T-CELLS RESPONSE IN EXPERIMENTAL AUTOIMMUNE UVEITIS OF VARYING SEVERITY

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SUMMARY

Aim: to investigate the dynamics of the T-cell immune response in rabbits with experimental autoimmune uveitis (EAU) of varying severity.
Materials and Methods: The experiment involved two groups of Chinchilla rabbits (15 rabbits in each group). The model of EAU was created. The clinical picture of intraocular inflammation of varying severity was assessed. The determination of the level of white blood cells (WBC), lymphocytes (Lymphs), CD3+, CD4+, CD8+, and CD16+ in the blood of rabbits was conducted.
Results: Group I – moderate and severe uveitis, Group II – uveitis of mild severity. WBC, Lymphs, CD3+, CD4+, CD16+ were elevated and statistically significant in both groups of animals compared to control parameters on all days of the experiment (3, 7, 10, 14, 21 days) ($p < 0.001$). CD8+ level had a significantly lower count than the control one ($p < 0.001$). When comparing the two groups, the immune response was more active in Group I, and the number of immune cells did not return to normal by the end of the experiment.
Conclusion: In the case of EAU, the immune response is characterized by the activation of the T-cell immune system, with the intensity of this response depending on the severity of the clinical presentation of uveitis. Various degrees of clinical severity in EAU were obtained using an experimental model employed in our study. A rapid response of the immune system helps to establish a diagnosis and predict the severity of autoimmune uveitis.
Key words: T-cells response, experiment, autoimmune uveitis

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INTRODUCTION

Timely etiotropic treatment of uveitis holds significant medical and social importance, due to the high prevalence of this condition, its impact on young individuals, and the high disability rates among patients – on average 30 % and 50–60 % in severe cases of the disease [1,2]. Numerous studies have revealed that in 2.8–10 % of cases, uveitis leads to blindness [1,3]. Since the pathogenesis of the disease is primarily driven by the development of immunological reactions in response to antigens and damage to the vascular layer of the eye, any type of infection or systemic illness in the body triggers immune system activation, potentially leading to uveitis [1]. The aim of the study was to investigate the dynamics of the T-cell immune response in rabbits with EAU of varying severity.

MATERIALS AND METHODS

The experiment involved two groups of Chinchilla rabbits (15 rabbits in each group), weighing 2.5–3.0 kilograms. These rabbits were kept under standard vivarium conditions and were provided with a standard diet. The experiment was conducted at the Danylo Halytsky Lviv National Medical University, in accordance with all internationally accepted norms and rules for working with experimental animals, following the provisions of the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes of 18.03.1986, the guidelines for the care and use of laboratory animals of "The general ethical principles of animal experiments", which were approved by the 3rd National Congress in Kyiv in 2007, and the WMA Declaration of Helsinki: "Guidelines for the use of experimental ani-

mals in experimental research" from 1964–2000, as well as Council Directive 2010/63/EU.

Two weeks before the experiments, the rabbits were in quarantine. Before the start of the experiment, blood was taken from all experimental rabbits for analysis of immune cells. These results were considered as normal. Since this study is experimental, no informed consent forms were provided.

To create an EAU model, all rabbits were intravenously injected into the marginal ear vein with 1.0 ml normal horse serum for biological nutrient media (AT Biolog, No. 5050 21) for a duration of 5 days, at 24-hour intervals. Ten days after the last injection, rabbits in Group I were intravitreally administered a provocative dose (0.1 ml) of undiluted normal sterile horse serum in both eyes, thus modeling uveitis of moderate and severe degrees. Rabbits in Group II were intravitreally administered a provocative dose (0.1 ml) of diluted normal sterile horse serum in both eyes (diluted with saline in a 1:2 ratio).

The assessment of the clinical picture of intraocular inflammation of varying severity was conducted based on biomicroscopy and ophthalmoscopy. Depending on the localization of the primary focus of inflammation, uveitis is classified into several types according to the data provided by the National Eye Institute, as presented in Table 1 [4].

When assessing clinical changes in the anterior part of the eye in EAU, it was deemed appropriate to use a scoring system. We find it advisable to combine existing classifications for a more comprehensive description of clinical changes in the eye in EAU (Table 2) [5–10].

To assess changes in the posterior segment of the eye, we utilized a classification that was proposed by Thurau S.R. with co-authors (Table 3) [11].

Table 1. Anatomical classification of uveitis based on Standardization of Uveitis Nomenclature (SUN) for reporting clinical data

Type of inflammation	Primary Site of Inflammation	Includes
Anterior uveitis	Anterior chamber	Iritis Iridocyclitis Anterior cyclitis
Intermediate uveitis	Vitreous	Pars planitis Posterior cyclitis Hyalitis
Posterior uveitis	Choroid or Retina	Focal, multifocal or diffuse choroiditis Chorioretinitis Retinochoroiditis Retinitis Neuroretinitis
Panuveitis	Anterior chamber, vitreous and retina or choroid	

Table 2. Clinical grading of EAU in rabbit by biomocroscopy

Grade	Anterior chamber	Cornea	Iris	Vitreum
0	No cells	Transparent	Normal	No evidence of vitreal haze. Posterior pole clearly visible
1^+	Faint cell reaction. Iris and lens details clear	Edema	Iris vessel engorgement, normal pupil shape and light reflex	Posterior pole details slightly hazy. Obscured view but definition to optic nerve head and retinal vessels
2^+	Moderate cell reaction. Iris and lens details clear	Light precipitation and edema	Light hyperemia and edema, normal pupil shape and light reflex	Posterior pole details very hazy. Obscured view but definition to retinal vessels
3^+	Intense cell reaction. Early fibrinous exudate at pupillary margin. Iris and lens details hazy	Moderate precipitation and edema	Moderate hyperemia and edema, posterior synechiae, irregular-shaped or constricted pupil	Posterior pole details barely visible. Optic nerve head visualized but borders are very blurry
4^+	Fibrin plugging pupil. Retroiridal hypopyon. Iris details hazy, no lens details	Severe precipitation and edema	Severe hyperemia and edema, posterior synechiae, no pupillary light reflex	Obscured fundal view. Fundal details not visible

EAU – Experimental autoimmune uveitis

The determination of the level of white blood cells (WBC), lymphocytes (Lymphs), $CD3^+$, $CD4^+$, $CD8^+$, and $CD16^+$ in the blood of rabbits was conducted in the immunological research laboratory of the State institution "The Filatov Institute of Eye Diseases and Tissue Therapy of NAMS of Ukraine", Ukraine, using the immunohistocytochemical PAP method with a set of monoclonal antibodies produced by Novus Biologicals (UK). Blood samples were collected before the experiment, prior to intravitreal injection of normal sterile horse serum, on days 3, 7, 10, 14, and 21 after the start of the experiment.

Statistical data analysis

The analysis of the research results was performed using the EZR software v. 1.61 (a graphical user interface for R statistical software v. 4.2.2, R Foundation for Statistical Computing, Vienna, Austria) [12]. The normality of quantitative variables was checked using the Shapiro-Wilk test. To present the results (due to non-normal distribution), the median value (Me) and interquartile range (Q1–Q3) were provided. The Kruskal-Wallis test was used for comparison, and post hoc comparisons were conducted using Conover (1999) [13]. In all cases of statistical comparison, two-tailed critical regions were used, and the critical level of significance was set at 0.05.

Table 3. Clinical grading of EAU in rabbit by fundoscopy

Score	Description
0.5 (trace)	Minimal vasculitis Few (1–2) very small, peripheral, focal, chorioretinal lesion
1^+	Mild vasculitis ≤ 5 small focal chorioretinal lesion ≤ 1 linear chorioretinal lesion
2^+	Severe vascultits (large thick infiltrates) Multiple (> 5) chorioretinal lesion and/or inflammatory infiltrates Severe vascultits (large thick infiltrates) ≤ 5 linear chorioretinal lesion
3^+	Patter of chorioretinal lesion Large, confluent chorioretinal lesion Subretinal neovascularization Haemorrhages
4^+	Large retinal detachment Retinal atrophy

EAU – Experimental autoimmune uveitis

RESULTS

As a result of modeling EAU in the rabbit, uveitis of varying severity was observed. In all rabbits in Group I, the examination revealed ciliary or mixed injection of the eye, corneal and iris edema, multiple precipitates, miosis. In some cases, hypopyon and multiple posterior synechiae were also noted. By ophthalmoscopy, the fundus could not be seen in detail due to dense opacities in the vitreous body. In the meantime, we observed significant changes in the fundus of the eye, namely, severe vasculitis, subretinal neovascularization, hemorrhages, retinal detachment and atrophy. The developed clinical picture was considered as uveitis of moderate and severe degrees and according to Table 2 and Table 3.

In Group II, all animals exhibited the clinical picture of uveitis of mild severity, corresponding to stages 0.5+, 1+, and 2+ according to classifications in Tables 2 and 3. Moderate cell reaction, light precipitation, some posterior synechiae were observed. The fundus details were observed to be slightly or very hazy. Obscured view, but definition to optic nerve head and in some cases retinal vessels. At the same time, we diagnosed mild or severe vasculitis.

Owing to our study, we obtained the following results of the count of immunological cells in the blood of rabbits from the two experimental groups with different disease severity on all experimental days (Tables 4–8, Graphs 1–6). The main immune response cells, such as WBC, Lymphs, $CD3^+$, $CD4^+$, $CD16^+$ were elevated and statistically significant in both groups of experimental animals with EAU, compared to control parameters on all days of the experiment ($p< 0.001$) This confirms the active involvement of the immune response in intraocular inflammation. The dynamics of these indicators in rabbits from

Table 4. Immunological cells parameters in the blood of rabbits in experimental groups with EAU on Day 3 (Me[Q_I – Q_{III}], p)

Immune cells	Norm	Group I	Group II	p
WBC	4.4 (4.3–4.5)^{1,2}	4.55 (4.5–4.6)^{0,2}	5.2 (5.11–5.28)^{0,1}	< 0.001
Lymphs	29 (28.0–29.0)^{1,2}	68 (67.25–69.0)^{0,2}	65 (63.5–66.0)^{0,1}	< 0.001
$CD3^+$	57.45 (57.0–57.7)^{1}	62 (61.25–63.0)^{0,2}	58 (57.0–58.75)^{1}	< 0.001
$CD4^+$	43.5 (43.3–43.8)^{1,2}	51 (49.0–56.25)^{0,2}	46 (45.0–46.0)^{0,1}	< 0.001
$CD8^+$	14 (14.0–14.0)^{1,2}	12 (11.0–12.0)^{0,2}	13 (13.0–14.0)^{0,1}	< 0.001
$CD16^+$	15 (15.0–16.0)^{1,2}	19 (18.25–19.75)^{0,2}	17 (16.0–17.0)^{0,1}	< 0.001

Notes: For comparison, the Kruskal-Wallis criterion was used, and posterior comparisons were conducted using Conover (1999):
^{0} – difference from the Norm is statistically significant, $p < 0.05$
^{1} – difference from the Group I is statistically significant, $p < 0.05$
^{2} – difference from the Group II is statistically significant, $p < 0.05$
EAU – Experimental autoimmune uveitis
WBC – white blood cells

the two main groups are associated with the dynamics of clinical changes. Specifically, the gradual regression of intraocular inflammation was to some extent confirmed by the decrease in the number of WBC, Lymphs, $CD3^+$, $CD4^+$, $CD16^+$ in the blood of rabbits. At the same time, the level of $CD8^+$ significantly decreased compared to the control parameters on all days of the experiment in both groups ($p < 0.001$). However, there was a tendency for an increase in this immunological indicator from the 14th day of the study.

For comparing changes in the immune response in two different experimental groups, we created comparative images (Graphs 1–6). In Group I of experimental animals, where uveitis of moderate and severe degrees was modeled, a significant increase in WBC, Lymphs, and $CD3^+$ was observed from the 3rd day of the experiment. Specifically, the levels were 4.55 g/l (4.5–4.6), 68 % (67.25–69.0), 62 % (61.25–63.0). The peak increase in the number of WBC, Lymphs, and $CD3^+$ occurred on the 7th day of the study, and their counts were 6.8 g/l (6.46–7.0),

Table 5. Immunological cells parameters in the blood of rabbits in experimental groups with EAU on Day 7 3 (Me[Q_i – Q_{iii}], p)

Immune cells	Norm	Group I	Group II	p
WBC	4.4 (4.3–4.5)^{1,2}	6.8 (6.475–7.0)^{0,2}	6.1 (5.950–6.225)^{0,1}	< 0.001
Lymphs	29 (28.0–29.0)^{1,2}	69 (68.75–71.25)^{0,2}	64 (63.0–66.0)^{0,1}	< 0.001
$CD3^+$	57.45 (57.0–57.7)^{1,2}	68 (66.75–69.0)^{0,2}	62 (61.75–63.0)^{0,1}	< 0.001
$CD4^+$	43.5 (43.3–43.8)^{1,2}	54 (54.0–55.25)^{0,2}	51 (49.75–51.25)^{0,1}	< 0.001
$CD8^+$	14 (14.0–4.0)^{1,2}	10 (9.0–10.0)^{0,2}	11 (10.0–11.0)^{0,1}	< 0.001
$CD16^+$	15 (15.0– 6.0)^{1,2}	22 (22.0–23.0)^{0,2}	20 (19.0–21.0)^{0,1}	< 0.001

Notes: For comparison, the Kruskal-Wallis criterion was used, and posterior comparisons were conducted using Conover (1999):
^{0} – difference from the Norm is statistically significant, $p < 0.05$
^{1} – difference from the Group I is statistically significant, $p < 0.05$
^{2} – difference from the Group II is statistically significant, $p < 0.05$
EAU – Experimental autoimmune uveitis
WBC – white blood cells

Table 6. Immunological cells parameters in the blood of rabbits in experimental groups with EAU on Day 10 (Me[Q_i – Q_{iii}], p)

Immune cells	Norm	Group I	Group II	p
WBC	4.4 (4.3–4.5)^{1,2}	6.2 (6.025–6.275)^{0,2}	5.8 (5.7–5.9)^{0,1}	< 0.001
Lymphs	29 (28.0–29.0)^{1,2}	52 (51.0–53.0)^{0,2}	49 (48.0–49.75)^{0,1}	< 0.001
$CD3^+$	57.45 (57.0–57.7)^{1,2}	64 (63.25–65.0)^{0,2}	62 (61.25–62.0)^{0,1}	< 0.001
$CD4^+$	43.5 (43.3–43.8)^{1,2}	58 (57.0–59.0)^{0,2}	51 (50.25–52.0)^{0,1}	< 0.001
$CD8^+$	14 (14.0–14.0)^{1,2}	9 (9.0–9.0)^{0,2}	10 (10.0–11.0)^{0,1}	< 0.001
$CD16^+$	15 (15.0–16.0)^{1,2}	23 (23.0–24.0)^{0,2}	20 (20.0–21.0)^{0,1}	< 0.001

Notes: For comparison, the Kruskal-Wallis criterion was used, and posterior comparisons were conducted using Conover (1999):
^{0} – difference from the Norm is statistically significant, $p < 0.05$
^{1} – difference from the Group I is statistically significant, $p < 0.05$
^{2} – difference from the Group II is statistically significant, $p < 0.05$
EAU – Experimental autoimmune uveitis
WBC – white blood cells

Table 7. Immunological cells parameters in the blood of rabbits in experimental groups with EAU on Day 14 (Me[Q_i – Q_{iii}], p)

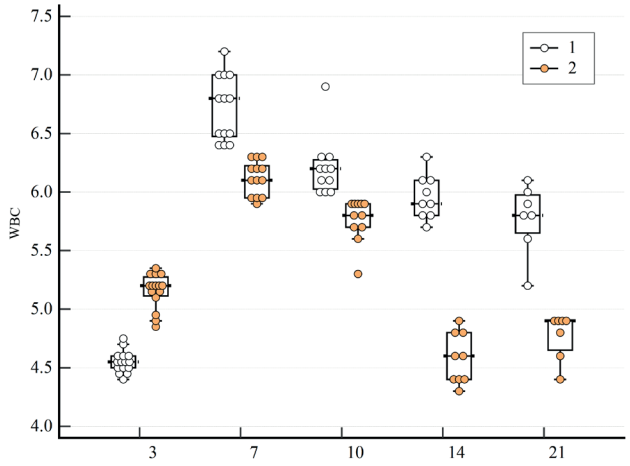
Immune cells	Norm	Group I	Group II	p
WBC	4.4 (4.3–4.5)^{1,2}	5.9 (5.8–6.1)^{0,2}	4.6 (4.4–4.8)^{0,1}	< 0.001
Lymphs	29 (28.0–29.0)^{1,2}	45 (44.0–46.0)^{0,2}	41 (40.0–42.25)^{0,1}	< 0.001
$CD3^+$	57.45 (57.0–57.7)^{1,2}	65 (64.0–65.5)^{0,2}	62 (60.75–62.0)^{0,1}	< 0.001
$CD4^+$	43.5 (43.3–43.8)^{1,2}	58 (57.0–59.0)^{0,2}	51 (50.75–52.0)^{0,1}	< 0.001
$CD8^+$	14 (14.0–14.0)^{1,2}	12 (11.0–12.0)^{0,2}	11 (10.75–11.0)^{0,1}	< 0.001
$CD16^+$	15 (15.0–16.0)^{1,2}	22 (21.0–22.25)^{0,2}	20 (18.75–20.0)^{0,1}	< 0.001

Notes: For comparison, the Kruskal-Wallis criterion was used, and posterior comparisons were conducted using Conover (1999):
^{0} – difference from the Norm is statistically significant, $p < 0.05$
^{1} – difference from the Group I is statistically significant, $p < 0.05$
^{2} – difference from the Group II is statistically significant, $p < 0.05$
EAU – Experimental autoimmune uveitis
WBC – white blood cells

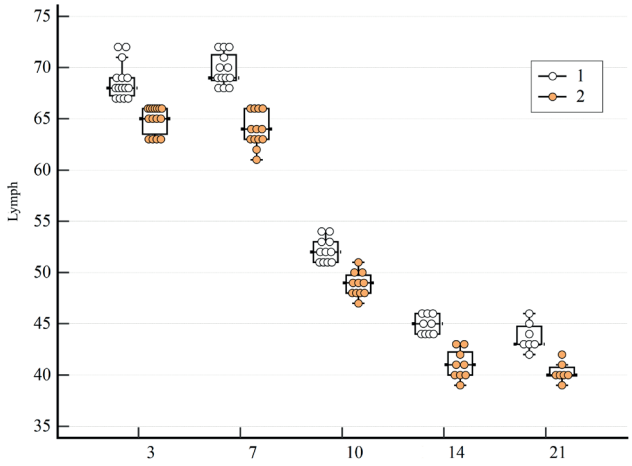
Table 8. Immunological cells parameters in the blood of rabbits in experimental groups with EAU on Day 21 (Me[Q_I – Q_{III}], p)

Immune cells	Norm	Group I	Group II	p
WBC	4.4 (4.3–4.5)[1,2]	5.8 (5.65–5.98)[0,2]	4.9 (4.65–4.9)[0,1]	< 0.001
Lymphs	29 (28.0–29.0)[1,2]	43 (43.0–44.75)[0]	40 (40.0–40.75)[0]	< 0.001
$CD3^+$	57.45 (57.0–57.7)[1,2]	61 (60.25–62.0)0	59 (58.25–60.0)[0]	< 0.001
$CD4^+$	43.5 (43.3–43.8)[1]	45 (45.0–46.0)[0,2]	43 (42.25–44.0)[1]	< 0.001
$CD8^+$	14 (14.0–14.0)[2]	14 (13.0–14.0)[2]	13 (12.25–13.0)[0,1]	< 0.001
$CD16^+$	15 (15.0–16.0)[1,2]	18 (17.25–18.75)[0,2]	16 (16.0–17.0)[0,1]	< 0.001

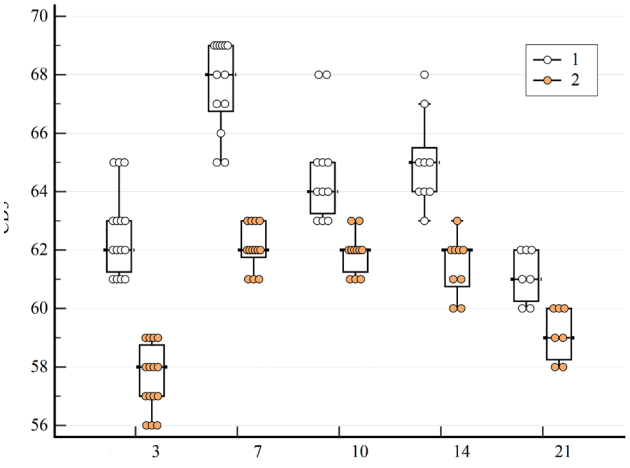
Notes: For comparison, the Kruskal-Wallis criterion was used, and posterior comparisons were conducted using Conover (1999):
[0] – difference from the Norm is statistically significant, p < 0.05
[1] – difference from the Group I is statistically significant, p < 0.05
[2] – difference from the Group II is statistically significant, p < 0.05
EAU – Experimental autoimmune uveitis
WBC – white blood cells



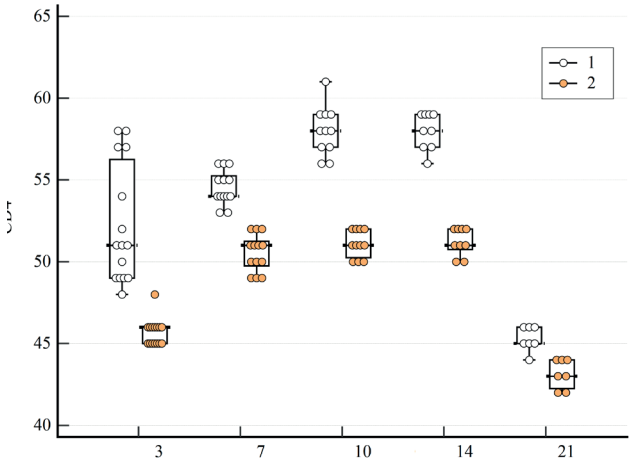
Graph 1. Comparative characterization of the dynamics of WBC count in the blood of rabbits in two experimental groups (1,2) with different disease severity
WBC – white blood cells



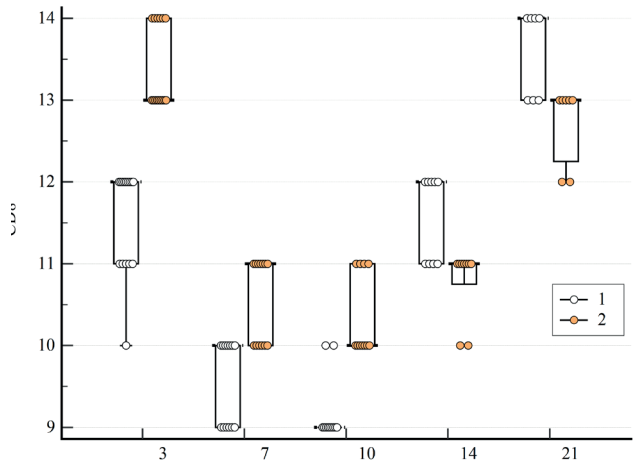
Graph 2. Comparative characterization of the dynamics of Lymph count in the blood of rabbits in two experimental groups (1,2) with different disease severit



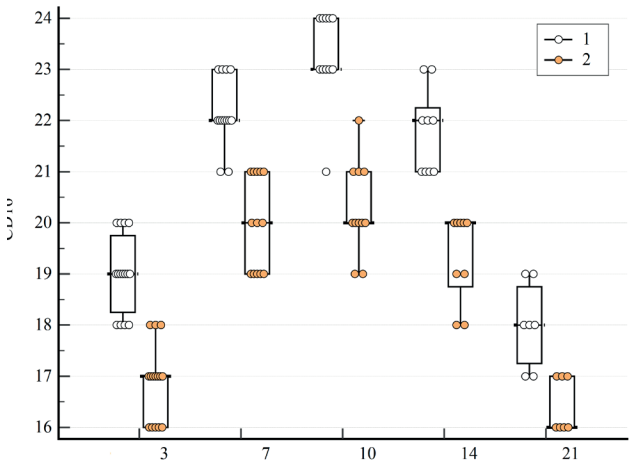
Graph 3. Comparative characterization of the dynamics of $CD3^+$ count in the blood of rabbits in two experimental groups (1,2) with with different disease severity
Lymphocytes $CD3^+$



Graph 4. Comparative characteri zation of the dynamics of $CD4^+$count in the blood of rabbits in two experimental groups (1,2) with with different disease severity
Lymphocytes $CD4^+$



Graph 5. Comparative characterization of the dynamics of $CD8^+$ count in the blood of rabbits in two experimental groups with with different disease severity
Lymphocytes $CD8^+$



Graph 6. Comparative characterization of the dynamics of $CD16^+$count in the blood of rabbits in two experimental groups with with different disease severity
Lymphocytes $CD16^+$

69 % (68.75–71.25) and 68 % (66.75–69.0), respectively. In addition, we observed the tendency to a decrease of these cells by the 10th day (WBC – 6.2 g/l (6.03–6.28), Lymphs – 52 % (51.0–53.0), $CD3^+$ – 64 % (63.25–65.0). Furthermore, the count of these cells did not return to normal by the end of the experiment and was at 5.8 g/l (5.65–5.98), 43 % (43.0–44.75), and 61 % (60.25 – 62.0) on the 21st day, respectively. In addition, $CD4^+$, $CD16^+$ were also consistently elevated on all days of the study (e.g. on the 3rd day, $CD4^+$ was 51 % (49.0–56.25), $CD16^+$ was 19 % (18.25–19.75); on the 7th day – 54 % (54.0 – 55.25), 22 % (22.0–23.0); on 10th day – 58 % (57.0–59.0), 23% (23.0–24.0); on 14th day – 58 % (57.0–59.0), 22 % (21.0–22.25), respectively, without returning to normal by the end of the experiment 45 % (45.0–46.0), 18 % (17.25–18.75).

Meanwhile, in Group II of rabbits, where mild uveitis was modeled, on the 3rd day of the experiment, a significant increase was observed in all investigated immune cells, except for $CD3^+$ (Table 4, Graphs 1–6). Specifically, the count of WBC was 5.2 g/l (5.11–5.28), Lymphs – 65 % (63.5–66.0), $CD4^+$ – 46 % (45.0–46.0), and $CD16^+$ – 17 % (16.0–17.0). From the 7th day, there was a statistically significant increase in the level of $CD3^+$ – 62 % (61.75–63.0), and further increases in the levels of $CD4^+$ –51 % (49.75–51.25), and $CD16^+$ – 20 % (19.0–21.0). Also, the counts of $CD3^+$, $CD4^+$, $CD16^+$ remained at an elevated level on days 7, 10, and 14 (Tables 4–8, Graphs 1–6). Furthermore, the count of all these cells did not return to normal by the end of the experiment.

As a consequence of examining of the $CD8^+$ level, we observed a significantly lower count than the control one ($p < 0.001$) in all rabbits in both experimental groups on all days of the experiment and differing between the two groups with different degrees of EAU severity (Table 4–8, Graphs 5,6). For example, on the 3rd day of the experiment, the $CD8^+$ level in Group I was 12 % (11.0–12.0), and in Group II, it was 13 % (13.0–14.0). On the 7th day, it was 10 % (9.0–10.0) in Group I, and 11 % (10.0–11.0) in Group II. On the 10th day, it was 9 % (9.0–9.0) in Group I, and 10(10.0–11.0) in Group II. On the 14th day, it was 12(11.0–12.0) in Group I, and 11 % (10.75–11.0) in Group II. Finally, on the 21st day, it was 14 % (14.0–14.0) in Group I, and 13 % (12.25–13.0) in Group II.

What is of paramount importance is that the number of all immunological cells used in this experimental study were statistically different in the two different groups of rabbits on all days of the experiment ($p < 0.001$), except Lymphs and $CD3^+$, that showed no difference on day 21. These manifestations confirm the active immune system response to the amount of antigen administered and the development of uveitis of varying severity.

DISCUSSION

The immune response plays a crucial role in the etiopathogenesis of uveitis. Immunological changes can both initiate inflammation (e.g. autoimmune uveitis, sympathetic ophthalmia) and accompany inflammation without a specific influence on its development [14]. Peripheral tolerance is maintained by antigen-specific T-regulatory cells. The introduction of an antigen into the anterior chamber of the eye leads to the activation of the T-cell immune response, which works to suppress immune hyperactivity, maintaining self-tolerance [15]. The eye enjoys immunological privilege, extending to the anterior chamber, the subretinal space, and the vitreous body [16,17]. When an antigen enters the intraocular environments, an active immune response occurs, triggering a cascade of inflammatory reactions, which leads to a disruption of the blood-ocular barrier [18,19]. As a result, immune defense cells migrate beyond the eye envi-

ronment, specifically into the bloodstream [20], allowing for an adequate assessment of the immune system's condition and its response to inflammatory changes in the eye when analyzing blood serum.

This discussion highlights the critical role of the immune response in the development and progression of uveitis. It also emphasizes the importance of studying immunological changes in the blood to better understand and monitor the immune system's response to intraocular inflammation.

Over the past half-century, numerous models of non-infectious intraocular inflammation have been described in various experimental animals, including mice, rats and rabbits, used to study different types of uveitis [21–24]. The most common method of creating an experimental autoimmune uveitis (EAU) model involves the introduction of autoantigens either systemically or directly into the eyeball. Therefore, these models are classified as systemically induced or local. In many studies, the most frequently described model involves the use of S-antigen [25–28], interphotoreceptor retinoid-binding protein, bacterial endotoxin and cytokines [29,30]. However, previous publications have not addressed the issue of the severity of EAU depending on the type of model and the amount of antigen introduced, confirmed by key clinical signs depending on the severity. In our experiment, we successfully recreated an EAU model of varying severity with confirmed immune response.

In our experimental study of EAU, the immune response was characterized by the activation of the T-cell immune system, specifically the induction of $CD3^+$, $CD4^+$, $CD16^+$. Additionally, at the core of the EAU mechanism, there is a disruption in the regulation of the immune response, which is attributed to $CD8^+$ cells (cytotoxic suppressors). As their numbers decrease, and, consequently, the levels of $CD4^+$, this results in an imbalance in the normal immune response.

This observation underscores the involvement of T-cell-mediated immunity in the pathogenesis of EAU and highlights the dysregulation of immune responses, leading to the imbalance of immune cell populations in the context of uveitis.

Furthermore, during our experimental study, we found that the intensity and severity of inflammation of the vascular membrane of the eye correlated with the activation of the T-cell immune system. When comparing the two groups of animals where EAU was modeled, the levels of all immune response cells in blood were significantly different throughout the experiment ($p < 0.001$). However, the level of $CD8^+$ decreased similarly in both groups on days 3, 7, 10, and 14 compared to the control data and began to increase from day 21. In the early stages of EAU, there was an association with a T-helper phenotype of the immune response. As inflammation regressed in the vascular membrane of the eye, the number of T-suppressor cells gradually increased. This was also confirmed by research conducted by German scientists [31]. These changes in the balance between $CD4^+$ and $CD8^+$ in the dynamics of the development and regression of EAU probably reflect the kinetics and regulation of the inflammatory process [32].

In summary, the study observed a relationship between the intensity of EAU and the activation of the T-cell immune system, with different stages of the disease being associated with shifts in the balance between T-helper and T-suppressor cells. Various degrees of clinical severity in EAU were obtained using an experimental model employed in our study. This insight contributes to our understanding of the dynamic nature of uveitis and its immunological underpinnings.

Thus, the assessment of the levels of $CD3^+$, $CD4^+$, $CD8^+$, $CD16^+$ in peripheral blood can serve as a criterion for evaluating the severity of non-infectious autoimmune uveitis, its course, and possibly prognosis. Currently, research continues to identify the most accurate algorithm for predicting the development of uveal tract inflammation. Therefore, timely immunological studies are of utmost importance. This, in turn, will enable the focus on immunomodulation as part of the comprehensive treatment of non-infectious autoimmune uveitis.

CONCLUSION

In the case of EAU, the immune response is characterized by the activation of the T-cell immune system, with the intensity of this response depending on the severity of the clinical presentation of uveitis. Various degrees of clinical severity in EAU were obtained using an experimental model employed in our study. A rapid response of the immune system helps to establish a diagnosis and predict the severity of autoimmune uveitis. These findings serve as templates for the development of new therapeutic approaches in humans.

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