UDC 613.2.099
DOI: 10.21668/health.risk/2025.3.11.eng

Read online

Research article

ALTERNARIA TOXINS AS RISK FACTORS FOR CELLULAR IMMUNITY DISORDERS AND CYTOKINE PROFILE IMBALANCE (BASED ON AN *IN VIVO* EXPERIMENTAL MODEL)

E.N. Trushina[1], N.A. Rieger[1], O.K. Mustafina[1], A.N. Timonin[1], I.V. Aksenov[1], V.A. Tutelyan[1,2,3]

[1]Federal Research Centre of Nutrition and Biotechnology, 2/14 Ust'inskii proezd, Moscow, 109240, Russian Federation
[2]I.M. Sechenov First Moscow State Medical University (Sechenov University), 8 Trubetskaya Str., build. 2, Moscow, 119048, Russian Federation
[3]Peoples' Friendship University of Russia named after Patrice Lumumba, 6 Miklukho-Maklaya Str., Moscow, 117198, Russian Federation

Possible contamination of food products with Alternaria toxins determines the need to investigate immunotropic impacts of toxic metabolites to clarify the dose-dependent effects of their exposure.

The aim of the study was to evaluate the effect of tenuazonic acid (TeA) and Alternaria toxins extract as risk factors causing cellular immunity disorders and cytokine profile imbalance in rats in an in vivo experiment.

The experiment was conducted on three groups of male Wistar rats with an average body weight of 238 g. The animals received a balanced semi-synthetic feed and purified water (ad libitum). The rats of the control group (1st group) were administered a single intragastric injection of a 10 % aqueous solution of ethyl alcohol (solvent) in the amount of 3 ml/kg body weight (b.w.); the 2nd group – a solution of the pure TeA at a dose of 30 mg/kg b.w.; the 3rd group – a solution of the extract of the cultivation medium of the producer fungus Alternaria alternata, containing a mixture of Alternaria toxins: TeA (at a dose of 30 mg/kg b.w.), alternariol (AOH) (0.276 mg/kg b.w.) and its methyl ester (AME) (0.902 mg/kg b.w.), tentoxin (TEN) (0.018 mg/kg b.w.). The solutions administered to rats in groups 2 and 3 contained an amount of ethyl alcohol adequate to that in the control group. Twenty-four hours after the solutions were administered the rats were removed from the experiment by decapitation with collection of blood samples. The hematological profile was determined on a Coulter ACT TM 5 diff OV hematological analyzer. Expression of CD45R, CD3, CD4, CD8a, CD161 receptors on rat peripheral blood lymphocytes was determined by direct immunofluorescence staining of whole blood cells using a panel of monoclonal antibodies on an FC-500 flow cytometer. The content of cytokines IFN-γ (interferon-γ), IL-1β (interleukin 1β), IL-2, IL-5, IL-6, IL-10, IL-17A, MCP-1 (monocyte chemoattractant protein-1), MIP-1α (macrophage inflammatory protein-1α) and TNF-α (tumor necrosis factor-α) in blood plasma was determined by multiplex immunoassay on a Luminex 200 analyzer.

The results of the study indicate a proinflammatory effect produced by intragastric administration of TeA to rats, both as a monocomponent and as part of the extract of the Alternaria alternata culture medium. The Alternaria toxins used in the study are capable of inducing systemic inflammatory reactions identified by an increase in the content of B-lymphocytes responsible for humoral immunity and an increase in the levels of proinflammatory, proapoptogenic cytokines in the blood plasma: TNF-α, IL-1β, IFN-γ, IL-6, IL-10, IL-2, IL-5, IL-17A and chemokines MIP-1α and MCP-1. Introduction of TeA leads to a decrease in the levels of anti-inflammatory IL-5 and IL-10, which may be a consequence of a decrease in the activity of Treg (T-regulatory) lymphocytes confirmed by a decrease in the IL-10/IL-17A ratio.

Keywords: *mycotoxins, Alternaria toxins, tenuazonic acid, cellular immunity, humoral immunity, lymphocytes, cytokines, inflammation.*

Eleonora N. Trushina – Candidate of Medical Sciences, Head of the Laboratory of Immunology (e-mail: trushina@ion.ru; tel.: +7 (495) 698-53-45, +7 (905) 729-12-01; ORCID: http://orcid.org/0000-0002-0035-3629).

Nikolay A. Rieger – Doctor of Medical Sciences, Professor, Chief Expert of the Laboratory of Immunology (e-mail: rieger_63@mail.ru; tel.: +7 (495) 698-53-68; ORCID: http://orcid.org/0000-0001-7149-2485).

Oksana K. Mustafina – Candidate of Medical Sciences, Senior Researcher of the Laboratory of Immunology (e-mail: mustafina@ion.ru; tel.: +7 (495) 698-53-45; ORCID: http://orcid.org/0000-0001-7231-9377).

Andrey N. Timonin – Candidate of Biological Sciences, Researcher of the Laboratory of Immunology (e-mail: andrey8407@mail.ru; tel.: +7 (495) 698-53-20; ORCID: http://orcid.org/0000-0001-6087-6918).

Ilya V. Aksenov – Candidate of Medical Sciences, Leading Researcher of the Laboratory of Nutrition Enzymology (e-mail: ilyaaksenoff@yandex.ru; tel.: +7 (495) 698-53-65; ORCID: http://orcid.org/0000-0003-4567-9347).

Viktor A. Tutelyan – Academician of the Russian Academy of Sciences, Doctor of Medical Sciences, Professor, Scientific Supervisor; Head of the Department of Nutrition Hygiene and Toxicology; Professor of the Department of Ecology and Food Safety (e-mail: tutelyan@ion.ru; tel.: +7 (495) 698-53-46; ORCID: http://orcid.org/0000-0002-4164-8992).

Mycotoxins produced by mold fungi from the *Alternaria* genus are widespread contaminants found in foods and forage [1]. AOH, AME, TeA and TEN are Alternaria toxins most frequently detected in foods and affecting human health. They are found in grain-based foods, vegetable oils, tomatoes, peppers, wines, fruit juices, infant and baby foods and forages [2–4]. A review [4] provides the results obtained by investigating contents of Alternaria toxins in food raw materials and processed products evidencing their high prevalence. Thus, TeA frequency in wheat equals 57–100 %; rice, up to 83 %; sunflower seeds, 51–100 %; sunflower oil, 21 %; tomatoes, up to 100 %; apples, up to 20 %; dried apricots, 38–100 %. Children in their first three years of life are a population group with the highest average daily intake of Alternaria toxins: AOH ≤ 271 ng/kg b.w.; AME ≤ 97 ng/kg b.w.; TEN ≤ 54 ng/kg b.w.; TeA ≤ 3603 ng/kg b.w. Major contributions to occurrence of alternariatoxins in foods for babies and infants are made by products based on processed fruits and tomatoes, berries, grain-based products and vegetable oils. High prevalence and toxic effects of Alternaria toxins proven by *in vitro* and *in vivo* studies allow considering them a significant health risk factor.

Mycotoxins enter the human body with food, by inhalation or by contacts through skin or mucosa. Clinical effects produced by Alternaria toxins vary from mild damage to organs and systems to a fatal outcome upon acute intoxication detected in domestic and laboratory animals [5]. Alternaria toxins were shown to have high cytotoxic, genotoxic and mutagenic potential by *in vitro* studies [6–8]. TeA is a secondary metabolite of *Alternaria* genus fungi; it has the most pronounced acute toxicity and is included in the Registry of Toxic Effects of Chemical Substances by the USA Food and Drug Administration (FDA[1] [9]. In 2022, the European Commission published a recommendation proposing to monitor presence of Alternaria toxins in foods with special attention paid to AOH, AME and TeA[2]. The European Food Safety Agency (EFSA) singled out four Alternaria toxins, namely, AOH, AME, TeA and TEN, relying on assessment of toxic effects produced by them. The TTC approach (toxicological concern threshold) was implemented by the EFSA for these four toxins on the basis of their chemical structure, 2.5 ng/kg b.w./day for genotoxic ATs (AOH and AME) and 1500 ng/kg b.w./day for non-genotoxic ATs (TeA and TEN) [10].

Innate and adaptive immunity pathways, barrier functions of the skin, respiratory and digestive systems as well as the liver and kidneys can be disrupted by Alternaria toxins and this can have a crucial effect on development or exacerbation of already existing diseases. Recent observations indicate negative effects produced by mold/mycotoxin exposure in individuals with pre-existing dysregulation of the immune system, due to exacerbation of underlying pathophysiology including allergic and non-allergic chronic inflammatory diseases, autoimmune disorders [11, 12]. Data on immune-modulating effects produced by Alternaria toxins are rather scarce and controversial. Since Alternaria toxins are a significant health risk factor, additional investigations are necessary to shed some light on immunotropic effects produced by toxic metabolites of *Alternaria* fungi as well as to more precisely define dose-dependent effects of their exposure in order to mitigate negative influence on human health and to develop safe standards for their contents in food products,

The aim of this study was to evaluate the effect of tenuazonic acid (TeA) and Alternaria toxins extract as risk factors causing

[1] Janardhanan K.K., Husain A. Studies on isolation, purification and identification of tenuazonic acid, a phytotoxin produced by Alternaria alternata (Fr.) Keissler causing leaf blight of Datura innoxia Mill. *Mycopathologia*, 1983, vol. 83, pp. 135–140. DOI: 10.1007/BF00437019

[2] European Commission Recommendation (EU) 2022/553 of 5 April 2022 on monitoring the presence of Alternaria toxins in food. *Official Journal of the European Union*, 2022. Available at: https://eur-lex.europa.eu/eli/reco/2022/553/oj/eng (April 23, 2025).

cellular immunity disorders and cytokine profile imbalance in rats in an *in vivo* experiment.

Materials and methods. The experiment was conducted on 30 male *Wistar* rats 4 weeks old, which were brought from the Stolbovaya Vivarium (a subsidiary of the FBMA's Scientific Center for Biomedical Technologies). The study was approved by the Ethics Committee of the Federal Research Centre of Nutrition and Biotechnology, and was conducted in conformity with the guidelines provided in the State Standard GOST 33216-2014 Guide on Upkeep and Care of Laboratory Animals. Rules for Upkeep and Care of Laboratory Rodents and Rabbits[3]. During the whole experiment, the animals were kept separately in plastic cages (one animal in each cage) with the cage floor covered with wood chips; illuminance was artificial (equal day and night periods); the animals received balanced semi-synthetic feed (the caloric contents were 4 kcal/g of dried mixture; 15 grams of dried mixture per rat/day) and purified water (*ad libitum*). After 5 weeks of upkeep, the animals were randomized per body weight (the average body weight was 238 grams) into three groups. The rats of the control group (1st group) were administered a single intragastric injection of a 10 % aqueous solution of ethyl alcohol (solvent) in the amount of 3 ml/kg body weight (b.w.); the 2nd group, an alcoholic solution of pure TeA (Category No. CS-BX-00058, Clearsynth, India) at a dose of 30 mg/kg b.w.; the 3rd group, an alcoholic solution of a cultivation medium extract (tomato paste; 24 °C for 2 weeks) of the producer fungus *Alternaria alternata*, containing a mixture of Alternaria toxins: TeA (at a dose of 30 mg/kg b.w.), alternariol (AOH) (0.276 mg/kg b.w.) and its methyl ester (AME) (0.902 mg/kg b.w.), tentoxin (TEN) (0.018 mg/kg b.w.). The solutions administered to rats in groups 2 and 3 contained an amount of ethyl alcohol adequate to that in the control group. The rats were fed 3 hours after administration of the solutions; twenty-four hours after the solutions were administered, the rats were removed from the experiment by decapitation with collection of blood samples into tubes with sodium salt of ethylenediaminetetraacetic acid (EDTA) (MiniMed, Russia). The last feeding took place a night prior to administration of the solutions and blood sampling. The TeA dose used in the experiment (30 mg/kg b.w.) equaled 1/6 LD_{50}[4] and was selected in accordance with the Methodical Guidelines on Establishing and Substantiating Safe Standards for Contents of Chemicals and Biological Agents in Food Products per Health Risk Criteria (recommended by the EEC Board on February 26, 2020. No. 4)[5] as well as studies by other authors [13, 14].

Hematological tests were conducted with Coulter ACT TM 5 diff OV hematology analyzer (Beckman Coulter, USA). The following indicators were established in each sample of whole peripheral blood: red blood count (RBC), white blood count (WBC), platelets (PTL), hemoglobin (HGB), hematocrit (HCT), thrombocrit (PCT), mean corpuscular volume (MCV), mean platelet volume (MPV), mean corpuscular hemoglobin (MCH), mean corpus-

[3] State Standard GOST 33216-2014. Guidelines for accommodation and care of animals. Species-specific provisions for laboratory rodents and rabbits: Interstate Standard, approved by the Interstate Council on Standardization, Metrology and Certification (Meeting Report dated December 22, 2014 No. 73-P). *Federal Research Center Original and Prospective Biomedical and Pharmaceutical Technologies: official web-site*. Available at: https://www.academpharm.ru/images/upload/ru/1241/zamenyayuschij_GOST_33216-2014.pdf (April 16, 2025) (in Russian).

[4] Smith E.R., Fredrickson T.N., Hadidian Z. Toxic effects of the sodium and the N,N'-dibenzylethylenediamine salts of tenuazonic acid (NSC-525816 and NSC-82260). *Cancer Chemother. Rep.*, 1968, vol. 52, no. 5, pp. 579–585.

[5] O metodicheskikh ukazaniyakh po ustanovleniyu i obosnovaniyu gigienicheskikh normativov soderzhaniya khimicheskikh primesei, biologicheskikh agentov v pishchevoi produktsii po kriteriyam riska dlya zdorov'ya cheloveka: Rekomendatsiya Kollegii EEK ot 26.02.2020 № 4 [On Methodical Guidelines on Establishing and Substantiating Safe Standards for Contents of Chemicals and Biological Agents in Food Products per Health Risk Criteria (recommended by the EEC Board on February 26, 2020. No. 4]. *YuIS Legalakt: laws, codes and regulatory documents of the Russian Federation*. Available at: https://legalacts.ru/doc/rekomendatsija-kollegii-evraziiskoi-ekonomicheskoi-komissii-ot-26022020-n-4/ (April 16, 2025) (in Russian).

cular hemoglobin concentration (MCHC), and the leukocyte formula (neutrophils, eosinophils, basophils, lymphocytes, and monocytes).

Expression of the receptors CD3, CD4, CD8a, CD161, and CD45R on lymphocytes in rats' peripheral blood was analyzed by direct immunofluorescence of whole blood cells using a panel of monoclonal antibodies conjugated with fluorescent dyes with FC-500 flow cytofluorometer (Beckman Coulter, USA). We established relative counts of CD45R+ (B-lymphocytes), CD3+ (T-lymphocytes), CD3+CD4+ (T-helpers), CD3+CD8+ (T-cytotoxic lymphocytes), CD161+ (NK-cells) and CD3+CD161+ (NKT-cells). The immune regulation index (IRI) was calculated as the CD4+/CD8+ proportion ratio.

Levels of the cytokines (pg/ml) IFN-γ (interferon-γ), IL-1β (interleukin-1β), IL-2, IL-5, IL-6, IL-10, IL-17A, MCP-1 (monocytic chemoattractant protein-1), MIP-1α (macrophage inflammatory protein-1α) and TNF-α (tumor necrosis factor-α) were established in blood plasma by multiplex immune analysis using the basic Bio-Plex Pro™ Reagent Kit V (Bio-Rad Laboratories Inc., USA) with Luminex 200 analyzer (Luminex Corporation, USA) relying on the xMAP technology with Luminex xPONENT Version 3.1. The IL-10/IL-17A ratio reflecting the balance between Treg and Th17 lymphocytes was established per the ratio of IL-10 to IL-17A levels in blood plasma for each blood sample.

All experimental data were statistically analyzed using SPSS 20.0 (SPSS, USA) и Microsoft Excel (Microsoft, USA) software packages. The normalcy was checked for the analyzed dependent variables using the Shapiro – Wilk test; Levene test was used to check if the variances of two or more groups were equal. In case the normalcy and homoscedasticity conditions were met, parametric methods were used; in case such methods were not eligible, we used their non-parametric analogs. The experiment model was one-factor with three normative levels corresponding to the 1st, 2nd, and 3rd group. In parametric analysis, we used one factor dispersion analysis (ANOVA) with three independent levels. Multiple comparison of the means using the Tukey's range test was applied as a post-choc method in case of a null hypothesis deviation. The Kruskal – Wallis test (H-test) was used as ANOVA analog in non-parametric analysis. In addition, we calculated the effect size (ε^2 for the H-test and η^2 for ANOVA). The effect size was estimated using the following classification: small (between 0 and 0.06), medium (between 0.06 and 0.14) and big (0.14 or above). The Dunn's test with Bonferroni – Holm correction was used as a non-parametric method for multiple comparison. The data analyzed by parametric methods were given as $M \pm m$, where M was a simple mean of a dependent variable and m was standard error. In non-parametric analysis, the data were given as quantiles *Q1*, *Me*, *Q3*. Differences were considered authentic at $p < 0.05$.

Results and discussion. ***Assessment of effects produced by TeA and mixture of Alternaria toxins on rats' hematological indicators.*** Results of hematological tests provided in Table 1 do not confirm any statistically authentic differences in the analyzed hematological indicators between the control and test groups.

Assessment of effects produced by TeA and mixture of Alternaria toxins on rats' cellular immunity. As shown in Table 2, no significant intergroup differences were established in relative levels of lymphocyte subpopulations in rats' peripheral blood, levels of B-lymphocytes being the only exception. Relative B-lymphocyte contents were authentically ($p < 0.05$) higher in the 3rd test group (the rats administered with an alcoholic solution of the cultivation medium extract of the producer fungus *Alternaria alternata*, containing a mixture of Alternaria toxins) against the control and the 2nd group (Table 2).

Cellular immunity indicators were found to be of low information value per the calculated effect sizes, except for relative levels of B-lymphocytes in peripheral blood. Therefore, this indicator does not only give an authentic statistical response but also represents a significant target study effect.

Table 1

Hematological indicators ($M \pm m$)

Indicator	Animal groups		
	Control (n = 10)	TeA (n = 10)	Extract of several Alternaria toxins (n = 10)
	1st group	2nd group	3rd group
RBC, 10^{12}/L	7.37 ± 0.20	7.50 ± 0.13	7.66 ± 0.13
HGB, %	140.1 ± 3.03	141.0 ± 2.66	148.6 ± 3.82
HCT, %	38.85 ± 0.98	39.23 ± 0.92	41.1 ± 0.87
MCV, μm^3	53.0 ± 0.52	52.3 ± 0.73	53.6 ± 0.43
MCH, pg	19.04 ± 0.28	18.83 ± 0.26	19.36 ± 0.20
MCHC, g/L	359.20 ± 3.18	360.11 ± 3.08	360.60 ± 2.71
WBC, 10^9/L	9.42 ± 0.74	7.58 ± 0.72	8.83 ± 1.04
Ne, %	6.93 ± 0.55	7.39 ± 0.92	7.96 ± 0.50
EO, %	1.31 ± 0.29	0.89 ± 0.21	1.23 ± 0.29
Bas,%	1.61 ± 0.23	1.19 ± 0.26	1.06 ± 0.72
Ly, %	88.36 ± 0.80	87.49 ± 1.68	87.67 ± 0.96
MO, %	2.24 ± 0.24	3.04 ± 0.49	2.08 ± 0.27
Ne, 10^9/L	0.62 ± 0.04	0.54 ± 0.056	0.69 ± 0.08
EO, 10^9/L	0.11 ± 0.03	0.06 ± 0.01	0.1 ± 0.02
Bas, 10^9/L	0.1 ± 0.02	0.08 ± 0.015	0.48 ± 0.39
Ly, 10^9/L	8.37 ± 0.72	6.77 ± 0.72	7.76 ± 0.92
MO, 10^9/L	0.22 ± 0.04	0.22 ± 0.03	0.20 ± 0.04
PTL, 10^9/L	531.30 ± 43.72	478.11 ± 30.71	470.00 ± 39.62
MPV, μm^3	6.44 ± 0.09	6.67 ± 0.18	6.53 ± 0.17
PCT, %	0.36 ± 0.04	0.32 ± 0.02	0.30 ± 0.02

Note: TeA is for tenuazonic acid; RBC, red blood count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; WBC, white blood count; Ne, neutrophils; EO, eosinophils; Bas, basophils; Ly, lymphocytes; MO, monocytes; PTL, platelet count; MPV, mean platelet volume; PCT, thrombocrit.

Table 2

Cellular immunity indicators (%, $M \pm m$)

Indicator	Animal group		
	Control (n = 10)	TeA (n = 10)	Extract of several Alternaria toxins (n = 10)
	1st group	2nd group	3rd group
CD45RA+ (B-lymphocytes)	23.89 ± 2.70	27.33 ± 3.65	36.43 ± 1.76*,**
CD3+ (T- lymphocytes)	58.31 ± 3.04	54.45 ± 4.74	55.54 ± 3.43
CD3+CD4+ (T-helpers)	52.38 ± 3.95	59.06 ± 3.48	60.64 ± 3.52
CD3+CD8+ (T-cytotoxic lymphocytes)	37.05 ± 3.78	34.71 ± 1.21	36.88 ± 2.38
CD4/CD8 (immune regulation index)	1.53 ± 0.22	1.72 ± 0.13	1.71 ± 0.17
CD161+ (NK-cells)	4.28 ± 1.06	4.44 ± 0.91	3.83 ± 1.33
CD3+CD161a+ (NKT-cells)	0.89 ± 0.28	0.74 ± 0.14	0.51 ± 0.08

Note: TeA is tenuazonic acid; * means significant differences ($p < 0.05$) from the 1st group and ** from the 2nd group.

Administration of both pure TeA and extract of several Alternaria toxins induced significant changes in the cytokine profile in both test groups against the control. Levels of IFN-γ, IL-1β, IL-2, IL-6, IL-17A, MCP-1, MIP-1α and TNF-α in blood plasma grew ($p < 0.05$) in the rats from both test groups together with a decline ($p < 0,05$) in levels of IL-5 and IL-10 against the control (the 1st group) (Figures 1–2).

We did not find any authentic differences in the cytokine profile between the 2nd and 3rd group (the test groups). However, contents of IL-2 and IL-17A tended ($p = 0.08$) to go down in blood plasma in the 3rd group (where the rats were administered extract of Alternaria toxins) (5.23 ± 0.54 pg/mL and 0.91 ± 0.17 pg/mL) against the effects produced by pure TeA (6.06 ± 1.30 pg/mL and 1.02 ± 0.09 pg/mL accordingly). It is noteworthy that levels of these cytokines, despite different composition of the administered preparations, were authentically higher ($p < 0.05$) than in the 1st group (control) (Figure 2). Total assessment of effects produced by the Alternaria toxins used in the experiment revealed TeA to have the most significant influence, both in its pure form and as a component in the extract, on the levels of the following cytokines: IL-5, IL-6, IL-10, IL-17A, MCP-1 and TNF-α (η^2 between 0.4 and 0.82), as well as on changes in the indicator IL-10/IL-17A ($\eta^2 = 0.79$). The ratio between the levels of IL-10 and IL-17A calculated for the control (3.04 ± 0.891) was almost thrice as high ($p < 0,.05$) as in both test groups (0.74 ± 0.26 and 0.85 ± 0.30 accordingly), as shown in Figure 3.

Therefore, intragastric TeA administration, both as a monocomponent or in a mix with other Alternaria toxins, has a considerable effect on production of cytokines participating in regulation of various immunity sections.

A small number of immunological studies with their focus on effects produced by Alternaria toxins have yielded rather controversial results. Some authors reported immunosuppression induced by even small doses of

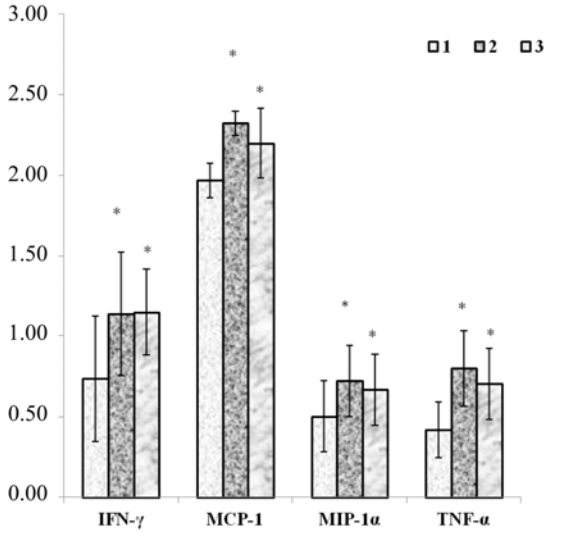

Figure 1. Changes in levels of IFN-γ, TNF-α cytokines and MCP-1, MIP-1α chemokines in rat blood plasma after administering TeA and extract of several Alternaria toxins (* means significant differences ($p < 0.05$) from the 1st group. *Legend*: Y-axis shows levels of cytokines and chemokines in blood plasma, pg/ml: 1 is control; 2 is TeA at a dose of 30 mg/kg b.w. (2nd group), 3 is TeA at a dose of 30 mg/kg b.w. with extract of several Alternaria toxins (3rd group); X-axis: IFN-γ is interferon gamma, TNF-α is tumor necrosis factor alpha, MCP-1 is monocytic chemoattractant protein-1, MIP-1α is macrophage inflammatory protein-1 alpha)

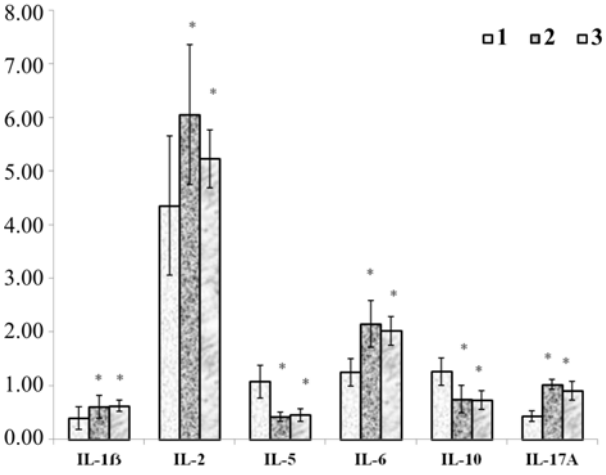

Figure 2. Changes in levels of interleukins in rats' blood plasma after administering TeA and extract of Alternaria toxins (* means significant differences ($p < 0.05$) from the 1st group. *Legend*: Y-axis shows levels of interleukins in blood plasma, pg/ml: 1 is control; 2 is TeA at a dose of 30 mg/kg b.w. (2nd group), 3 is TeA at a dose of 30 mg/kg b.w. with extract of several Alternaria toxins (3rd group); X-axis: IL-1β, IL-2, IL-5, IL-6, IL-10 and IL-17A)

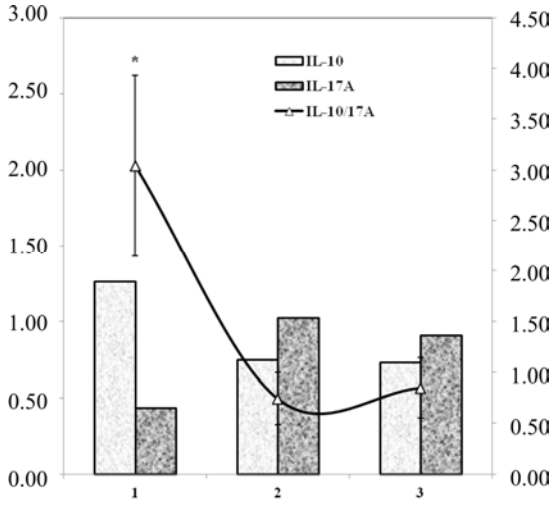

Figure 3. Changes in the ratio IL-10/IL-17A influenced by TeA and the extract of Alternaria toxins (* means significant differences ($p < 0.05$) from the test groups (the 2nd and 3rd group) for the IL-10/IL-17A curve. *Legend*: Y-axis (to the right) shows IL-10/IL-17A values; to the left, levels of interleukins in blood plasma, pg/mL; X-axis: 1 is control; 2 is TeA at a dose of 30 mg/kg b.w. (2nd group), 3 is TeA at a dose of 30 mg/kg b.w. with extract of several Alternaria toxins (3rd group))

mycotoxins making the body more susceptible to infections [11, 15]. Atrophy of lymphoid organs, the spleen in particular, was reported in publications aiming to assess immune toxicity and genotoxicity of ATX-I, AOH or AME in experiments on Sprague Dawley rats [16–18]. Other researchers published some data confirming visibly enlarged Peyer's patches in the small intestine upon administration of the extract of *Alternaria* culture in Sprague Dawley rats and this could be considered a potential sign of active immune response involving inflammation [14].

Our study established an authentic ($p < 0.05$) increase in the relative contents of B-lymphocytes in peripheral blood of the rats from the 3rd group that were administered with TeA at a dose of 30 mg/kg b.w. in a mixture of Alternaria toxins (Table 2). B-lymphocytes are a main cell population supporting humoral immune response. They are formed in bone marrow; after leaving it, transitory and mature naïve B-lymphocytes are localized in the spleen and local lymph nodes creating germ centers. Proliferation of B-lymphocytes and their differentiation into plasmatic cells ensures antibody synthesis. B-cells also participate in cellular immunity acting as antigen-representing cells and produced co-stimulating effects on T-lymphocytes. In addition, B-lymphocytes produce various cytokines thereby coordinating inflammation and can act as regulatory cells in cellular and humoral immunity response [19].

Changes detected in the cytokine profile in the test groups allow assuming high likelihood of toxic effects produced by the analyzed Alternaria toxins on rats' immune status. Activated production and growing levels of IFN-γ, IL-1β, IL-2, IL-6, IL-17A, MCP-1, MIP-1α and TNF-α in rats' blood plasma give evidence of activated regulation of inflammation induced by the analyzed mycotoxins.

Approximately 87 % of TeA has been found to remain unchanged and be excreted from the body predominantly with urine [14]. However, low system absorption does not exclude toxicological or pro-inflammatory effects produced by exposure to Alternaria toxins. An established toxic TeA effect is its ability to induce production of reactive oxygen species and to inhibit protein synthesis in cytoplasm on ribosomes[6] [20]. Protein synthesis slowdown has been established to be mediated by a decline in ornithine decarboxylase activity, which ultimately leads to apoptosis induced by DNA damage [21]. Blocking of polyamine sysnthesis due to inhibition of ornithine decarboxylase activity depletes the intracellular polyamine pool and inhibits T-cell proliferation [22]. T-helpers are found to express a high level of ornithine decarboxylase relative to Treg lymphocytes and, which is especially important, expres-

[6] Shigeura H.T., Gordon C.N. The biological activity of tenuazonic acid. *Biochemistry*, 1963, vol. 2, pp. 1132–1137. DOI: 10.1021/bi00905a039

sion of polyamine-associated enzymes enhances considerably in activated Th17 (T-helpers 17) and is inhibited in Treg lymphocytes upon toxic exposures [23, 24]. Given all the above-stated, in our study, toxic effects produced by TeA were revealed to involve elevated IL-17A, declining IL-5, IL-10 levels and, consequently, an authentic decline in the IL-10/IL-17A ratio in the rats from the test groups. Growing levels of IFN-γ, IL-1β, IL-2, IL-6, IL-17A, TNF-α and chemokines MCP-1, MIP-1α regulating immune response were identified in rats' blood plasma in the test groups due to the same reasons (see Figures 1– 3).

Conclusion. Our findings give evidence of pro-inflammatory effects produced by intragastric TeA administration in rats, both as a monocomponent or in a mixture of the extract of the *Alternaria alternate* cultivation medium. The Alternaria toxins used in the study are capable of inducing systemic inflammatory reactions identified by an increase in the content of B-lymphocytes responsible for humoral immunity and an increase in the levels of proinflammatory, proapoptogenic cytokines in the blood plasma: TNF-α, IL-1β, IFN-γ, IL-6, IL-10, IL-2, IL-5, IL-17A and chemokines MIP-1α and MCP-1. Introduction of TeA leads to a decrease in the levels of anti-inflammatory IL-5 and IL-10, which may be a consequence of a decrease in the activity of Treg (T-regulatory) lymphocytes confirmed by a decrease in the IL-10/IL-17A ratio. These results allow using immunological indicators as markers of effects upon exposure to Alternaria toxins in providing scientific substantiation for sanitary-hygienic standards and assessing safety of food products.

Acknowledgments. The authors express their gratitude to A.D. Konev, a research assistant of the nutritional enzymology laboratory of the Federal Research Center of Nutrition and Biotechnology for his assistance in obtaining the alternaria toxin extract and working with animals.

Funding. The research work on preparing the manuscript was supported by funding as a subsidy for the implementation of a state assignment (topic No. FGMF-2023-0006).

Competing interests. The authors declare no competing interests.

References

1. Efimochkina N.R., Sedova I.B., Sheveleva S.A., Tutelyan V.A. Toxigenic properties of mycotoxin-producing fungi. *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya*, 2019, no. 45, pp. 6–33. DOI: 10.17223/19988591/45/1 (in Russian).

2. M de Sá S.V., Monteiro C., Fernandes J.O., Pinto E., Faria M.A., Cunha S.C. Emerging mycotoxins in infant and children foods: A review. *Crit. Rev. Food Sci. Nutr.*, 2023, vol. 63, no. 12, pp. 1707–1721. DOI: 10.1080/10408398.2021.1967282

3. Ji X., Zhou Y., Xiao Y., Lyu W., Wang W., Shao K., Yang H. A tiered approach of hazard-prioritization and risk-ranking for chemical hazards in food commodities: Application for selected mycotoxins. *Food Res. Int.*, 2024, vol. 178, pp. 113946. DOI: 10.1016/j.foodres.2024.113946

4. Aksenov I.V., Sedova I.B., Chalyy Z.A., Tutelyan V.A. Alternaria toxins as a risk factor for population health. *Health Risk Analysis*, 2023, no. 4, pp. 146–157. DOI: 10.21668/health.risk/2023.4.14.eng

5. Singh K., Kumari A. Overview. In book: *Mycotoxins and Mycotoxicoses*. Singapore, Springer Publ., 2022, pp. 15–26. DOI: 10.1007/978-981-19-2370-8_2

6. Ismail A.M., Elshewy E.S., El-Ganainy S.M., Magistà D., Hamouda A.F., Alhudaib K.A., Ebrahim W., Almaghasla M.I. Mycotoxins from Tomato Pathogenic *Alternaria alternata* and Their Combined Cytotoxic Effects on Human Cell Lines and Male Albino Rats. *J. Fungi (Basel)*, 2023, vol. 9, no. 3, pp. 282. DOI: 10.3390/jof9030282

7. Den Hollander D., Holvoet C., Demeyere K., De Zutter N., Audenaert K., Meyer E., Croubels S. Cytotoxic Effects of Alternariol, Alternariol Monomethyl-Ether, and Tenuazonic Acid and Their Relevant Combined Mixtures on Human Enterocytes and Hepatocytes. *Front. Microbiol.*, 2022, vol. 13, pp. 1–12. DOI: 10.3389/fmicb.2022.849243

8. Louro H., Vettorazzi A., López de Cerain A., Spyropoulou A., Solhaug A., Straumfors A., Behr A.-C., Mertens B. [et al.]. Hazard characterization of *Alternaria* toxins to identify data gaps and improve risk assessment for human health. *Arch. Toxicol*., 2024, vol. 98, no. 2, pp. 425–469. DOI: 10.1007/s00204-023-03636-8

9. Asam S., Rychlik M. Potential health hazards due to the occurrence of the mycotoxin tenuazonic acid in infant food. *Eur. Food Res. Technol*., 2013, vol. 236, pp. 491–497. DOI: 10.1007/s00217-012-1901-x

10. Agriopoulou S., Stamatelopoulou E., Varzakas T. Advances in occurrence, importance, and mycotoxin control strategies: prevention and detoxification in foods. *Foods*, 2020, vol. 9, no. 2, pp. 137. DOI: 10.3390/foods9020137

11. Kraft S., Buchenauer L., Polte T. Mold, Mycotoxins and a Dysregulated Immune System: A Combination of Concern? *Int. J. Mol. Sci*., 2021, vol. 22, no. 22, pp. 12269. DOI: 10.3390/ijms222212269

12. Beerweiler C.C., Masanetz R.K., Schaub B. Asthma and allergic diseases: Cross talk of immune system and environmental factors. *Eur. J. Immunol*., 2023, vol. 53, no. 6, pp. e2249981. DOI: 10.1002/eji.202249981

13. Yekeler H., Bitmiş K., Ozçelik N., Doymaz M.Z., Calta M. Analysis of toxic effects of *Alternaria* toxins on esophagus of mice by light and electron microscopy. *Toxicol. Pathol*., 2001, vol. 29, no. 4, pp. 492–497. DOI: 10.1080/01926230152499980

14. Puntscher H., Aichinger G., Grabher S., Attakpah E., Krüger F., Tillmann K., Motschnig T., Hohenbichler J. [et al.]. Bioavailability, metabolism, and excretion of a complex *Alternaria* culture extract versus altertoxin II: a comparative study in rats. *Arch. Toxicol*., 2019, vol. 93, no. 11, pp. 3153–3167. DOI: 10.1007/s00204-019-02575-7

15. Crudo F., Partsch V., Braga D., Blažević R., Rollinger J.M., Varga E., Marko D. Discovery of the *Alternaria* mycotoxins alterperylenol and altertoxin I as novel immunosuppressive and antiestrogenic compounds in vitro. *Arch. Toxicol*., 2025, vol. 99, no. 1, pp. 407–421. DOI: 10.1007/s00204-024-03877-1

16. Zhu X., Chen Y., Tang X., Wang D., Miao Y., Zhang J., Li R., Zhang L., Chen J. General toxicity and genotoxicity of altertoxin I: a novel 28-day multiendpoint assessment in male Sprague-Dawley rats. *J. Appl. Toxicol*., 2022, vol. 42, no. 8, pp. 1310–1322. DOI: 10.1002/JAT.4297

17. Miao Y., Wang D., Chen Y., Zhu X., Tang X., Zhang J., Zhang L., Chen J. General toxicity and genotoxicity of alternariol: a novel 28-day multi-endpoint assessment in male Sprague-Dawley rats. *Mycotoxin Res*., 2022, vol. 38, no. 4, pp. 231–241. DOI: 10.1007/s12550-022-00466-y

18. Tang X., Chen Y., Zhu X., Miao Y., Wang D., Zhang J., Li R., Zhang L., Chen J. Alternariol monomethyl ether toxicity and genotoxicity in male Sprague-Dawley rats: 28-Day *in vivo* multi-endpoint assessment. *Mutat. Res. Genet. Toxicol. Environ. Mutagen.*, 2022, vol. 873, pp. 503435. DOI: 10.1016/J.MRGENTOX.2021.503435

19. Lushova A.A., Zheremyan E.A., Astakhova E.A., Spiridonova A.B., Byazrova M.G., Filatov A.V. B-lymphocyte subsets: functions and molecular markers. *Immunologiya*, 2019, vol. 40, no. 6, pp. 63–76. DOI: 10.24411/0206-4952-2019-16009 (in Russian).

20. Wang H., Guo Y., Luo Z., Gao L., Li R., Zhang Y., Kalaji H.M., Qiang S., Chen S. Recent Advances in *Alternaria* Phytotoxins: A Review of Their Occurrence, Structure, Bioactivity, and Biosynthesis. *J. Fungi (Basel)*, 2022, vol. 8, no. 2, pp. 168. DOI: 10.3390/jof8020168

21. Pendeville H., Carpino N., Marine J.C., Takahashi Y., Muller M., Martial J.A., Cleveland J.L. The ornithine decarboxylase gene is essential for cell survival during early murine development. *Mol. Cell. Biol*., 2001, vol. 21, no. 19, pp. 6549–6558. DOI: 10.1128/MCB.21.19.6549-6558.2001

22. Wu R., Chen X., Kang S., Wang T., Gnanaprakasam J.R., Yao Y., Liu L., Fan G. [et al.]. De novo synthesis and salvage pathway coordinately regulate polyamine homeostasis and determine T cell proliferation and function. *Sci. Adv.*, 2020, vol. 6, no. 51, pp. eabc4275. DOI: 10.1126/sciadv.abc4275

23. Puleston D.J., Baixauli F., Sanin D.E., Edwards-Hicks J., Villa M., Kabat A.M., Kamiński M.M., Stanckzak M. [et al.]. Polyamine metabolism is a central determinant of

helper T cell lineage fidelity. *Cell*, 2021, vol. 184, no. 16, pp. 4186–4202.e20. DOI: 10.1016/j.cell.2021.06.007

24. Wagner A., Wang C., Fessler J., DeTomaso D., Avila-Pacheco J., Kaminski J., Zaghouani S., Christian E. [et al.]. Metabolic modeling of single Th17 cells reveals regulators of autoimmunity. *Cell*, 2021, vol. 184, no. 16, pp. 4168–4185.e21. DOI: 10.1016/j.cell.2021.05.045

Trushina E.N., Rieger N.A., Mustafina O.K., Timonin A.N., Aksenov I.V., Tutelyan V.A. Alternaria toxins as risk factors for cellular immunity disorders and cytokine profile imbalance (based on an in vivo experimental model). Health Risk Analysis, 2025, no. 3, pp. 104–113. DOI: 10.21668/health.risk/2025.3.11.eng

Received: 17.06.2025
Approved: 11.08.2025
Accepted for publication: 26.09.2025