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THE EFFECT OF T-2 TOXIN ON PERCENTAGES OF T-LYMPHOCYTES AND THEIR CYTOKINES

Abstract. *The immune system is one of the main toxicity targets of the T-2 toxin. In view of scant research data demonstrating the effect of T-2 on cellular and humoral responses in gut-associated lymphoid tissue (GALT), this study set out to investigate the effects of chronic exposure to low doses of the T-2 toxin (200 µg T-2 toxin kg⁻¹ feed) on percentages of $CD4^+$ and $CD8^+$ T lymphocytes, $CD4^+$ / $CD8^+$ double-positive T-lymphocytes, $CD21^+$ B cells, and IL-2, IFN-Y, IL-4 and IL-10 mRNA expression levels in porcine ileal Peyer's patches. The investigated material comprised ileum sections sampled from piglets (aged 8-10 weeks, body weight of 15-18 kg) on days 14, 28 and 42 of the experiment.*

After 42 days of exposure to T-2, a significant drop in the quantity of the IL-10 product was observed ($R=0.94$; S.E. 0.49-0.79; $p<0.001$). A gradual decrease in the amount of IL-4 and IFN- Y cytokine transcripts was found throughout the experiment, but the reported trend was not significant. On experiment days 14 and 42, a significant increase in the percentage of $CD8^+$ T lymphocytes was observed in comparison with the control ($p=0.04$ and $p=0.05$, respectively), whereas on day 28, a significant decrease in the percentage of the above subpopulation was noted ($p=0.00$). The percentage of $CD21^+$ B cells in the experimental group decreased steadily in comparison with the control, and the observed drop was significant on days 28 and 42 ($p=0.06$ and 0.00, respectively). On days 14 and 28, the percentages of $CD4^+$ and $CD8^+$ T lymphocytes were lower in the experimental animals than in the control group, and the drop reported on day 28 was statistically significant ($p=0.03$).

Key words: *T-2 toxin, pigs, Peyer's patches, lymphocyte subpopulation, qPCR, immunology, cytokines*

Introduction. Immune function modulation by natural factors can influence the progression of various diseases, including the acquired immune deficiency syndrome, infections, allergies, autoimmune diseases and neoplasia.

Mycotoxins are secondary metabolites produced by fungi, which contaminate crop plants and cause significant economic losses each year. The consumption of food and feed containing mycotoxins possess a potential threat for human and animal

health [1]. T-2 toxin is a widespread type-A trichothecene mycotoxin produced mostly by Fusarium sporotrichioides, which is found in cereal grains throughout Europe [2]. Long-term exposure to type-A trichothecenes leads to loss of appetite, a decrease in body weight, changes in the oral cavity and the esophagus. Similarly as other trichothecenes, T-2 is an inhibitor of protein synthesis [3]. Due to its hematotoxic effects, it impairs the immune response. The exposure to the T-2 toxin causes leukopenia and cell depletion in lymphoid organs, it inhibits erythropoiesis in the bone marrow and spleen [4-6]. T-2 intoxication can significantly impair antibody production [7-9], it reduces the proliferative response of lymphocytes [8-10] and hinders the development of dendritic cells [11-12].

Peyer's patches and mesenteric lymph nodes are lymphoid tissues which participate in the intestinal absorption of xenobiotics. They are the main sites for the induction of the immune response which leads to non-specific resistance of mucous membranes [13, 14]. Lymphocytes play two key roles in the gastrointestinal system. Firstly, the produce IgA which penetrate into the intestinal lumen and play the main role in antimicrobial protection. Secondly, they regulate the immune response to antigens entering the gastrointestinal tract to prevent excessive activation of the immune system [15]. Every few hours, vast quantities of antigens enter the digestive system with ingested food, therefore, the regulation of the immune response (mostly suppression) has to involve a highly precise mechanism. There is a general scarcity of data concerning the effects of T-2 on percentages of lymphoid tissue cells and mRNA expression levels of proinflammatory and anti-inflammatory cytokines.

As noted by the authors in their previous work, lymphocyte popylations differ in their sensitivity to the T-2 toxin. CD4 / CD8 double-positive T cells from the thymus of young mice are highly sensitive to this toxin [16-18]. CD44 low and CD45low cells which are B lymphocyte precursors are also highly sensitive to T-2 [19].

In view of the fact that the immune system is one of main toxicity targets of toxin T-2 and to compensate for the scarcity of research investigating the effect of T-2 on cellular and humoral responses in gut-associated lymphoid tissue (GALT), this experiment set out to analyze the effects of chronic exposure to low doses of the T-2 toxin on changes in the percentages of $CD4^+$ and $CD8^+$ T lymphocytes, $CD4^+$ / $CD8^+$ double-positive T-lymphocytes, $CD21^+$ B cells, and IL-2, IFN- Y, IL-4 and IL-10 mRNA expression in porcine ileal Peyer's patches.

This article analyzes the percentages of selected lymphocyte subpopulations in Peyer's patches and the mRNA expression profiles of IL-2, IFN- Y, IL-4 and IL-10 in pigs orally administered synthetic T-2 toxin.

The consumption of mycotoxins in amounts that do not produce clinical symptoms of mycotoxicosis can impair immune functions and resistance to infections. Trichothecenes, including T-2 toxin, modulate immune functions by disrupting intracellular signal transduction pathways in lymphocytes through their effect on the expression of immunoregulatory genes and through apoptosis [20]. T-2 is the most toxic trichothecene which inhibits protein synthesis following decreased DNA and RNA synthesis [21]. T-2 affects cell division mechanisms in the gastric

mucosa, skin, lymphoid and erythroid cells, and it can also decrease antibody, immunoglobulin and cytokine levels [22, 23]. T-2 is believed to be the main cause of alimentary toxic aleukia (ATA) in humans [24]. T-2 and other trichothecenec are rapidly absorbed in the intestines, they are metabolized and nearly entirely excreted (80-90%) within 48 hours [25], although their toxic effects may be exacerbated by hepatic and intestinal blood flow [26].

Previous studies have demonstrated that orally administered T-2 toxin first attacks Peyer's patches, followed by mesenteric lymph nodes and, lastly, the thymus. Due to the intestinal absorption, the symptoms of intoxication after oral administration of T-2 develop over time [4]. Peyer's patches, which occur principally in the ileum, play an important role in the induction and propagation of immune responses in the intestinal mucosa. According to Reynolds, Peyer's patches in pigs may also play the role of primary lymphoid organs for B cells [27].

The immunomodulatory effects of natural and environmental toxins, including T-2, have potential implications for human and animal health, and they generate incorrect inflammatory and autoimmune responses. The mucosal defense mechanism induces an immune response in Peyer's patches and stimulates B lymphocytes to produce secretory IgA in the intestinal lamina propria [18, 29]. Immunity is thus determined by antigen detection efficiency, and Peyer's patches are responsible for producing the immune response.

In the discussed experiment, pigs were not subjected to additional antigen stimulation, and a natural level of stimulation provided by ingested feed and commensal intestinal bacteria was maintained. The aim of the study was to evaluate the effect of a 200 µg dose of synthetic T-2 toxin kg^{-1} feed (approximately 20 µg T-2 kg^{-1} BW) administered orally to gilts over a period of 42 days. The applied dose was smaller than used by Rafai et al [30] in whose experiment, piglets were administered feed containing a 29 µg dose of T-2 kg^{-1} BW for 21 days. The percentages of lymphocyte subpopulation and interleukin mRNA expression levels were analyzed in samples of ileal Peyer's patches containing unfractionated cells. Cytokine production was evaluated by comparing the mRNA expression levels of different interleukins across the entire lymphocyte population of Peyer's patches. Cytokine mRNA levels were analyzed by Real-Time qPCR which supports detailed observations of changes in expression levels, a measure of the cells ability to respond to a given stimulus.

Lymphoid structures contain population of various cells, including cells of the innate immunity system (dendritic cells DC), monocytes, natural killer cells (NK) [31], NK-T cells, TYδ lymphocytes) and the acquired immunity system, such as B lymphocytes, cytotoxic T lympfocytes (CTL), T helper cells (Th) and regulatory T cells [32]. Those subpopulations have specific immune functions, and they produce various immunomodulatory molecules (Saalmuller et al.1999). Some of them are expressed on the cell membrane, while others are secreted as cytokines. According to their function, cytokines are classified as proinflammatory (e.g. IL-1, IL-6 and TNF-a) [33], T helper (e.g. IFNγ, IL-2, IL-4, IL-17 [34] and immunosuppressive cytokines (e.g. IL-10, IL-35 and TGF –β). This experiment analyzed changes in the percentages of $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells, $CD4^+$ / $CD8^+$ double-positive T cells

and CD 21^{+}B cells. The above cell types are vital for immune response generation and immunoregulation. The key role is played by $CD4^{+}$ cells which participate in the initiation and maintenance of the immune response. $CD8^{+}$ effector T cells eliminate infected cells and exert a cytotoxic effect. The radio of CD4 to CD8 lymphocytes determines the profile of the induced immune response. CD4 antigens, which are found mostly on Th cells, are capable of recognizing and binding specific antigens with MHC class II-expressing cells, and they are activated in response to extracellular antigens. CD8 antigens are present mainly on suppressor and cytotoxic lymphocytes (Ts/Tc), recognize the antigens of MHC class I particles and inhibit the immune response underexposure to intracellular antigens.

The observed decrease in the percentages of B lymphocyte populations (CD21+) in Peyer's patches is similar to that observed [4]. The cited authors reported a drop in $CD19^{+}$B cell populations in Peyer's patches of mice orally administered T-2. Extrathymic $CD4^{+}$ / $CD8^{+}$ DP T cells are a subset of memory T cells [29]. The results of our experiment support previous observations that $CD4^{+}$ / $CD8^{+}$ DP T cells are sensitive to the T-2 toxin [4, 17].

The polarization of the immune response is a critical process because Th cells contribute to the cellular and humoral immunity. Th cells facilitate the activation, proliferation and differentiation of B cells and precursors of cytotoxic T lymphocytes, both directly and through various cytokines, and they stimulate macrophages. The profile of released cytokines is a characteristic feature of a given subset of $CD4^{+}$ T cells. Due to the antagonistic effects of Th1 and Th2, disruptions in the functional or quantitative balance between different cytokine profiles can contribute to disease. The mRNA expression levels of IL-2, IFN-γ, IL-4 and IL-10 are used to measure the activity of Th1 and Th2 cells. Cytokines such as IL-4 protect B lymphocytes in germinal centers against apoptosis [30], they stimulate antibody production and control infections caused by extracellular pathogens. In this experiment, a significant drop in the percentage of the CD 21^{+} subpopulation was correlated with a decrease in IL-4 mRNA expression [30], which is indicative of the toxic effects of T-2. Th1 cells produce IL-2 and IFN-γ which activate cytotoxic lymphocytes (CTL) and macrophages, i.e. cells that actively control infections caused by intracellular pathogens. IFN-γ and IL-4 not only activate cellular molecules and humoral immunity, but they are also key to the negative suppression of Th2 and Th1, respectively. Unlike $CD4^{+}$ T cells which produce cytokines that regulate and coordinate the activity of cells participating in the immune response, $CD8^{+}$ T cells (Table 2) was correlated with an increase in IFN-γ mRNA accumulation. The above probably resulted from a shift in Th cell polarization towards Th1 cells and stimulation of the Tc lymphocyte subpopulation. IFN-γ controls Ig isotyhe switching in B lymphocytes and inhibits the proliferation of Th2 cells by shifting the immuneresponse towards Th1 cells [31], therefore a decrease in IFN-γ mRNA expression points to disruptions in immunoglobulin class switching. IL-10 inhibits the production of several proinflammatory cytokines [32, 33] the observed drop in IL-10 mRNA expression may lead to uncontrolled production of IL-1 and TNF and, consequently, inflammation of the intestinal mucosa.

The cytokine profile and/or the cytokine mRNA expression profile during mycotoxicosis provide valuable information about immunostimulation and immunosuppression mechanisms in animals. Cytokines regulate intestinal immune responses, in particular during interactions with food antigens. They play an important role in intestinal tissue damage observed in inflammatory bowel disease (IBD). Our results indicate that chronic exposure of pigs to subclinical doses of orally administered T-2 toxin leads to changes in immune cell polarization, a key factor in immune response regulation. Our findings also suggest that prolonged low-dose exposure to the T-2 toxin can influence memory T cells and exert an adverse effect on the humoral response mediated by B lymphocytes and the secondary immune response in pigs.

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