

DOI 10.24425/pjvs.2023.145049

*Original article*

# Depletion of T and B cells in lymphoid tissues of mice induced by oclacitinib, a Janus kinase inhibitor

**A. Jasiocka-Mikołajczyk, T. Maślanka**

Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine,  
University of Warmia and Mazury, Oczapowskiego 13, 10-718 Olsztyn, Poland

## Abstract

The main purpose of the study was to determine the safety of oclacitinib (OCL), a Janus kinase inhibitor, with respect of its effect on CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as B cells in the lymphoid tissue. The mice were treated orally with OCL at a dose of 2.7 mg/kg for 14 days and peripheral blood, head and neck lymph nodes (HNLNs), mediastinal lymph nodes (MLNs) and spleen were collected. The study found that OCL induced depletion of CD4<sup>+</sup> T cells in the HNLNs and MLNs, while it did not affect the absolute count of CD8<sup>+</sup> T cells in these tissues. Also OCL caused a loss of B cells in the HNLNs, although not in the MLNs. Moreover, OCL depleted B cells in the peripheral blood, but did not affect the absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Thus, it can be concluded that OCL may induce a depletive effect on CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as B cells in the lymphoid tissue. This effect should be seen as an unfavorable one, especially in patients with infections. Therefore, a clinical implication is that in such patients, the benefit/risk ratio should be thoroughly considered by clinicians. Moreover, OCL reduced the absolute count of eosinophils, basophils, neutrophils and monocytes. However, it is uncertain whether this effect should be considered to be of clinical importance because the levels of these cells were within the physiological range. It is possible that the depletive effect of OCL toward T and B cells, as well as eosinophils and basophils may contribute to the beneficial effects of the drug in the treatment of skin allergic diseases.

**Keywords:** B cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, Janus kinase inhibitor, lymphoid tissue, oclacitinib

## Introduction

Oclacitinib (OCL; Apoquel®) is an immunomodulatory oral agent that has been registered for control of pruritus associated with allergic dermatitis and control of atopic dermatitis (AD) in dogs. OCL has a unique mechanism of action that acts to inhibit the Janus kinase (JAK) enzymes and mainly displays activity against JAK1 enzyme over JAK2 or JAK3 in cellular assays (Gonzales et al. 2014). JAK inhibitors inhibit intracellular signalling controlled by numerous cytokines involved in the pathogenesis of allergic, autoimmune and inflammatory diseases. In all of these cases, innate immunity stimulation activates adaptive immunity, resulting in the production of autoreactive T cells as well as the stimulation and differentiation of B cells. Therefore, JAK inhibitors have the capacity to target multiple pathways of those diseases (Benucci et al. 2023).

The present work is a continuation of our research (Jasięcka-Mikołajczyk et al. 2018) on the influence of OCL on T cells, which demonstrated under *in vitro* condition, among others, that the exposure of canine peripheral blood lymphocytes to OCL caused a dramatic loss of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Moreover, the results of this research indicated that OCL may exert the proapoptotic action on these cells. In another study (De Caro Martins et al. 2022), it was demonstrated that a long-term treatment with OCL of dogs with AD did not affect the percentage of CD8<sup>+</sup> T cells in peripheral blood, but increased the percentage of CD4<sup>+</sup> T cells. In Summary of Product Characteristics of Apoquel® the producer asserts that the drug did not affect the total population of lymphocytes in peripheral blood in dogs. Also Denti et al. (2022) did not reveal any reduction in the total amount of lymphocytes in peripheral blood of dogs with AD after OCL administration. However, it needs to be mentioned that the lack of the influence exerted by a specific substance on the abundance of lymphocytes in peripheral blood may not necessarily prove that the drug has no effect on the counts of these cells in lymphoid organs. Generally, there is a significant gap in our knowledge in the scope of experimental studies (needless to mention clinical investigations) which would evaluate the effect of OCL on the absolute counts of T and B cells in lymphoid tissues in animals and humans. The available literature does not provide any data on the influence of OCL on: (a) the absolute counts of total lymphocyte population as well as T and B cell subsets in lymph nodes and spleen in animals and humans; (b) the absolute count of T and B cell subset in peripheral blood.

Because the *in vitro* studies have demonstrated that OCL may have a depletive effect on canine CD4<sup>+</sup> and

CD8<sup>+</sup> T cells (Jasięcka-Mikołajczyk et al. 2018), a hypothesis was put forward, suggesting that this drug can cause depletion of these cells as well as B cells in lymphoid tissues and peripheral blood. In general, CD4<sup>+</sup> T cells modulate the immune response to an infection by differentiating into two distinct subsets. T-helper type 1 (Th1) cells primarily enhance the cell mediated immune response to intracellular pathogens through synthesis of pro-inflammatory Th1 cytokines, especially IFN- $\gamma$  and interleukin IL-2. T-helper type 2 (Th2) cells primarily enhance the humoral immune response to extracellular pathogens and regulate the Th1 response through synthesis of anti-inflammatory Th2 cytokines, especially IL-4 and IL-10 (Gottlieb et al. 2010). It should be pointed out that several findings strongly suggest that Th1 and Th2 cells contribute to the pathogenesis of canine AD (Olivry et al. 1999, Nuttall et al. 2002, Schlotter et al. 2011). Among CD8-bearing T cells, the dominant ones are the cells which act as a crucial component of the cellular immune response, which is necessary for the control of a variety of bacterial and viral infections. These cells also represent a major arm of the cell-mediated antitumor immune response (Barry and Bleackley 2002). Effector cytotoxic CD8<sup>+</sup> T cells have the ability to kill target cells through perforin and Fas ligands (Hamann et al. 1997). Although CD4<sup>+</sup> T cells play a dominant role in the pathogenesis of AD, a few studies have revealed that CD8<sup>+</sup> T cells are also involved in the development of the disease in dogs (Olivry et al. 1997, Sinke et al. 1997, Majewska et al. 2016). In turn, B cells are the main cells of humoral immunity. The main functions of B cells are to produce antibodies, act as antigen-presenting cells and secrete cytokines (Wang et al. 2023). Stimulated by antigens, B cells can differentiate into antibody-producing plasma cells and memory B cells to perform specific humoral immunity (Wang et al. 2023). These cells are also involved in the pathophysiology of AD in dogs. The inflammatory reaction in this disease is caused by an imbalance between Th2 and Th1 cells, resulting in activation of B cells, which are stimulated to produce IgE. Binding of allergen-specific IgE to mast cells causes degranulation of these cells and the secreted inflammatory mediators lead to inflammation (Majewska et al. 2022).

Considering the key importance of T and B cells in the effective immune response to pathogens, it was deemed justifiable to verify the proposed hypothesis. For ethical reasons, it was impossible to conduct such a study on the lymph nodes and spleen in dogs. Thus, the experiment was carried out on a typical species used in immunological investigations, i.e. on mice. This study will provide more data regarding the safety of using OCL in dogs and other animals, which, in turn,

can improve the benefit-risk assessment of using this immunosuppressive agent in patients, especially suffering from bacterial and viral infections. In summary, the main research and application purpose of this study was to determine the safety of OCL with respect of its effect on T and B cells.

Another outcome of the study reported here is gaining better knowledge on the influence of OCL on the absolute count of peripheral blood leukocyte subsets. Apart from lymphocytes, other leukocytes also participate in both the immune response to infections and to the pathophysiology of AD. The number of eosinophils in the blood and some tissues is known to increase during specific immune responses, including host responses to helminth parasite infections (Weller and Spencer 2017, Ramirez et al. 2018), in turn an increase in basophils can mediate protective immunity against helminths and ticks (Wada et al. 2010). On the other hand, eosinophils are the essential effector of allergic inflammation. Several allergic dermatological diseases, including AD, are associated with both peripheral and/or tissue eosinophilia (Stosović and Bogić 1998, Ramirez et al. 2018). Also, basophils play an important role in allergic responses and have been implicated, among others, as a source of histamine and pro-inflammatory eicosanoids in AD (James et al. 1993); they play a significant role in the pathogenesis of itch in AD (Hashimoto et al. 2023). The above leads to the conclusion that a decrease in the abundance of circulating eosinophils or basophils during OCL treatment could be considered as an additional mechanism of action of this drug and, on the other hand, as an undesirable influence on the cells engaged in the elimination of some pathogens. Thus, the determination of the effect of OCL on the number of eosinophils and basophils can provide new data regarding the mechanism of action of this drug and its unwanted effects.

## Materials and Methods

### Animals and ethics statement

The animals were housed and treated in accordance with the rules of the Local Ethics Commission for Animal Experiments in Olsztyn (affiliated to the National Ethics Commission for Animal Experimentation, Polish Ministry of Science and Higher Education). All of the procedures were approved by the Local Ethics Commission (Ethical permission No. 3/2023). The experiments were carried out on 14-week-old Balb/c mice. The mice were bred and maintained under standard lab conditions [12/12 h light/dark cycle, controlled temperature (21 ± 2°C) and humidity (55 ± 5%), and ad libitum access to food and water] in the Animal Facility

of the Faculty of Veterinary Medicine, University of Warmia and Mazury in Olsztyn.

### Drug administration

The mice were randomly divided into two groups, i.e. the control group (n=12) and OCL group (n=12). The animals from OCL group were treated with OCL (Cayman Chemical, Ann Arbor, USA) at a dose of 2.7 mg/kg body weight, administered orally via oral gavage needle for 14 days every 12 h. The dose of the drug was calculated so that it would reflect the typical dosage of OCL intended for dogs (Summary of Product Characteristics of Apoquel®). To convert the dose used in dogs to the equivalent dose in mice appropriate formula for dose translation was applied (Reagan-Shaw et al. 2007). OCL was diluted in 0.5% methylcellulose (Sigma-Aldrich, Schelldorf, Germany) and the total volume of administered drug was 200 µl. The animals from the control group received orally 200 µl of 0.5% methylcellulose at the same time schedule as OCL-treated mice. The mice were euthanized by asphyxiation with CO<sub>2</sub> 12 h after the last administration of OCL or 0.5% methylcellulose and the peripheral blood, the head and neck lymph nodes (HNLNs; submandibular gland, parotid gland, deep cervical lymph nodes), the mediastinal lymph nodes (MLNs) and spleen were individually collected.

### Cell recovery

The HNLNs, MLNs and spleen were removed and subjected to Dounce homogenization. The resulting cell suspensions were filtered through nitex fabric (Fairview Fabrics, Hercules, CA, USA), washed with FACS (fluorescence-activated cell-sorting) buffer (FB; Dulbecco's PBS devoid of Ca<sup>2+</sup> and Mg<sup>2+</sup> with 2% v/v heat-inactivated FBS, both from Sigma-Aldrich), and centrifuged (300 x g for 5 min. at 5°C; the same parameters were used for all cell-washing procedures). Cells were re-suspended in FB, counted and stained for flow cytometric analysis.

Blood was drawn from the inferior vena cava into EDTA coated tubes. Erythrocytes were removed using Red Blood Cell Lysing Buffer (RBCLB) (Maślanka et al. 2016). The samples (i.e. 100 µl of whole blood) were treated with 1 ml of RBCLB, incubated for 5 min at 4°C, and washed twice with 2 ml of FB. The remaining cells were re-suspended in FB and stained for flow cytometric analysis. Routine complete blood count (CBC) was performed using a hematology analyzer Advia 2120i (Siemens Healthcare Diagnostics, Erlangen, Germany). The reference values were provided by the manufacturer (Siemens Healthcare Diagnostics).

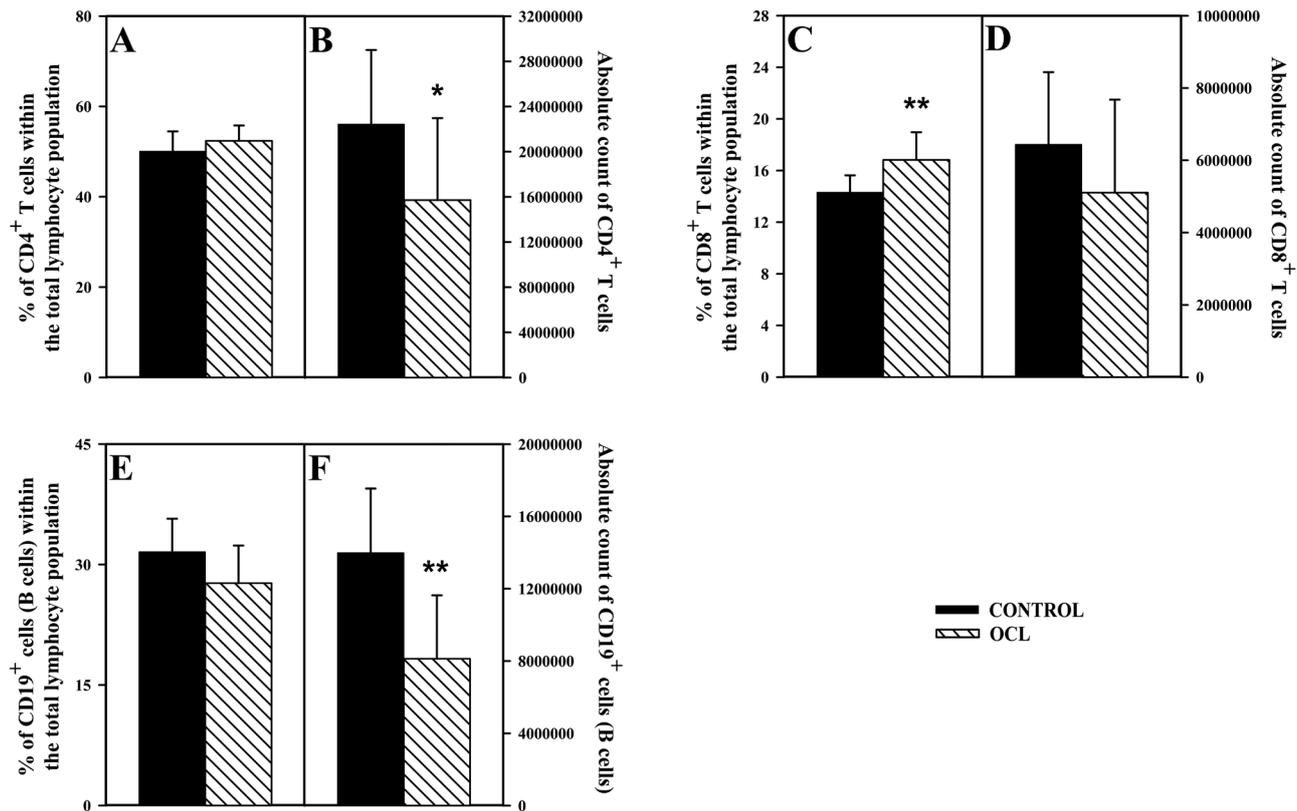


Fig. 1. Effect of oclacitinib on the percentage and absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and CD19<sup>+</sup> cells in the head and neck lymph nodes (HNLNs) in mice. The parameters were determined in the HNLNs of non-treated (control) and oclacitinib-treated mice (OCL). The percentage is expressed as a percentage of CD4<sup>+</sup> (A) and CD8<sup>+</sup> (C) T cells, and CD19<sup>+</sup> (E) cells within the total lymphocyte population. The absolute count represents the number of CD4<sup>+</sup> (B) and CD8<sup>+</sup> (D) T cells, and CD19<sup>+</sup> (F) cells in the whole HNLNs collected from individual mice. Results are expressed as the mean ( $\pm$  S.D.) of two independent experiments with 6 mice per group (n=12 per group, \* p<0.05, \*\* p<0.001).

### Staining for flow cytometry analysis

Cells prepared as described above were stained for surface antigens with fluorochrome-conjugated anti-mouse monoclonal antibodies: CD4 FITC (1:400; clone H129.19; isotype IgG2a,  $\kappa$ ), CD8a APC-Cy7 (1:400; clone 53-6.7; isotype IgG2a,  $\kappa$ ) and CD19 AF-700 (1:400; clone 1D3; isotype IgG2a,  $\kappa$ ) (all from BD Biosciences, San Jose, CA, USA). After 30 min incubation (on ice and in the dark), the cells were washed in 2 ml of FB and analyzed by flow cytometry.

### FACS acquisition and analysis

Flow cytometry analysis was performed using a FACSCelesta cytometer (BD Biosciences). The data were acquired by FACSDiva version 9.0 software (BD Biosciences) and analyzed by FlowJo software (Tree Star Inc., Stanford, USA). Absolute cell counts of lymphocyte subsets (i.e. number of cells from a particular subpopulation per  $\mu$ l of blood or per tissue sample) were calculated using the dual platform method, i.e. the absolute cell count was determined by calculating the data obtained from CBC (peripheral

blood) or a cell counting chamber (the total yield from the HNLNs, MLNs and spleen) by the percentage of particular cell subsets (the data from flow cytometric immunophenotyping).

### Statistical analysis

All data are presented as the mean  $\pm$  SD. For comparison of control vs. OCL group Student's unpaired *t*-test was used. Differences were deemed significant when the *p* values were <0.05. The data were graphed with Sigmaplot software (version 12, Systat Software, Inc, Chicago, USA).

## Results

### The effects of OCL on the percentage and absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the HNLNs and MLNs

The research showed that the absolute count of CD4<sup>+</sup> T cells in the in the HNLNs and MLNs of mice treated with OCL was significantly (*p*=0.021, *p*=0.04,

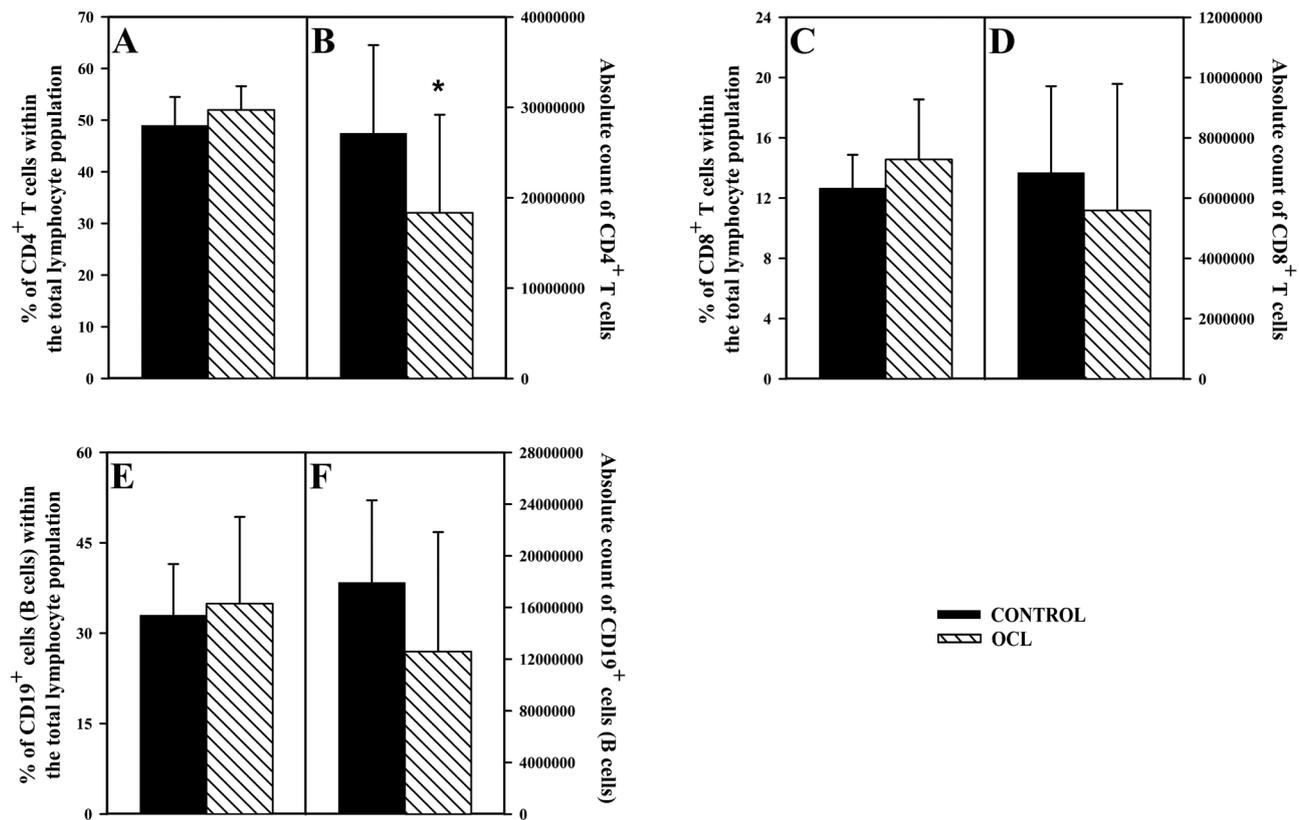


Fig. 2. Effect of oclacitinib on the percentage and absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and CD19<sup>+</sup> cells in the mediastinal lymph nodes (MLNs) in mice. The parameters were determined in the MLNs of non-treated (control) and oclacitinib-treated mice (OCL). The percentage is expressed as a percentage of CD4<sup>+</sup> (A) and CD8<sup>+</sup> (C) T cells, and CD19<sup>+</sup> (E) cells within the total lymphocyte population. The absolute count represents the number of CD4<sup>+</sup> (B) and CD8<sup>+</sup> (D) T cells, and CD19<sup>+</sup> (F) cells in the whole MLNs collected from individual mice. Results are expressed as the mean ( $\pm$  S.D.) of two independent experiments with 6 mice per group (n=12 per group, \* p<0.05).

respectively; Figs. 1B and 2B) lower compared to the control mice, while the percentages of these cells were not affected (Figs. 1A and 2A). On the contrary, the treatment with OCL did not affect the absolute count of CD8<sup>+</sup> T cells in the HNLNs and MLNs (Figs. 1D and 2D) but caused a significant (p=0.001) increase in the percentage of CD8<sup>+</sup> T cells in the HNLNs (Fig. 1C). The research did not reveal statistically significant differences in the percentage of CD8<sup>+</sup> T cells in the MLNs between both groups of mice, however, there was some trend towards increasing the value of this parameter in OCL-treated mice (p=0.144; Fig. 2C).

#### The effects of OCL on the percentage and absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the spleen and peripheral blood

The studies showed that OCL caused a significant decrease in the absolute count of CD4<sup>+</sup> (p=0.01; Fig. 3B) and CD8<sup>+</sup> (p=0.037; Fig. 3D) T cells in the spleen, but did not affect the percentages of these cells (Fig. 3A and B). The statistical analysis did not reveal any significant differences between OCL-treated and control mice with respect to the absolute counts

of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the peripheral blood, although some trend toward decreasing their number was observed (p=0.088 and p=0.102, respectively; Fig. 4B and D). Interestingly, the treatment with OCL resulted in a significant increase in the percentage of CD4<sup>+</sup> T cells in peripheral blood (p=0.049; Fig. 4A) and did not affect the percentage of CD8<sup>+</sup> T cells (Fig. 4C).

#### The effects of OCL on the percentage and absolute count of B cells in the HNLNs, MLNs, spleen and peripheral blood

The research did not reveal any statistically difference in the values of the percentage of CD19<sup>+</sup> cells (B cells) in the HNLNs, MLNs, spleen and peripheral blood of OCL-treated mice compared to the corresponding control values (Figs. 1E, 2E, 3E and 4E). On the contrary, the absolute count of CD19<sup>+</sup> cells in the HNLNs and peripheral blood in mice administered with OCL was lower than that in control mice (p=0.001 and p=0.027, respectively; Figs. 1F and 4F). In turn, the drug did not influence the absolute count of these cells in MLNs and in the spleen of mice, although a certain

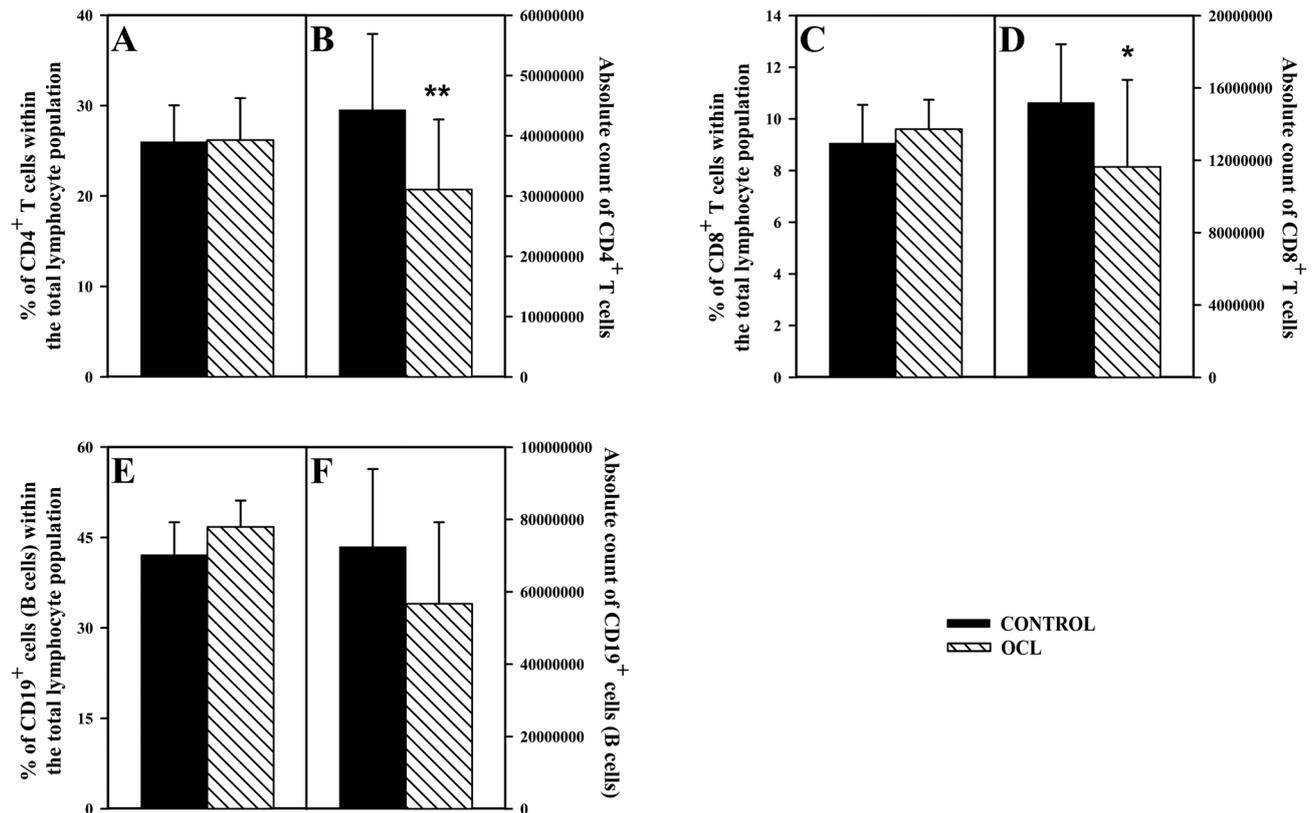


Fig. 3. Effect of oclacitinib on the percentage and absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and CD19<sup>+</sup> cells in the spleen in mice. The parameters were determined in the spleen of non-treated (control) and oclacitinib-treated mice (OCL). The percentage is expressed as a percentage of CD4<sup>+</sup> (A) and CD8<sup>+</sup> (C) T cells, and CD19<sup>+</sup> (E) cells within the total lymphocyte population. The absolute count represents the number of CD4<sup>+</sup> (B) and CD8<sup>+</sup> (D) T cells, and CD19<sup>+</sup> (F) cells in the spleen collected from individual mice. Results are expressed as the mean ( $\pm$  S.D.) of two independent experiments with 6 mice per group (n=12 per group, \* p<0.05, \*\* p<0.01).

trend (p=0.1 and p=0.082, respectively) towards decreasing this parameter was observed (Figs. 2F and 3F). Thus, the results indicate that OCL caused a depletion of CD19<sup>+</sup> cells in the HNLNs and peripheral blood, but not in the MLNs and spleen.

#### The effects of OCL on the percentage and absolute count of leukocytes in the peripheral blood

In order to determine the effect of OCL on the absolute count of leukocytes, a routine CBC was performed. In animals treated with OCL a significant decrease in the absolute count of lymphocytes (p=0.031), monocytes (p=0.023), neutrophils (p=0.007), eosinophils (p=0.046) and basophils (p=0.005) in the peripheral blood was observed (Fig. 5A, B, C, D and E). However, the values of all these parameters in OCL-treated mice were still in the reference range. There was no effect of OCL on the percentage of lymphocytes, monocytes, neutrophils, eosinophils and basophils in the peripheral blood (data not showed).

#### Discussion

The study found that OCL induced depletion of CD4<sup>+</sup> T cells in the HNLNs and MLNs, while did not affect CD8<sup>+</sup> T cell in these tissues. The reduction of the absolute number of CD4<sup>+</sup> T cells was profound, with the average depletion of these cells reaching about 30% in the HNLNs and 33% in MLNs. Moreover, the drug caused a relative profound depletion of B cells in the HNLNs, although not in the MLNs, as their absolute number was reduced by 42%. The loss in the absolute count of CD4<sup>+</sup> T cells and B cells in the HNLNs was reflected by the corresponding increase in the percentage of CD8<sup>+</sup> T cells. This increase should be interpreted only as the relative one, as it constituted mathematical consequence of the depletion of CD4<sup>+</sup> T cells and B cells and, therefore, was devoid of clinical importance. Thus, the result indicate that with respect to the effect of OCL on lymph node T cells, the drug exerted a depletive effect, but selective for CD4<sup>+</sup> T cells. However, such selectivity was not observed in the spleen as OCL reduced the number of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, with the average depletion of these cells reaching 30% and 23%,

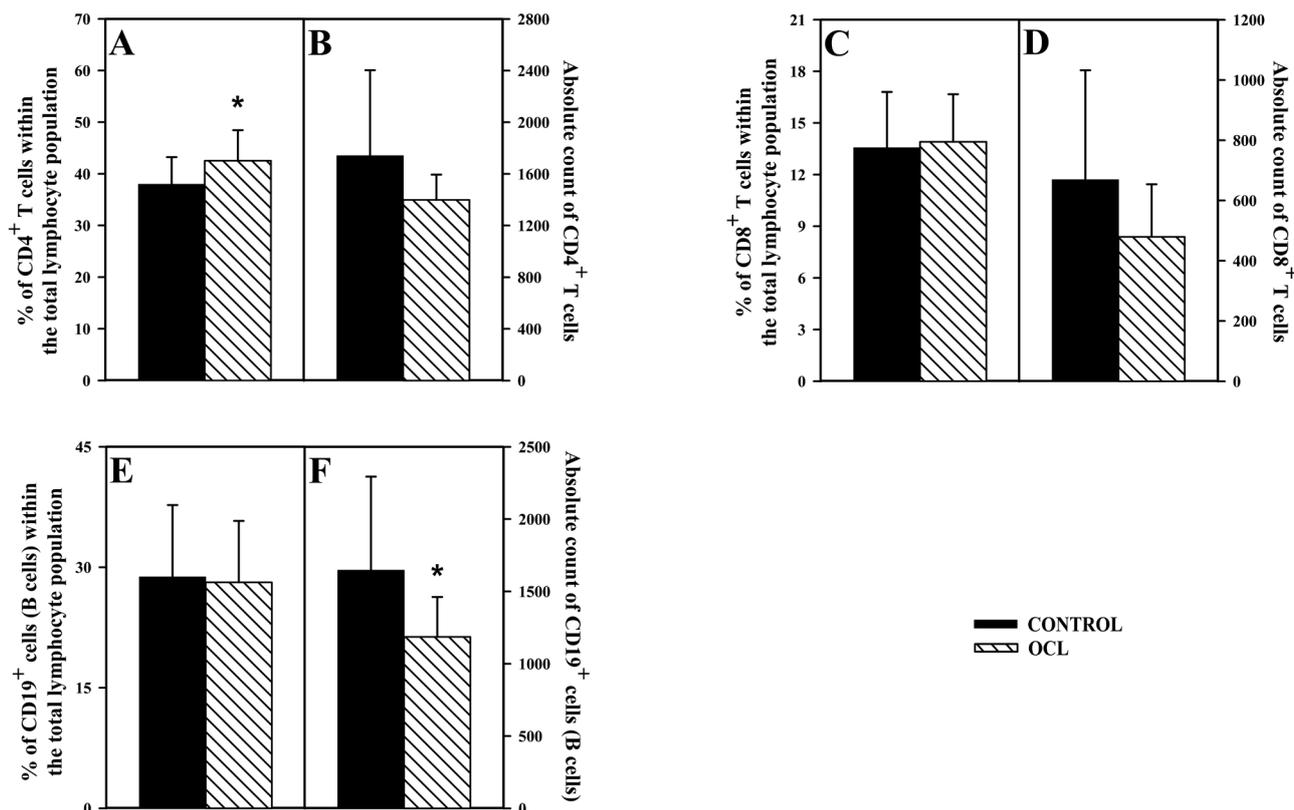


Fig. 4. Effect of oclacitinib on the percentage and absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells, and CD19<sup>+</sup> cells in the peripheral blood in mice. The parameters were determined in the spleen of non-treated (control) and oclacitinib-treated mice (OCL). The percentage is expressed as a percentage of CD4<sup>+</sup> (A) and CD8<sup>+</sup> (C) T cells, and CD19<sup>+</sup> (E) cells within the total lymphocyte population. The absolute count represents the number of CD4<sup>+</sup> (B) and CD8<sup>+</sup> (D) T cells, and CD19<sup>+</sup> (F) cells per µl of the peripheral blood collected from individual mice. Results are expressed as the mean (± S.D.) of two independent experiments with 6 mice per group (n=12 per group, \* p<0.05).

respectively. These effects were not reflected by the percentage data because CD4<sup>+</sup> and CD8<sup>+</sup> T cell losses were of comparable scale.

The present study did not demonstrate any effect of OCL on the absolute count of CD4<sup>+</sup> and CD8<sup>+</sup> T cells in the peripheral blood, although some toward decreasing their number was observed. On the contrary, the drug reduced the absolute number of B cells with the average depletion of these cells reaching about 28%. Most likely the loss of B cells was behind the OCL-induced increase in the percentage of CD4<sup>+</sup> T cells.

In conclusion, the entirety of the results achieved in this experiment suggests that OCL can cause depletion of lymphocytes T and B in lymph nodes and in the spleen. The results also indicate that the lack of any effect of this drug on a specific population of lymphocytes in peripheral blood does not need to reflect its impact on this population in lymphoid tissues.

Our earlier study strongly suggests that this depletive effect of OCL is more likely to be responsible for the proapoptotic effect of the drug than its antiproliferative effect (Jasiecka-Mikołajczyk et al. 2018, 2021). This is also implied by results of other research, in which it was demonstrated that OCL did not

significantly affect T cells proliferation (Banovic et al. 2019). It has also been shown that other JAK inhibitors induce apoptosis of cultured murine CD4<sup>+</sup> T cells (Cetkovic-Cvrlje et al. 2012) and certain lymphoma cell lines (Lee et al. 2018).

To the best of our knowledge, this is the first report on the effect of OCL on the T and B cells in the lymph nodes and spleen. Moreover, the available literature does not contain any data on the absolute count of these cell subsets in the peripheral blood, however, there are three reports related to this issue. In Summary of Product Characteristic of Apoquel® the manufacturer claims that treatment with OCL of dog did not affect the absolute count of the total lymphocyte population of the peripheral blood. Also, Denti et al. (2022) did not reveal any change in this parameter in dogs treated with the drug. However, the present study found that OCL induced a decrease in the absolute count of the total lymphocyte population in peripheral blood with the average depletion of these cells reaching about 27%, and this depletion resulted from the loss of B cells, but not T cells. This discrepancy may be associated with differences in the response to the drug between different animal species. With respect to the effect of OCL on the

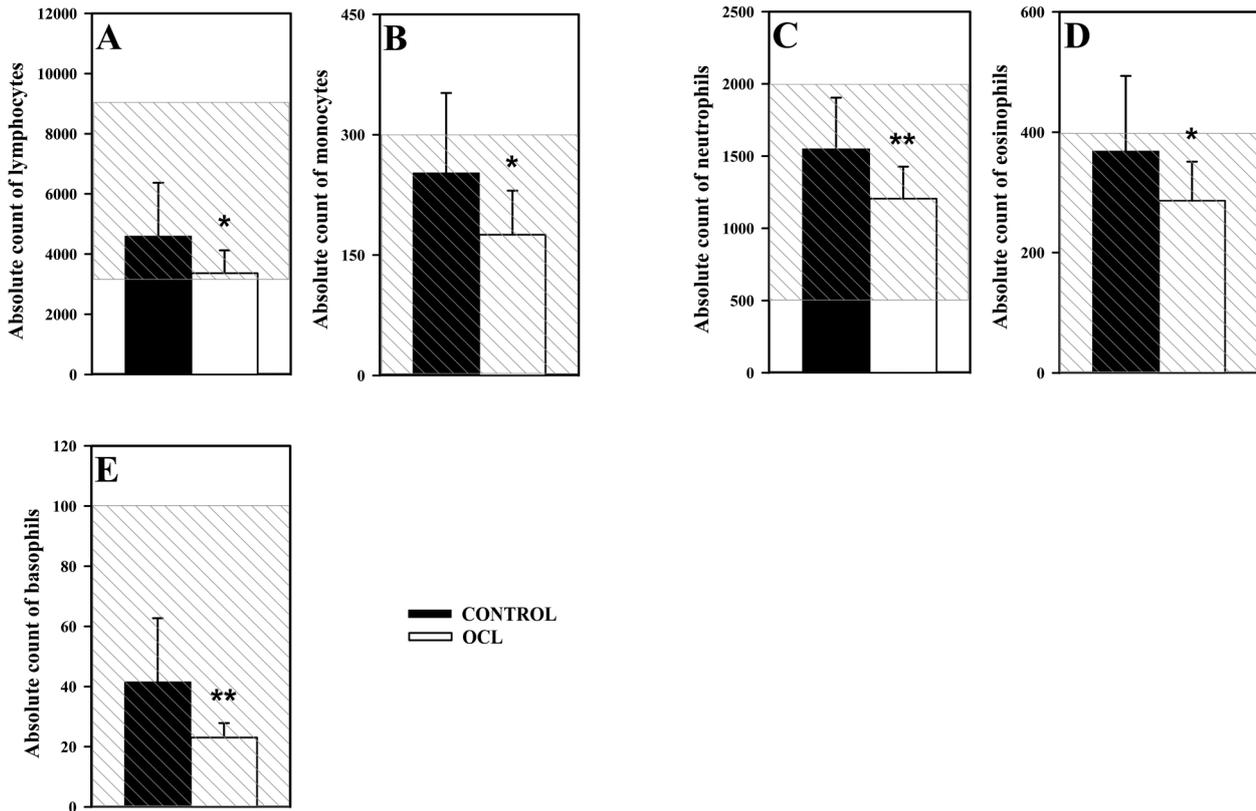


Fig. 5. Effect of oclacitinib on the absolute count of lymphocytes (A), monocytes (B), neutrophils (C), eosinophils (D) and basophils (E) in the peripheral blood in mice. The absolute count represents the number of each subsets of the leukocytes per  $\mu\text{l}$  of the peripheral blood collected from individual mice. Results are expressed as the mean ( $\pm$  S.D.) of two independent experiments with 6 mice per group ( $n=12$  per group, \*  $p<0.05$ , \*\*  $p<0.01$ ). The oblique lines indicate the reference range of particular leukocyte subsets.

percentage of peripheral blood  $\text{CD4}^+$  and  $\text{CD8}^+$  T cells the results obtained are in agreement with those by De Caro et al. (2022). They also found that treatment with OCL of dogs resulted in the increased percentage of  $\text{CD4}^+$ , but not  $\text{CD8}^+$ , T cells, although this effect was also relatively weakly expressed.

In a previous *in vitro* study on canine PBMCs (Jasińska-Mikołajczyk et al. 2018) it was demonstrated that OCL depleted  $\text{CD4}^+$  and  $\text{CD8}^+$  T cells, and these losses were considerably (over 50%) higher than those found in the current study. Basically, the results obtained here are in agreement with findings reported in the cited paper although the depletive effect of OCL on these cells differed depending on the analyzed tissue and was not as profound as that observed in the *in vitro* study. This should not be puzzling as the toxic effect of drugs in *in vitro* settings is usually expressed more intensely than in *in vivo* experiments. Under *in vivo* conditions OCL is metabolized in the body and affect cells only for certain time, while under *in vitro* conditions the cells are exposed to the drug all the time. Moreover, some regulatory mechanisms can be activated by the body in response to the drug and can modify its effect.

It is a common knowledge that T and B cell immunodeficiencies increase susceptibility to infections. Thus, the depleting effect of OCL on T and B cells

should be seen as an unfavorable one, especially in patients with bacterial and viral infections. Therefore, in such patients, the benefit/risk ratio should be thoroughly considered by the clinicians.

The main mechanism of OCL action rely on the inhibition of cytokine-mediated signaling via blockade of the JAK-STAT signaling pathway. However our previous studies found that the drug may have a more complex mechanism of action (Jasińska-Mikołajczyk et al. 2021). Considering the fact that T and B cells are involved in the pathophysiology of AD in dogs (Olivry et al. 1999, Nuttall et al. 2002, Schlotter et al. 2011, Majewska et al. 2022), the depletive effect of the drug toward these cells may contribute to the beneficial effects of OCL in the treatment of skin allergic diseases.

It was found in this study that that OCL induced a decrease in the absolute count of eosinophils, basophils, neutrophils and monocytes, but it should be emphasized that the values of all these parameters were still in the references range. It is fully in agreement with information included in the Summary of Product Characteristic of Apoquel®. The manufacturer of this product declares that treatment with OCL induced a decrease in the absolute count of eosinophils, neutrophils and monocytes, but all the values of these parameters remained within the laboratory reference range.

Also Denti et al. (2022) demonstrated a decrease in the absolute number of neutrophils, eosinophils and monocytes in dogs administered OCL. OCL induced loss, to a certain extent, of eosinophils, basophils, neutrophils and monocytes, i.e. the cells playing a significant role in control of bacterial, viral or helminth infections. However, it is uncertain whether this effect should be considered to be of clinical importance as the present and previous reports (Summary of Product Characteristic of Apoquel®, Denti et al. 2022) clearly demonstrated that OCL does not decrease the number of these cells below the physiological values. On the other hand, eosinophils and basophils play an important role in the pathogenesis of allergic diseases including AD (James et al. 1993, Stosović and Bogić 1998, Ramirez et al. 2018, Hashimoto et al. 2023). Therefore, the OCL-induced a depletive effect on these cells, especially former ones, may be involved in shaping the anti-allergic properties of the drug.

In summary, the present results indicate that with respect to the effect of OCL on lymph node T cells, the drug exerted a depletive effect, but selective for CD4<sup>+</sup> T cells. However, such selectivity was not observed in the spleen as the drug caused a loss of CD4<sup>+</sup> and CD8<sup>+</sup> T cells. Moreover, OCL induced the depletion of B cells in HNLNs and peripheral blood. Therefore, it can be concluded that OCL may induce a depletive effect on CD4<sup>+</sup> and CD8<sup>+</sup> T cells as well as B cells in the lymphoid tissue. This effect should be seen as an unfavorable one, especially in patients with bacterial and viral infections. Therefore, in such patients, the benefit/risk ratio should be thoroughly considered by the clinicians. The study found that OCL also induced a reduction in the absolute count of eosinophils, basophils, neutrophils and monocytes. However, it is uncertain whether this effect should be considered to be of clinical importance because the levels of these cells were within the physiological range. It is possible that the depletive effect of OCL toward T and B cells, as well as eosinophils and basophils may contribute to the beneficial effects of the drug in the treatment of skin allergic diseases.

## Acknowledgements

The project was supported by a grant (No. 15.610.008-110) from the University of Warmia and Mazury in Olsztyn.

## References

Apoquel® Summary of Product Characteristics available here: [www.ema.europa.eu/en/documents/product-information/apoquel-epar-product-information\\_en.pdf](http://www.ema.europa.eu/en/documents/product-information/apoquel-epar-product-information_en.pdf).

- Banovic F, Tarigo J, Gordon H, Barber JP, Gogal RM Jr (2019) Immunomodulatory *in vitro* effects of oclacitinib on canine T-cell proliferation and cytokine production. *Vet Dermatol* 30: 17-e6.
- Barry M, Bleackley RC (2002) Cytotoxic T lymphocytes: all roads lead to death. *Nat Rev Immunol* 2: 401-409.
- Benucci M, Bernardini P, Coccia C, De Luca R, Levani J, Economou A, Damiani A, Russo E, Amedei A, Guiducci S, Bartoloni E, Manfredi M, Grossi V, Infantino M, Perricone C (2023) JAK inhibitors and autoimmune rheumatic diseases. *Autoimmun Rev* 22: 103276.
- Cetkovic-Cvrlje M, Olson M, Ghate K (2012) Targeting Janus tyrosine kinase 3 (JAK3) with an inhibitor induces secretion of TGF- $\beta$  by CD4<sup>+</sup> T cells. *Cell Mol Immunol* 9: 350-360.
- De Caro Martins G, da Costa-Val AP, Coura FM, Diamantino GM, Nogueira MM, de Oliveira Melo-Junior OA, Giunchetti RC, da Silveira-Lemos D, Melo MM (2022) Immunomodulatory effect of long-term oclacitinib maleate therapy in dogs with atopic dermatitis. *Vet Dermatol* 33: 142-e40.
- Denti D, Caldin M, Ventura L, De Lucia M (2022) Prolonged twice-daily administration of oclacitinib for the control of canine atopic dermatitis: a retrospective study of 53 client-owned atopic dogs. *Vet Dermatol* 33: 149-e42.
- Gonzales AJ, Bowman JW, Fici GJ, Zhang M, Mann DW, Mitton-Fry M (2014) Oclacitinib (APOQUEL®) is a novel Janus kinase inhibitor with activity against cytokines involved in allergy. *J Vet Pharmacol Ther* 37: 317-324.
- Gottlieb SL, Martin DH, Xu F, Byrne GI, Brunham RC (2010) Summary: The natural history and immunobiology of *Chlamydia trachomatis* genital infection and implications for Chlamydia control. *J Infect Dis* 201 Suppl 2: S190-204.
- Hamann D, Baars PA, Rep MH, Hooibrink B, Kerkhof-Garde SR, Klein MR, van Lier RA (1997) Phenotypic and functional separation of memory and effector human CD8<sup>+</sup> T cells. *J Exp Med* 186: 1407-1418.
- Hashimoto T, Yokozeki H, Karasuyama H, Satoh T (2023) IL-31-generating network in atopic dermatitis comprising macrophages, basophils, thymic stromal lymphopoietin, and periostin. *J Allergy Clin Immunol* 151: 737-746.
- James JM, Kagey-Sobotka A, Sampson HA (1993) Patients with severe atopic dermatitis have activated circulating basophils. *J Allergy Clin Immunol* 91: 1155-1162.
- Jasiecka-Mikołajczyk A, Jaroszewski JJ, Maślanka T (2018) Oclacitinib depletes canine CD4<sup>+</sup> and CD8<sup>+</sup> T cells in vitro. *Res Vet Sci* 121: 124-129.
- Jasiecka-Mikołajczyk A, Jaroszewski JJ, Maślanka T (2021) Oclacitinib, a Janus Kinase Inhibitor, Reduces the Frequency of IL-4- and IL-10-, but Not IFN- $\gamma$ -, Producing Murine CD4<sup>+</sup> and CD8<sup>+</sup> T Cells and Counteracts the Induction of Type 1 Regulatory T Cells. *Molecules* 26: 5655.
- Lee S, Shah T, Yin C, Hochberg J, Ayello J, Morris E, van de Ven C, Cairo MS (2018) Ruxolitinib significantly enhances in vitro apoptosis in Hodgkin lymphoma and primary mediastinal B-cell lymphoma and survival in a lymphoma xenograft murine model. *Oncotarget* 9: 9776-9788.
- Majewska A, Dembele K, Dziendzikowska K, Prostek A, Gajewska M (2022) Cytokine and Lymphocyte Profiles in Dogs with Atopic Dermatitis after Allergen-Specific Immunotherapy. *Vaccines (Basel)* 10: 1037.

- Majewska A, Gajewska M, Dembele K, Maciejewski H, Prostek A, Jank M (2016) Lymphocytic, cytokine and transcriptomic profiles in peripheral blood of dogs with atopic dermatitis. *BMC Vet Res* 12: 174.
- Maślanka T, Otrocka-Domagala I, Zuśka-Prot M, Mikiewicz M, Przybysz J, Jasiocka A, Jaroszewski JJ (2016) IκB kinase β inhibitor, IMD-0354, prevents allergic asthma in a mouse model through inhibition of CD4(+) effector T cell responses in the lung-draining mediastinal lymph nodes. *Eur J Pharmacol* 775: 78-85.
- Nuttall TJ, Knight PA, McAleese SM, Lamb JR, Hill PB (2002) Expression of Th1, Th2 and immunosuppressive cytokine gene transcripts in canine atopic dermatitis. *Clin Exp Allergy* 32: 789-795.
- Olivry T, Dean GA, Tompkins MB, Dow JL, Moore PF (1999) Toward a canine model of atopic dermatitis: amplification of cytokine-gene transcripts in the skin of atopic dogs. *Exp Dermatol* 8: 204-211.
- Olivry T, Naydan DK, Moore PF (1997) Characterization of the cutaneous inflammatory infiltrate in canine atopic dermatitis. *Am J Dermatopathol* 19: 477-486.
- Ramirez GA, Yacoub MR, Ripa M, Mannina D, Cariddi A, Saporiti N, Ciceri F, Castagna A, Colombo G, Dagna L (2018) Eosinophils from Physiology to Disease: A Comprehensive Review. *Biomed Res Int* 2018: 9095275.
- Reagan-Shaw S, Nihal M, Ahmad N (2007) Dose translation from animal to human studies revisited. *FASEB J* 22: 659-661.
- Schlötter YM, Rutten VP, Riemers FM, Knol EF, Willemse T (2011) Lesional skin in atopic dogs shows a mixed Type-1 and Type-2 immune responsiveness. *Vet Immunol Immunopathol* 143: 20-26.
- Sinke JD, Thepen T, Bihari IC, Rutten VP, Willemse T (1997) Immunophenotyping of skin-infiltrating T-cell subsets in dogs with atopic dermatitis. *Vet Immunol Immunopathol* 57: 13-23.
- Stosović R, Bogić M (1998) The role of eosinophilic leukocytes in allergic inflammation. *Srp Arh Celok Lek* 126: 130-137.
- Wada T, Ishiwata K, Koseki H, Ishikura T, Ugajin T, Ohnuma N, Obata K, Ishikawa R, Yoshikawa S, Mukai K, Kawano Y, Minegishi Y, Yokozeki H, Watanabe N, Karasuyama H (2010) Selective ablation of basophils in mice reveals their nonredundant role in acquired immunity against ticks. *J Clin Invest* 120: 2867-2875.
- Wang S, Li H, Lian Z, Deng S (2023) The Role of m6A Modifications in B-Cell Development and B-Cell-Related Diseases. *Int J Mol Sci* 24: 4721.
- Weller PF, Spencer LA (2017) Functions of tissue-resident eosinophils. *Nat Rev Immunol* 17: 746-760.