON group compared to controls. The laterality of ON exerted an impact only on the relative number of lymphocytes, proportion of CD8 and CD4:CD8 ratio in total patients. Patients had increased absolute numbers of peripheral blood T cells sensitive to adrenaline and acetylcholine compared to controls. There were significant direct correlations of the level of the T cells sensitive to adrenaline and acetylcholine with the leukocyte cell count and CD3, CD8, CD19 and CD16 lymphocyte subset counts in patients with ON and its sequelae.*

Keywords:

optic neuritis, immunogram, adrenoreceptor and acetylcholine receptor expressing T cells

Introduction

Optic neuritis (ON) is an optic nerve inflammation that can result in acute or subacute impairment of visual function [1]. It is seen most commonly in young adults and frequently is the first manifestation of a central nervous system (CNS) disease [2]. The disease can be idiopathic or associated with recurrent demyelinating diseases, classically and most commonly, multiple sclerosis (MS) but also the more recently recognized antibody-associated diseases neuromyelitis optica spectrum disorder (NMOSD) and myelin oligodendrocyte glycoprotein antibody disease (MOGAD) [3, 4, 5]. Other atypical entities of ON include chronic/relapsing inflammatory optic neuropathy (CRION), and sarcoidosis-associated ON [4]. No universal opinion exists on the nomenclature of subtypes of ON; the nomenclature is still under clarification. Advances in immunology like serologic testing techniques with the determination of specific serologic antibody biomarkers for demyelinating diseases have improved our knowledge of some subtypes of ON. Specific serological and radiological biomarkers have been established not for all subtypes [5]. More than two decades ago, Bitsch and colleagues [6] assessed the role of CD4-positive and CD8-positive T cells and amyloid precursor protein expression in axonal injury and demyelination in multiple sclerosis [6]. Feldman et al [7] evaluated the role of CD19(+) B cells in the pathogenesis of the early phase of acute ON and Guy et al [8] analyzed T-lymphocyte subpopulations in the peripheral blood of patients with acute unilateral ON. The pathophysiology and natural history of idiopathic ON are, however, not well understood [9]. Studying expression patterns of immune cell subpopulations and genes are of importance for revealing the role and influence of each of them in the early ongoing ON pathological process [7]. Studies are scarce on the expression of adrenaline and acetylcholine receptors on the surface of immunocompetent blood cells in inflammatory processes [10]. Lymphocytes differ in terms of density and sensitivity of adrenergic receptors [11] and muscarinic and nicotinic acetylcholine receptors [12]. The involvement of these immune system components in the pathophysiology of inflammation in optic nerve pathology has not been sufficiently investigated. It is believed that early diagnosis and timely management are essential for restoring and preserving vision and prevention of relapse in ON [4].

The purpose of this study was to examine the immunologic status and sensitivity of peripheral blood T cells to adrenaline and acetylcholine neurotransmitters in patients with ON and its sequelae.

Material and Methods

The study cohort included 45 patients (25 women and 20 men; 82 affected eyes) with idiopathic ON who were examined at the Laboratory for Studies of Ocular Function and whose samples were obtained and processed at Immunology laboratory, Filatov Institute of Eye Diseases and Tissue Therapy. Patients were divided into 3 groups. Group 1 consisted of 27 patients (38 eyes) with primary acute ON (median [interquartile range (IQR)] time from initial symptoms to the diagnosis, 12 (7–30) days) and clinical findings of neuritis (papillitis). Group 2 consisted of 9 patients (13 eyes) with partial optic atrophy (POA) following ON and the median (IQR) time from initial symptoms to the diagnosis of 1080 (180–1825) days. These patients included 5 patients (5 eyes) with unilateral ON and 4 patients (8 eyes) with bilateral ON. Group 3 consisted of 9 patients (14 eyes) with ON which resulted in complications (macular edema, macular degeneration, and posterior hyaloid detachment with macular traction) and the median (IQR) time from initial symptoms to the diagnosis of 700 (150–1440) days. These patients included 4 patients (4 eyes) with unilateral ON and 5 patients (10 eyes) with bilateral ON. The average patient age was 37.8 ± 11.3 years (mean ± standard deviation). The control group was composed of 27 volunteers of matched age without ocular disease or general medical condition.

The study followed the ethical standards stated in the Declaration of Helsinki, the European Convention on Human Rights and Biomedicine and relevant laws of Ukraine. Written informed consent was obtained from all participants. Measures were taken to preserve the anonymity of patients.

Patients underwent a clinical ophthalmological examination, which included assessment of visual acuity, tonometry, slit-lamp biomicroscopy, ophthalmoscopy through a dilated pupil, axial length of the eye, current threshold for eliciting phosphenes, critical flicker fusion frequency, and Humphrey 24-2 Swedish Interactive Thresholding Algorithm (SITA) visual field testing (Carl Zeiss Meditec). In addition, optical coherence tomography (OCT) of the macula area, optic nerve and peripapillary area was performed if required to clarify the diagnosis. Patients were consulted by a neuropathologist and underwent magnetic resonance imaging (MRI) and computed tomography (CT) of the brain. Patients with MS were excluded from the study.

Histo/immunocytochemical techniques with monoclonal antibodies were used to determine lymphocyte subsets and lymphocyte activation markers. With this in mind, a 4-5-ml sample of heparinized blood was obtained from the cubital vein with a disposable vacuum system, and twice diluted with 0.9% NaCl. The objective magnification was set to 80× and a 15x eyepiece was used. Cells containing the horseradish peroxidase-conjugated antigen show a reddish brown rim encircling the cell. To assess the specific sensitivity of lymphocytes to adrenaline and acetylcholine, we employed our complex methodology for assessing the individual's sensitivity to medicaments which has been developed at Immunology laboratory, Filatov Institute of Eye Diseases and Tissue Therapy [13, 14]. The methodology involves obtaining lymphocytes from an individual, culturing lymphocytes with examined drugs immunohistochemically using a peroxidase anti-peroxidase method with monoclonal antibodies [15]. A

summary of the method procedure is as follows. First, peripheral blood mononuclear cells from whole blood are separated through density gradient centrifugation using Ficoll separating solution with density of 1.077 g/mL and washed twice to obtain lymphocyte suspension. Second, (a) lymphocyte cell suspension (0.05 ml) is mixed with NaCl 0.9% (0.05 ml); (b) lymphocyte cell suspension (0.05 ml) is mixed with adrenaline 0.18% (0.05 ml; sterile solution, ready for use, manufactured by JSC Darnytsia, Kyiv, Ukraine); and (c) lymphocyte cell suspension (0.05 ml) is mixed with acetylcholine chloride 0.1% (0.05 ml; sterile solution, manufactured by Sinbias LLC, Kyiv) (dry substance with diluted with physiological saline); and these three mixture samples are incubated in parallel at 37oC for one hour. The protocols for concentrations of the used solutions have been developed previously by others [14]. Thereafter, T cells (CD 3) were determined immunohistochemically using a routine method with monoclonal antibodies. CD3 counts were determined for study samples (with adrenaline and acetylcholine) and control samples (with physiological saline). Immunocytochemical techniques with monoclonal antibodies were used to examine lymphocyte activation markers.

Spreadsheets and STATISTICA 8.0 (StatSoft, Tulsa, OK) software were used for statistical analysis. Nominal data were described using numbers with percentages. Quantitative data were tested for normality using the Shapiro–Wilk's test. If normally distributed, data were presented as mean (M) ± standard deviation (SD) and the independent samples t-test was used for comparisons. Non-normally distributed qualitative data were presented as median (IQR) and the Mann-Whitney U test was used to test for significant differences. The level of significance $p \leq 0.05$ was assumed. Spearman and Pearson correlation analyses were used for non-parametric and parametric data respectively. Univariate and multivariate analysis (ANOVA) were used to identify the factors characterizing the interrelationships among the variables. Analysis results were presented graphically using plots and as F-test values and p-values.

Results

The number of leukocytes per liter of peripheral blood in group 1 (with primary acute ON) was 8.5*106/l (95% CI, (7.3-9.7)*106/l), which was 41.6% higher than in group 2 (6.12*106/l, 95% CI (3.4-8.8))*106/l; p = 0.039) and group 3 (5.9*106/l, 95% CI (4.6-7.2))*106/l; p = 0.04) and 57% higher than in group 4 (5.4*106/l, 95% CI (5.1-5.7))*106/l; p = 0.000 (Fig. 1). In addition, ANOVA demonstrated the effect of the course of optic neuritis on the level of leukocytes (F = 4.38; p = 0.024), but no effect of unilaterality or bilaterality of the process on the level of leukocytes (F=0.64; p = 0.43). In addition, no interaction between these factors was observed.

Univariate analysis demonstrated the effect of ON laterality and the presence of complications following optic

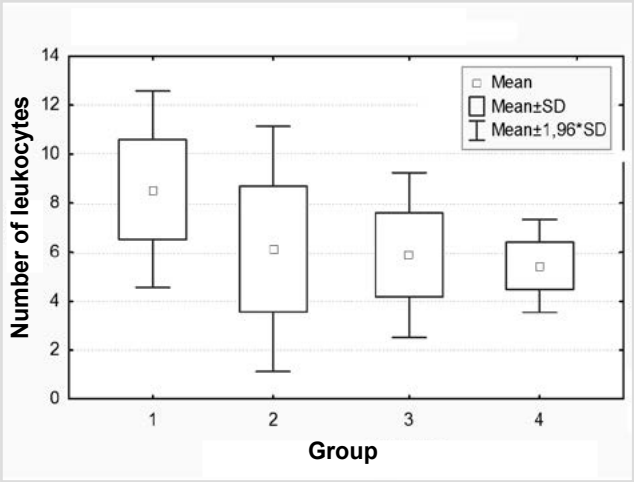

Fig. 1. Number of leukocytes per liter of peripheral blood in patients with optic neuritis and its sequelae

neuritis on the white blood cell (WBC) count (F = 9.2; p = 0.0039). Overall, the relative WBC count in unilateral ON ((31.0 ± 9.3) %, 95% CI, (27.9-35.1)%) was 32% lower than in bilateral ON ((41.2 ± 13.1)%, 95% CI, (35.3-47.2)%) (p = 0.004). T-cell immunity analysis found that absolute numbers of CD3, CD4, and CD8 cells in group 1 were 1.8-fold (p = 0.0008), 2.05-fold (p = 0.00002), and 1.44-fold (p = 0.004), respectively, increased compared to the control group (group 4), with median values for controls being 1007 cell/ml, 645 cell/ml and 274 cell/ml, respectively. The absolute number of CD3 cells in group 2 was 1.5-fold smaller compared to group 1 (p = 0.008) (Table 1).

Absolute numbers of CD3 and CD4 cells in group 3 were not different from those in the control group, and 1.9-fold ($p < 0.05$) and 1.8-fold, respectively, smaller compared to group 1 (Table 1). There was no significant difference in relative percentage of CD3 and CD4 cells among the groups. The relative percentage of CD8 cells for group 2 was 33% decreased compared to the control group (p = 0.03), with the mean relative percentage for the control group being 18.2 ± 5.4 % (Table 1). The bilaterality of ON was found to affect the relative percentage of CD8 cells for the total study sample (F = 5.4; p = 0.02). The relative percentage of CD8 cells for bilateral ON was 15.7% lower than for unilateral ON ((12.8 ± 3.8) % versus (15.2 ± 3.9) %). The CD4:CD8 ratio for group 1 was not significantly different from the norm (2.5 ± 0.9) (Table 1), and for group 2 was 56% higher (p = 0.038) compared to the norm (Table 1). The bilaterality of ON was found to affect the CD4:CD8 ratio (F = 6.1; p = 0.016) (Fig. 2) due to the above-mentioned effect of this factor on the relative percentage of CD8. The CD4:CD8 ratio for bilateral ON was found to be 20% increased.

With regard to humoral immunity features, in group 1, absolute numbers of CD19 B cells were 2.05-fold increased (p = 0.00009) compared to the control group (the median value for the control group was 236 cell/ml) and 1.9-fold increased (p = 0.004) compared to group 3

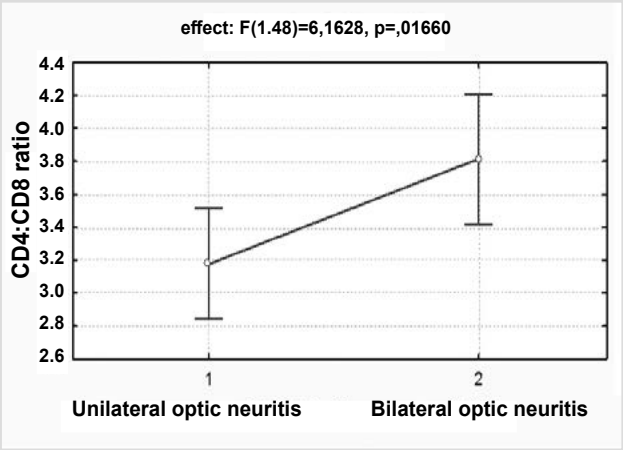

Fig. 2. Effect of the bilaterality of optic neuritis on the CD4:CD8 ratio in total patients

(Table 2). In addition, IgA levels were 1.5-fold increased in group 1 (p = 0.001) and 1.47-fold increased in group 2 (p = 0.002) compared to the control group (1.9 ± 0.5 g/l; Table 2). Compared to controls, only patients in group 3 had increased IgM levels (1.4-fold; p = 0.01) (Table 2). IgG levels were 12.5% lower in group 1 (p = 0.02) and 18.8% lower in group 2 (p = 0.07) compared to the control group (14.4 ± 2.3 g/l; Table 2).

Group 1 showed an almost significant tendency to increase in the absolute number of natural killers (p = 0.06) compared to the control group (the median value for the control group was 196 cell/ml) (Table 3). The absolute number of phagocytic neutrophils in group 1 was 9.6% higher (p = 0.0002) than in the control group (the median value for the control group was 2926 cell/ml), 75% higher (p = 0.001) than in group 2, and 43% higher (p = 0.03) than in group 3. In addition, ANOVA demonstrated the effect of the course of ON (F = 4.01; p = 0.013), but no effect of the laterality (F=1.1; p=0.3) on the relative level of peripheral blood T cells sensitive to adrenaline. Absolute numbers of peripheral blood T cells sensitive to adrenaline in groups 1, 2 and 3 were 3.4-fold (p = 0.06), 2.4-fold (p = 0.02), and 1.7-fold (p = 0.03), respectively, greater than in the control group (the median value for the control group was 115 cell/ml). The ratios for the relative numbers of peripheral blood T cells sensitive to adrenaline in groups were mostly similar to the ratios for the absolute numbers.

The absolute number of peripheral blood T cells sensitive to adrenaline in group 1 was 2.06-fold (p = 0.007) greater than group 3 (Table 4). ANOVA demonstrated the effect of the course of ON (F = 3.7; p = 0.03), but no effect of the laterality (F = 0.25; p=0.6) on the relative level of peripheral blood T cells sensitive to acetylcholine. Absolute numbers of peripheral blood T cells sensitive to acetylcholine in groups 1 and 2 were 2.8-fold (p = 0.0005) and 2.6-fold (p = 0.017), respectively, greater than in the control group (the median value for the control group was 129 cell/ml) (Table 4). The absolute number of peripheral blood T cells sensitive to acetylcholine in group 1 was 1.9-fold (p = 0.001) greater than group 3 (Table 4). The ratios for the relative numbers of peripheral blood T cells sensitive to acetylcholine in groups were mostly similar to the ratios for the absolute numbers.

There were significant (p < 0.05) direct correlations of the level of adrenoreceptor expressing T cells with the leukocyte cell count (r = 0.59) and CD3 (r = 0.74), CD4 (r = 0.74), CD8 (r = 0.69), CD19 (r = 0.63) and CD16 (r = 0.69) lymphocyte subset counts. In addition, there were significant (p < 0.05) direct correlations of the level of acetylcholine receptor-expressing T cells with

Table 1. Characteristics of cell-mediated immunity in patients with optic neuritis and its sequelae

Nosological form	Group	CD3		CD4		CD8		CD4:CD8 ratio
		Cell/ml Median (IQR)	% M±SD	Cell/ml Median (IQR)	% M±SD	Cell/ml Median (IQR)	% M±SD	M±SD
Acute ON n=27	1	1805 (1188–2080)	60±8.4	1325 (886–1390)	45.8±3.9	395 (282–500)	14.4±4.5	3.5±1.1
POA following ON n=9	2	1227 (954–1517)	59.6±1.6	872 (731–1208)	45.0±2.0	257 (207–293)	12.0±2.5	3.9±0.8
Other sequelae of ON n =9	3	954 (1635–1893)	59.9±4.2	731 (730–1020)	46.3±4.2	207 (207–388)	13.7±4.0	3.5±0.4
Controls n=27	4	1007 (772–1104)	64.8±5.8	645 (575–736)	43.1±5.7	274 (198–322)	18.2±5.4	2.5±0.9
Significance of difference (P-value)		P_{1-4}=0.0008 P_{1-3}=0.008	-	P_{1-4}=0.00002 P_{2-4}=0.02 P_{1-2}=0.008	-	P_{1-4}=0.004 P_{1-3}=0.01	P_{2-4}=0.03	P_{2-4}=0.038

Note: IQR, interquartile range; M, mean value; n, number of patients; ON, optic neuritis; POA, partial optic atrophy; SD, standard deviation;

Table 2. Characteristics of humoral immunity in patients with optic neuritis and its sequelae

Nosological form	Group	CD19 (B lymphocytes)		Ig A	Ig M	Ig G
		Cell/ml, Median (IQR)	% M±SD	g/l M±SD	g/l M±SD	g/l M±SD
Acute ON n =27	1	486 (339-609)	17.1±2.9	2.9±0.5	1.1±0.2	12.6±2.0
POA following ON n=9	2	266 (207-506)	14.0±2.0	2.8±0.2	1.1±0.1	13.3±2.1
Other sequelae of ON n =9	3	254 (450-514)	13.5±4.0	2.5±1.2	1.3±0.3	11.7±2.2
Controls n =27	4	236 (178-278)	15.7±3.4	1.9±0.5	0.9±0.2	14.4±2.3
Significance of difference (P-value)		P_{1-4}=0.00009 P_{1-3}=0.004	P_{1-2}=0.05	P_{1-4}=0.001 P_{2-4}=0.02	P_{3-4}=0.01	P_{1-4}=0.02 P_{3-4}=0.007

Note: IQR, interquartile range; M, mean value; n, number of patients; ON, optic neuritis; POA, partial optic atrophy; SD, standard deviation

Table 3. Levels of natural killers and phagocytic neutrophils in patients with optic neuritis and its sequelae

Nosological form	Group	CD 16 (natural killers)		Phagocytic neutrophils	
		Cell/ml, Median (IQR)	% M±SD	Cell/ml, Median (IQR)	% M± SD
Acute ON n =27	1	352 (146–330)	10.4±3.3	3207 (2791–4392)	74.8±11.8
POA following ON n=9	2	305 (143–506)	13.7±6.2	1834 (1442–3952)	68.6±13.4
Other sequelae of ON n =9	3	178 (143–205)	11.0±2.7	2240 (1422–3255)	64.8±9.9
Controls n =27	4	196 (102–233)	12.4±3.9	2926 (2162–2954)	67±5.2
Significance of difference (P-value)		-	-	P_{1-4}=0.0002 P_{1-2}=0.001 P_{1-3}=0.03	P_{1-4}=0.0002

Note: IQR, interquartile range; M, mean value; n, number of patients; ON, optic neuritis; POA, partial optic atrophy; SD, standard deviation

the leukocyte cell count (r = 0.55) and CD3 (r = 0.77), CD4 (r = 0.74), CD8 (r = 0.69), CD19 (r = 0.66) and CD16 (r = 0.72) lymphocyte subset counts. There was no significant difference in correlation coefficients between the adrenoreceptor expressing T cells-lymphocyte subset counts relationships and acetylcholine receptor-expressing T cells- lymphocyte subset counts relationships.

Discussion

In the present-day literature, the immunological status of patients with ON is specified mostly in the presence of demyelinating process affecting the optic nerve. In MS it is suggested that autoreactive CD4+ T cells are activated in the peripheral blood by nonself antigens that resemble CNS myelin proteins. Thereafter, these cells migrate through the blood–brain barrier in response to chemotactic signals, stimulate CD14+ macrophage by secretion of inflammatory cytokines, such as IFN-γ and IL-1, to induce myelin damage. Humoral immunity is also involved in the process, with CD19+ B-cells being activated at the early stage of ON [7]. In the current study, patients with acute idiopathic ON showed increased cell immunity activity, with increased absolute numbers of CD3, CD4, and CD8 cells compared to the control group. In addition, there was no significant difference in CD4:CD8 ratio between patients with acute idiopathic ON and controls. This is in agreement with the findings of Guy and colleagues [16], who found that, mean ratio of inducer (CD4) to suppressor

Table 4. Levels of the peripheral blood T cells sensitive to adrenaline and acetylcholine in patients with optic neuritis and its sequelae

Nosological form	Group	Sensitivity of peripheral blood T cells to neurotransmitters			
		Adrenaline		Acetylcholine	
		Cell/ml	% M±SD	Cell/ml	% M±SD
Acute ON n =27	1	394 (284–460)	14.4±3.8	366 336–394	13.5±2.6
POA following ON n=9	2	291 (174–424)	13.4±3.5	334 (190–349)	14.0±2.5
Other sequelae of ON n =9	3	191 (113–287)	11.1±5.5	192 (190–219)	11.7±2.9
Контроль n =27	4	115 (86–203)	7.3±2.9	129 (106–199)	7.8±1.9 (6.0–9.4)
Significance of differences (P-value)		$p_{1-4=}$0.006 $p_{2-4=}$0.02 $p_{3-4=}$0.03 $p_{1-3=}$0.007	$p_{1-4=}$0.0001 $p_{2-4-=}$0.004	$p_{1-4=}$0.0005 $P_{2-4=}$0.017 $p_{1-3=}$0.001	$p_{1-4=}$0.00002 $p_{2-4=}$0.0009 $p_{3-4=}$0.03 $p_{1-3=}$0.05

Note: IQR, interquartile range; M, mean value; n, number of patients; ON, optic neuritis; POA, partial optic atrophy; SD, standard deviation

(CD8) T-lymphocytes was 2.07 ± 0.51 for the group with ON, which was statistically indistinguishable from a value of 1.78 ± 1.04 for the control group. Moreover, we noted increased levels of activity of humoral immunity components (increased absolute numbers of B lymphocytes and increased IgA and IgM levels) in acute ON compared to the control group. Others [7] also reported evidence implicating humoral immune involvement in ON. We, however, noted lower IgG levels and increased phagocytic neutrophil levels in the peripheral blood of patients with acute ON compared to the control group. To the best of our knowledge, these findings have not been reported in the available literature. Therefore, we observed activation of cell-mediated as well as humoral immunity in patients with acute ON.

In the current study, we compared patients with acute ON with those with POA following ON and those with loss of posterior pole structure following ON in terms of their immune status. A reduction in and normalization of absolute numbers of CD3 and CD4 T cells compared to acute ON were characteristic features of cell-mediated immunity in patients with POA following ON and those with loss of posterior pole structure following ON. The percentage of CD8 cells in patients with POA following ON was lower than in controls. It is these characteristic proportions of immunocompetent cell ratios that mirrored in increased CD4:CD8 ratios in ON groups, which is associated with a reduced level of cytotoxic T cells. Thus, in patients with POA following ON, the percentage of CD8 cells increased, resulting in a 56% increase in the CD4:CD8 ratio. Therefore, following ON, T-lymphocyte level and T-helper level normalized, but the T-cell ratio changed due to a reduced level of cytotoxic T cells. Our previous study on uveitis complicated by intraocular hypertension [17] has demonstrated that the CD4:CD8 ratio was higher in patients with Fuchs heterochromic uveitis or Posner-Schlossman syndrome than in those with uncomplicated idiopathic uveitis.

Following ON, the level of CD19 B-cells was reduced compared to acute ON, but the levels of IgA and IgM were still high. However, in patients with acute ON, patients with POA following ON and those with loss of posterior pole structure following ON, their IgG levels were lower than in controls. This rigidity in changes in immunoglobulin levels mirrors the features of humoral immunity regulation.

The relative level of peripheral blood T cells sensitive to adrenaline and acetylcholine was increased compared controls in all ON groups, and was higher in patients with acute ON and patients with POA following ON compared to those with loss of posterior pole structure following ON. In our previous work on posterior uveitis [10], we noted that absolute and relative adrenoreceptor and acetylcholine receptor expression levels in patients with posterior uveitis were higher in patients with posterior uveitis compared to controls. Absolute adrenoreceptor and acetylcholine receptor expression values were higher in the recurrent process compared to the period of remission. However, in patients with posterior uveitis, adrenoreceptor and acetylcholine receptor expression values in the period of remission were still higher than normal values [10]. We also found direct correlations of the level of the T cells sensitive to these neurotransmitters with CD3, CD8, CD19 and CD16 lymphocyte subset counts. This demonstrates the role of T cells with neurotransmitter receptor expression, with this expression manifesting in concert with other

components of cell-mediated and humoral immunity during inflammatory process.

Conclusion

First, the levels of CD3, CD4, and CD8 cells (the components of cell-mediated immunity) in acute ON group were increased 2.0-fold, 1.8-fold, and 1.4-fold, respectively, compared to controls, but were normalized in groups of post-ON POA or complications. In addition, a reduction in the level of CD8 cells in patients with POA following ON resulted in a 56% increase in the CD4:CD8 ratio.

Second, with regard to humoral immunity components, the levels of CD19 and IgA were increased 2.05-fold and 1.5-fold, respectively, whereas the level of IgG was decreased by 12.5% in acute ON group compared to controls. In addition, the level of IgA was increased 1.5-fold in patients with POA following ON compared to controls.

Third, the laterality of ON exerted an impact only on the relative number of lymphocytes, proportion of CD8 and CD4:CD8 ratio in total patients. The relative number of lymphocytes was 32% larger, the proportion of CD8, 15.7% smaller, and the CD4:CD8 ratio, 20% larger, in bilateral ON than in unilateral ON.

Fourth, patients in groups 1, 2 and 3 had increased absolute numbers of peripheral blood T cells sensitive to adrenaline, being 3.4-fold, 2.4-fold, and 1.7-fold more than controls, respectively. In addition, patients in groups 1 and 2 had increased absolute numbers of peripheral blood T cells sensitive to acetylcholine, being 2.8-fold and 2.6-fold more than controls, respectively.

Finally, we found significant direct correlations of the level of the T cells sensitive to adrenaline and acetylcholine with the leukocyte cell count and CD3, CD8, CD19 and CD16 lymphocyte subset counts in patients with ON and its sequelae.

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Disclosures

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Abbreviations: *CRION, chronic/relapsing inflammatory optic neuropathy; MS, multiple sclerosis; ON, optic neuritis; POA, partial optic atrophy*