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Original article

The effect of T-2 toxin on percentages of $CD4^+$, $CD8^+$, $CD4^+CD8^+$ and $CD21^+$ lymphocytes, and mRNA expression levels of selected cytokines in porcine ileal Peyer's patches

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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-γ, 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-γ cytokine transcripts was found throughout the experiment, but the reported trend was not significant. On experimental 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 p=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

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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 (Oswald and Coméra 1998). T-2 toxin is a widespread type-A trichothecene mycotoxin produced mostly by *Fusarium sporotrichioides,* which is found in cereal grains throughout Europe (CAST 2003, Obremski et al. 2008). 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 (Meissonnier et al. 2008). 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 (Nagata et al. 2001, Grizzle et al. 2004, Parent-Massin 2004). T-2 intoxication can significantly impair antibody production (Niyo et al. 1988, Kamalavenkatesh et al. 2005, Li et al. 2006a,b), it reduces the proliferative response of lymphocytes (Rafai et al. 1995, Kamalavenkatesh et al. 2005) and hinders the development of dendritic cells (Hymery et al. 2006, Hymery et al. 2009).

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 (Brandtzaeg and Pabst 2004, Burkey et al. 2009). Lymphocytes play two key roles in the gastrointestinal system. Firstly, they 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 (Wittig and Zeitz 2003). 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 populations 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 (Holladay et al. 1993, Islam et al. 1998, Smith et al. 1994). $CD44^{low}$ and $CD45^{low}$ cells which are B lymphocyte precursors are also highly sensitive to T-2 (Holladay et al. 1995).

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-γ, IL-4 and IL-10 mRNA expression in porcine ileal Peyer's patches.

Materials and Methods

Animals and the experimental procedure

The study was performed on 30 Polish Large White female pigs (aged 2 months, body weight 15-18 kg) obtained from a commercial fattening farm in Baldy, Poland. The animals were housed and handled in accordance with the procedures laid down by the local Ethics Commission No. 55/2008 (affiliated with the National Ethics Commission for Animal Experimentation of the Polish Ministry of Science and Higher Education). The pigs were assigned to two groups. Group A ($n=15$) which consisted of healthy animals served as the control. Group B ($n=15$) comprised healthy pigs which were fed the T-2 toxin (SIGMA-ALDRICH, Cat No. T4887) at 0.2 mg kg^{-1} feed day^{-1}.

The investigated material comprised sections of the ileum sampled from pigs on days 14, 28 and 42 of the experiment. Five randomly selected pigs from each group were euthanized on each of the above experimental days. The excised segment of the ileum was opened, and the Peyer's patch was identified. The mucosa was scraped and minced. It was placed in 1.5 ml of ice-cold phosphate buffered saline (PBS, pH 7.4, 0.1 M). The minced tissues were shaken for 1 min in PBS, the sediment was allowed to settle for 2 min, and the suspensions were removed. The "extraction" was repeated with 1.5 ml of ice-cold PBS. The pooled suspensions were filtered through polyester wool in 2 ml disposable syringes. The number of lymphocytes was established in a hemocytometer.

For the gene expression analysis, representative fragments of Peyer's patches were excised and im-

Table 1. Specification of Real-Time PCR primers used in the study.

Primer name	Sequence (5'→3')	Target tissue Peyer's patch		Reference
		conc. (pmol)	*E*	
IL-2	F: atctctccaggatgctcacatttaa	5.0	0.93	Duvigneau et al. 2005
	R: tccagagctttgagttcttctactaa	2.5		
IL-4	F: gtctgcttactggcatgtacca	5.0	0.90	Duvigneau et al. 2005
	R: gctccatgcacgagttctttct	2.5		
IL-10	F: cggcgctgtcatcaatttctg	2.5	0.94	Duvigneau et al. 2005
	R: cccctctcttggagcttgcta	5.0		
IFNγ	F: cgatcctaaaggactattttaatgcaa	5.0	0.98	Duvigneau et al. 2005
	R: ttttgtcactctcctctttccaat	2.5		
CycA	F: gcgtctccttcgagctgtt	5.0	0.95	Hyland et al. 2006
	R: ccattatggcgtgtgaagtc	5.0		
β-actin	F: acatcaaggagaagctctgctacg	5.0	0.94	Waclawik et al. 2006
	R: aggggcgatgatcttgatcttca	5.0		

mediately immersed in the RNALater™ solution (Sigma, Germany) according to the manufacturers recommendations, and stored at -20°C.

Lymphocyte subpopulation study

The percentages of the lymphocyte subpopulations obtained from Peyer's patches were determined with the use of mouse monoclonal antibodies against porcine CD4, CD8, CD21 (CD4 VMRD, 74-12-4, IgG2b; CD8 VMRD, 76-2-11, IgG2a; CD21 VMRD, BB6-11C9, IgG1) and secondary (Biotinylated rat anti-mouse IgG2b, DB Pharmingen 550333, streptavidin-PE, DB Pharmingen 554061, FITC rat anti-mouse IgG2a, DB Pharmingen 553390) antibodies. Payer's patch leukocytes for cytometry were obtained in accordance with the procedure described by Kaleczyc et al. (2010).

The samples were analyzed in a flow cytometer (FACScalibur; Becton Dickinson, San Jose, California), and the results were analyzed in the Cell Quest™ program (Becton Dickinson). Lymphocytes were gated based on forward/side scatter cytograms, and lymphocyte subpopulations were identified based on the fluorescence intensity of dot-plot quadrant statistics.

Total RNA isolation and cDNA synthesis

Total RNA was extracted from RNALater™ preserved tissues (approx. 20 mg per sample; n=5 in each experimental group) using the Total RNA Mini isolation kit (A&A Biotechnology, Poland) according to the manufacturer's protocol. To prevent genomic DNA contamination, RNA samples were incubated with RNase-free DNase I (Roche Diagnostics, Germany). The quality and quantity of total RNA from all samples were estimated using the BioPhotometer (Eppendorf; Germany), and based on the results, cDNA was synthesized with the use of the RevertAid™ First Strand cDNA Synthesis Kit (Fermentas, Canada). The cDNA synthesis reaction mixture for each sample contained 1 µg of total RNA and 0.5 µg of oligo(dT)$_{18}$ primers, and the reaction was performed according to the procedure recommended by the manufacturer. The first strand of synthesized cDNA was suspended in sterile H_2O and stored at -20°C for further analysis.

Real-Time qPCR

Real-Time PCR primers for target and reference mRNAs were established based on the literature, and they were specific for the *Sus scrofa* species (Table 1). The assay was performed in the ABI 7500 Real-Time PCR System thermocycler (Applied Biosystems, USA) in singleplex mode, and all the samples were analyzed in duplicates. Each PCR reaction tube contained 10 µL of the FastStart SYBR Green Master ROX mix (Roche Diagnostics), 2.5 to 5 pmol of each primer (forward and reverse, Table 1), 1 µL of previously synthesized cDNA as a template, and PCR-grade H_2O to a final volume of 20 µL. The reaction was performed under standard thermal conditions: 95°C for 10 min, followed by 45 cycles at 95°C for 15 s, and 60°C for 1 min. On the plate, negative water controls (NTCs) were included to rule out the possibility of cross-contamination. To verify the quality of PCR products, a melting curve analysis followed by agarose gel electrophoresis were performed after each run. Additionally, representative subsamples

Table 2. Percentages of $CD4^+$, $CD8^+$, $CD4^+CD8^+$ and $CD21^+$ cells in ileal Peyer's patches in control pigs (A) and in pigs administered T-2 toxin (B). Mean values (n=5) and standard deviations are presented.

	Cell surface receptor	Group		*p* value
		Control (A)	Experimental (B)	
Day 14	$CD4^+$	7.36 ± 1.76	7.70 ± 3.21	0.85
	$CD8^+$	5.10 ± 0.59	12.58 ± 6.26*	0.04
	$CD4^+CD8^+$	19.05 ± 13.50	6.35 ± 4.61	0.06
	$CD21^+$	32.87 ± 11.70	21.23 ± 6.48	0.06
Day 28	$CD4^+$	8.89 ± 2.28	5.85 ± 0.64	0.07
	$CD8^+$	6.09 ± 0.78	3.68 ± 0.85*	0,00
	$CD4^+CD8^+$	8.58 ± 3.21	5.29 ± 0.83*	0.03
	$CD21^+$	40.50 ± 9.13	21.88 ± 10.69*	0.01
Day 42	$CD4^+$	6.92 ± 2.08	6.31 ± 1.28	0.55
	$CD8^+$	6.49 ± 1.39	8.57 ± 1.79*	0.05
	$CD4^+CD8^+$	8.72 ± 1.66	9.39 ± 3.34	0.07
	$CD21^+$	52.56 ± 4.99	20.53 ± 17.13*	0.00

* – differences were significant at $p \leq 0.05$

of the PCR products of each primer pair were sequenced under contract (Genomed, Poland) and analyzed for homology with reference sequences available in the GenBank (NCBI-NIH).

Data analysis and statistics

The percentages of lymphocyte subpopulations in ileal Peyer's patches were analyzed statistically by one-way ANOVA and by calculating standard deviation, the mean value and the significance of differences at $p \leq 0.05$. The statistical analysis was performed with the use of STATISTICA 9 software (StatSoft, USA) and Tukey's post-hoc test.

The expression ratio (R) of each target mRNA relative to endogenous controls (CycA, β-actin) was computed using REST 2009 software (Pfaffl et al. 2002). The calculations were based on the efficiency of Real-Time PCR targeting a single gene (E), and the quantitative cell-cycle difference (ΔCq) between a sample and the control ($\Delta Cq_{control\text{-}sample}$) according to the mathematical model of: Ratio (R) = $[(E_{target})^{\Delta Cq\ target}] \cdot [geoMEAN(E_{references})^{\Delta Cqreferences}]^{-1}$ implemented in the software (REST 2009). To estimate reaction efficiencies, a dilution series of the cDNA template (10-fold dilution factor; n = 3) was spiked into separate tubes and run for every primer pair. Cq vs. cDNA was plotted to calculate the respective slope values. The corresponding efficiencies for each primer pair (Table 1) were calculated according to the equation: $E = 10^{[-1/slope]}$ (Pfaffl 2001), and used for calculations in REST 2009. The significance of differences in individual mRNA expressions between the control and the treated samples was assessed in group means by randomization tests in REST 2009 software (Pfaffl et al. 2002). A total of 5000 randomizations were performed throughout the experiment.

Statistical differences in target mRNA levels at various points of the experiment were tested using one-way ANOVA, followed by Tukey's post-hoc HSD, and they were regarded as significant at $p < 0.05$. Before analysis, the R values were log-transformed and tested for normal distribution (Shapiro-Wilk W test) and homogeneity of variance (Levene's test) to fulfill parametric test requirements. The results were processed using STATISTICA 9 software (StatSoft, USA).

Results

The effects of the T-2 toxin on the percentages of lymphocyte subpopulations in porcine Payer's patches

The results of the study indicate that the T-2 toxin can modify the percentages of T and B lymphocyte subpopulations in Peyer's patches. The effects of chronic exposure to low T-2 doses on percentages of lymphocyte subpopulations isolated from porcine ileal Peyer's patches were determined by immunophenotyping and cytometric analysis. The percentages of $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells, $CD4^+/CD8^+$ double-positive T cells, and $CD21^+$ B cells in ileal Peyer's patches of pigs orally

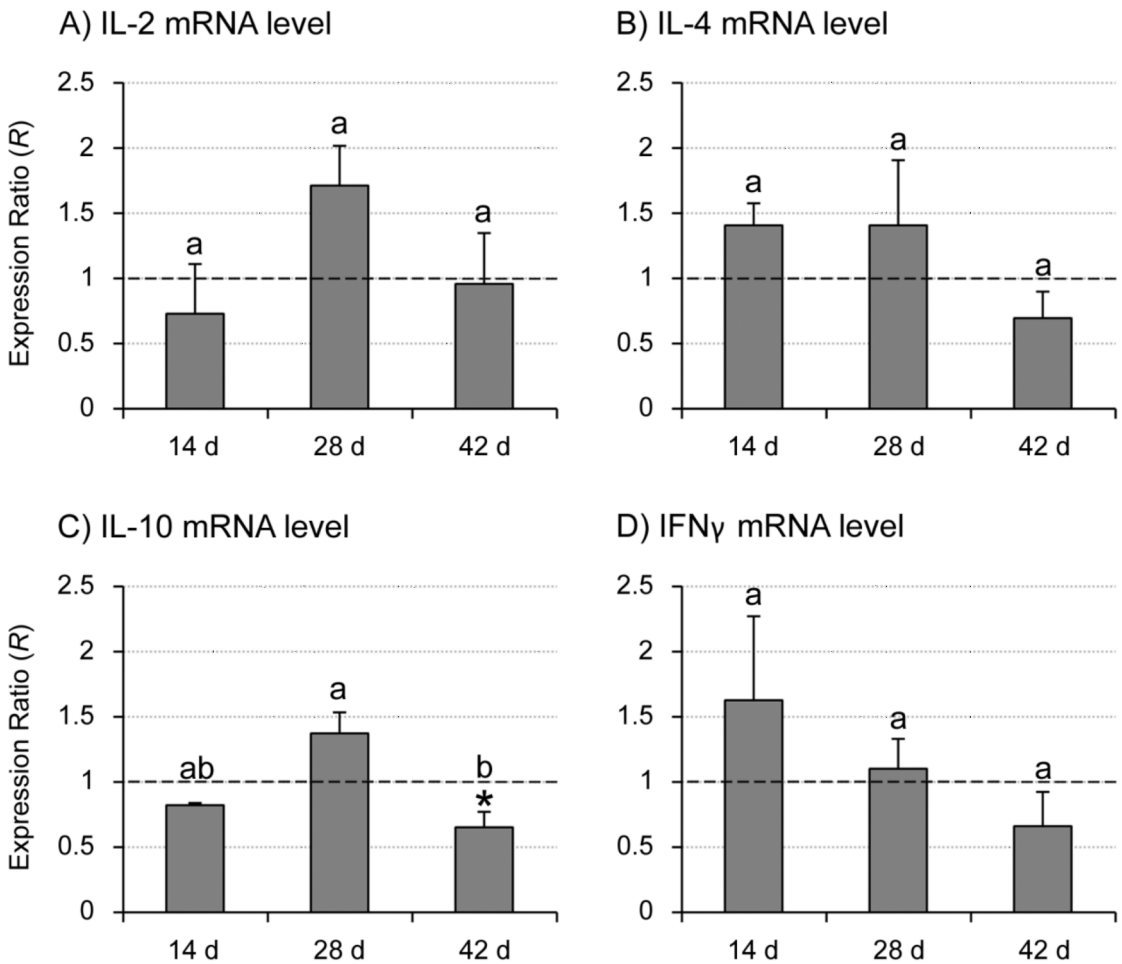

Fig. 1. Kinetics of Th1-like (IL-2, IFN-γ) and Th2-like (IL-4, IL-10) mRNA expression of A) IL-2, B) IL-4, C) IL-10, and D) IFN-γ genes in ileal Peyer's patches of pigs after 14, 28, or 42 days of exposure to T-2 toxin. Bars represent mean values of expression ratios (*R*) with their respective standard errors of the mean (S.E.; $n = 5$), normalized by β-actin and CycA mRNA as reference genes, and relative to control group ($R=1.00$; dashed line). Asterisks indicate significant differences in mRNA levels between control and T-2-treated groups at respective points in time (*, $p < 0.05$; REST2009). Different letters denote significant differences across time-points within the treated group of pigs (ANOVA followed by Tukey's HSD, $p < 0.05$).

administered synthetic T-2 toxin at 200 μg kg^{-1} feed for 14, 28 and 42 days are given in Table 2. Significant differences were observed in the percentages of the examined subpopulations between the experimental group (B) and the control group (A) on different days of the experiment. On 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 percentages 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 noted drop was significant on days 28 and 42 (p=0.06 and p=0.00, respectively). On days 14 and 28, the percentages of CD4$^+$/CD8$^+$ T double-positive lymphocytes were lower than those found in the control, and the decrease reported on day 28 was statistically significant (p=0.03).

The effects of the T-2 toxin on mRNA expression of IL-2, IL-4, IL-10 and IFN-γ cytokines in porcine Payer's patches

Real-time PCR efficiencies for each analyzed primer set were high (>0.95), and reaction specificity was confirmed with melting curve analysis and agarose gel electrophoresis. Real-Time PCR was normalized by β-actin and CycA mRNA as reference genes relative to the control group. Figure 1 shows the mRNA expression ratios (*R*) of IL-2, IL-4, IL-10 and IFN-γ cytokines in ileal Peyer's patches of pigs administered synthetic T-2 toxin (20 μg kg^{-1} BW) for 14, 28

and 42 days. In most cases, the exposure to T-2 did not significantly affect the mRNA expression of the studied genes during 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-γ cytokine transcripts was observed throughout the experiment, but the reported trend was not significant.

Discussion

This article analyzes the percentages of selected lymphocyte subpopulations in Peyer's patches and the mRNA expression profiles of IL-2, IFN-γ, 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 (Pestka et al. 2004). T-2 is the most toxic trichothecene which inhibits protein synthesis following decreased DNA and RNA synthesis (Gutleb et al. 2002). 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 (Niyo et al. 1988, Minervini et al. 2005). T-2 is believed to be the main cause of alimentary toxic aleukia (ATA) in humans (Canady et al. 2001). T-2 and other trichothecenes are rapidly absorbed in the intestines, they are metabolized and nearly entirely excreted (80-90%) within 48 hours (Prelusky et al. 1986), although their toxic effects may be exacerbated by hepatic and intestinal blood flow (Sudakin 2003).

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 (Nagata et al. 2001). 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 (Reynolds 1987).

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 (Brandtzaeg et al. 1999, Levast et al. 2010). 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 that used by Rafai et al. (1995) 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 subpopulations 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 populations of various cells, including cells of the innate immunity system (dendritic cells (DC), monocytes, natural killer cells (NK) (Gerner et al. 2009), NK-T cells, Tγδ lymphocytes) and the acquired immunity system, such as B lymphocytes, cytotoxic T lymphocytes (CTL), T helper cells (Th) and regulatory T cells (Tregs) (Sakaguchi et al. 2010). 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-α) (Wee et al. 2011), T helper (e.g. IFNγ, IL-2, IL-4, IL-17) (Serre et al. 2010) and immunosuppressive cytokines (e.g. IL-10, IL-35 and TGF-β) (Shalev et al. 2011). This experiment analyzed changes in the percentages of $CD4^+$ T helper cells, $CD8^+$ cytotoxic T cells, $CD4^+/CD8^+$ double-positive T cells and $CD21^+$ 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 ratio 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, which are present mainly on suppressor and cytotoxic lymphocytes (Ts/Tc), recognize the antigens of MHC class I particles and inhibit the immune response under exposure to intracellular antigens.

The observed decrease in the percentages of B lymphocyte populations ($CD21^+$) in Peyer's patches is similar to that observed by Nagata et al. (2001). 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 (Saalmüller et al. 2002). The results of our experiment support previous observations that $CD4^+/CD8^+$ DP T cells are sensitive to the T-2 toxin (Islam et al. 1998, Nagata et al. 2001).

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 (Andersen et al. 1999), they stimulate antibody production and control infections caused by extracellular pathogens. In this experiment, a significant drop in the percentage of the $CD21^+$ subpopulation was correlated with a decrease in IL-4 mRNA expression (Andersen et al. 1999), 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 lymphocytes have a cytotoxic function. On experimental day 14, a significant increase in the percentage of $CD8^+$ T cells (Table 2) was correlated with an increase in IFN-γ mRNA accumulation (Fig. 1). 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 isotype switching in B lymphocytes and inhibits the proliferation of Th2 cells by shifting the immune response towards Th1 cells (Farrar and Schreiber 1993, Billau 2009), therefore a decrease in IFN-γ mRNA expression points to disruptions in immunoglobulin class switching. IL-10 inhibits the production of several proinflammatory cytokines (Saraiva and O'Garra 2010), and the observed drop in IL-10 mRNA expression (Fig. 1) 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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References

Andersen JK, Takamatsu H, Pullen L, Parkhouse RM (**1999**) Systematic characterization of porcine ileal Peyer's patch, II. A role for CD154 on T cells in the positive selection of immature porcine ileal Peyer's patch B cells. Immunology 98: 622-629.

Billiau A, Matthys P (**2009**) Interferon-γ: a historical perspective. Cytokine Growth Factor Rev 20: 97-113.

Brandtzaeg P, Farstad IN, Johansen FE, Morton HC, Norderhaug IN, Yamanaka T (**1999**) The B-cell system of human mucosae and exocrine glands. Immunol Rev 171: 45-87.

Brandtzaeg P, Pabst R (**2004**) Let's go mucosal: communication on slippery ground. Trends Immunol 25: 570-577.

Burkey TE, Skjolaas KA, Minton JE (**2009**) Board-invited review: porcine mucosal immunity of the gastrointestinal tract. J Anim Sci 87: 1493-1501.

Canady RA, Coker RD, Egan SK, Krska R, Olsen M, Resnik S, Schlatter J (**2001**) T-2 and HT-2 toxins. In: Safety Evaluation of Certain Mycotoxins in Food. WHO Food Additives Series 47, FAO Food and Nutrition Paper 74, WHO, Geneva, Switzerland, pp 557-597.

CAST (**2003**) Mycotoxins: risks in plant, animal, and human systems, Task Force Report, No. 139. Council for Agricultural Science and Technology, Ames, Iowa, pp 1-191.

Duvigneau JC, Hartl RT, Groiss S, Gemeiner M (**2005**) Quantitative simultaneous multiplex real-time PCR for the detection of porcine cytokines. J Immunol Methods 306: 16-27.

Farrar MA, Schreiber RD (**1993**) The molecular cell biology of interferon-γ and its receptor. Annu Rev Immunol 11: 571-611.

Gerner W, Käser T, Saalmüller A (**2009**) Porcine T lymphocytes and NK cells – an update. Dev Comp Immunol 33: 310-320.

Grizzle JM, Kersten DB, McCracken MD, Houston AE, Saxton AM (**2004**) Determination of the acute 50% lethal dose T-2 toxin in adult bobwhite quail: additional studies on the effect of T-2 mycotoxin on blood chemistry and the morphology of internal organs. Avian Dis 48: 392-399.

Gutleb AC, Morrison E, Murk AJ (**2002**) Cytotoxicity assays for mycotoxins produced by Fusarium strains: a review. Environ Toxicol Pharmacol 11: 309-320.

Holladay SD, Blaylock BL, Comment CE, Heindel JJ, Luster MI (**1993**) Fetal thymic atrophy after exposure to T-2 toxin: selectivity for lymphoid progenitor cells. Toxicol Appl Pharmacol 121: 8-14.

Holladay SD, Smith BJ, Luster MI (**1995**) B lymphocyte precursor cells represent sensitive targets of T2 mycotoxin exposure. Toxicol Appl Pharmacol 131: 309-315.

Hyland KA, Brown DR, Murtaugh MP (**2006**) Salmonella enterica serovar choleraesuis infection of the porcine jejunal Peyer's patch rapidly induces IL-1β and IL-8 expression. Vet Immunol Immunopathol 109: 1-11.

Hymery N, Léon K, Carpentier FG, Jung JL, Parent-Massin D (**2009**) T-2 toxin inhibits the differentiation of human monocytes into dendritic cells and macrophages. Toxicol In Vitro 23: 509-519.

Hymery N, Sibiril Y, Parent-Massin D (**2006**) In vitro effects of trichothecenes on human dendritic cells. Toxicol In Vitro 20: 899-909.

Islam Z, Nagase M, Yoshizawa T, Yamauchi K, Sakato N (**1998**) T-2 toxin induces thymic apoptosis in vivo in mice. Toxicol Appl Pharmacol 148: 205-214.

Kaleczyc J, Podlasz P, Winnicka A, Wasowicz W, Sienkiewicz W, Zmudzki J, Lakomy M (**2010**) Characterization of Autonomic Nerve Markers and Lymphocyte Subsets in the Ileal Peyer's Patch of Pigs Infected Experimentally with Brachyspira hyodysenteriae. J Comp Pathol 143: 248-257.

Kamalavenkatesh P, Vairamuthu S, Balachandran C, Manohar BM, Raj GD (**2005**) Immunopathological effect of the mycotoxins cyclopiazonic acid and T-2 toxin on broiler chicken. Mycopathologia 159: 273-279.

Levast B, De Monte M, Melo S, Chevaleyre C, Berri M, Salmon H, Meurens F (**2010**) Differences in transcriptomic profile and IgA repertoire between jejunal and ileal Peyer's patches. Dev Comp Immunol 34: 102-106.

Li M, Cuff CF, Pestka JJ (**2006a**) T-2 toxin impairment of enteric reovirus clearance in the mouse associated with suppressed immunoglobulin and IFN-gamma responses. Toxicol Appl Pharmacol 214: 318-325.

Li M, Harkema JR, Islam Z, Cuff CF, Pestka JJ (**2006b**) T-2 toxin impairs murine immune response to respiratory reovirus and exacerbates viral bronchiolitis. Toxicol Appl Pharmacol 217: 76-85.

Meissonnier GM, Laffitte J, Raymond I, Benoit E, Cossalter AM, Pinton P, Bertin G, Oswald IP, Galtier P (**2008**) Subclinical doses of T-2 toxin impair acquired immune response and liver cytochrome P450 in pigs. Toxicology 247: 46-54.

Minervini F, Fornelli F, Lucivero G, Romano C, Visconti A (**2005**) T-2 toxin immunotoxicity on human B and T lymphoid cell lines. Toxicology 210: 81-91.

Nagata T, Suzuki H, Ishigami N, Shinozuka J, Uetsuka K, Nakayama H, Doi K (**2001**) Development of apoptosis and changes in lymphocyte subsets in thymus, mesenteric lymph nodes and Peyer's patches of mice orally inoculated with T-2 toxin. Exp Toxicol Pathol 53: 309-315.

Niyo KA, Richard JL, Tiffany LH (**1988**) Effect of T-2 mycotoxin ingestion on phagocytosis of *Aspergillus fumigatu*s conidia by rabbit alveolar macrophages and on hematologic, serum biochemical, and pathologic changes in rabbits. Am J Vet Res 49: 1766-1773.

Obremski K, Zielonka Ł, Gajęcka M, Jakimiuk E, Bakuła T, Baranowski M, Gajęcki M (**2008**) Histological estimation of the small intestine wall after administration of feed containing deoxynivalenol, T-2 toxin and zearalenone in the pig. Pol J Vet Sci 11: 339-345.

Oswald IP, Coméra C (**1998**) Immunotoxicity of mycotoxins. Rev Med Vet-Toulouse 149: 585-590.

Parent-Massin D (**2004**) Haematotoxicity of trichothecenes. Toxicol Lett 153: 75-81.

Pestka JJ, Zhou HR, Moon Y, Chung YJ (**2004**) Cellular and molecular mechanisms for immune modulation by deoxynivalenol and other trichothecenes: unraveling a paradox. Toxicol Lett 153: 61-73.

Pfaffl MW (**2001**) A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 29: e45.

Pfaffl MW, Horgan GW, Dempfle L (**2002**) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30: e36.

Prelusky DB, Hamilton RM, Trenholm HL, Miller JD (**1986**) Tissue distribution and excretion of radioactivity following administration of 14C-labeled deoxynivalenol to White Leghorn hens. Fundam Appl Toxicol 7: 635-645.

Rafai P, Tuboly S, Bata A, Tilly P, Ványi A, Papp Z, Jakab L, Túry E (**1995**) Effect of various levels of T-2 toxin in the immune system of growing pigs. Vet Rec 136: 511-514.

Reynolds JD (**1987**) Peyer's patches and the early development of B lymphocytes. Curr Top Microbiol Immunol 135: 43-56.

Saalmüller A, Pauly T, Höhlich BJ, Pfaff E (**1999**) Characterization of porcine T lymphocytes and their immune response against viral antigens. J Biotechnol 73: 223-233.

Saalmuller A, Werner T, Fachinger V (**2002**) T-helper cells from naive to committed. Vet Immunol Immunopathol 87: 137-145.

Sakaguchi S, Miyara M, Costantino CM, Hafler DA (**2010**) FOXP3+ regulatory T cells in the human immune system. Nat Rev Immunol 10: 490-500.

Saraiva M, O'Garra A (**2010**) The regulation of IL-10 production by immune cells. Nat Rev Immunol 10: 170-181.

Serre K, Mohr E, Gaspal F, Lane PJ, Bird R, Cunningham AF, MacLennan IC (**2010**) IL-4 directs both CD4 and CD8 T cells to produce Th2 cytokines in vitro, but only CD4 T cells produce these cytokines in response to alum-precipitated protein in vivo. Mol Immunol 47: 1914-1922.

Shalev I, Schmelzle M, Robson SC, Levy G (**2011**) Making sense of regulatory T cell suppressive function. Semin Immunol 23: 282- 292.

Smith BJ, Holladay SD, Blaylock BL (**1994**) Hematopoietic alterations after exposure to T-2 mycotoxin. Toxicon 32: 1115-1123.

Sudakin DL (**2003**) Trichothecenes in the environment: relevance to human health. Toxicol Lett 143: 97-107.

Waclawik A, Rivero-Muller A, Blitek A, Kaczmarek MM, Brokken LJ, Watanabe K, Rahman NA, Ziecik AJ (**2006**) Molecular cloning and spatiotemporal expression of prostaglandin F synthase and microsomal prostaglandin E synthase-1 in porcine endometrium. Endocrinology 147: 210-221.

Wee JL, Greenwood DL, Han X, Scheerlinck JP (**2011**) Inflammatory cytokines IL-6 and TNF-α regulate lymphocyte trafficking through the local lymph node. Vet Immunol Immunopathol 144: 95-103.

Wittig BM, Zeitz M (**2003**) The gut as an organ of immunology. Int J Colorectal Dis 18: 181-187.