DOI 10.1002/mnfr.200700131 Mol. Nutr. Food Res. 2007, *51*, 1406 – 1412

Research Article

Fumonisin B1 alters cell cycle progression and interleukin-2 synthesis in swine peripheral blood mononuclear cells

Daniela E. Marin[2]*, Marie-Estelle Gouze[1]*, Ionelia Taranu[1, 2] and Isabelle P. Oswald[1]

[1] Laboratoire de Pharmacologie-Toxicologie, UR 66, Institut National de la Recherche Agronomique, Toulouse, France
[2] INCDBNA, National Research and Development Institut for Biology and Animal Nutrition, Balotesti, Romania

Fumonisin B_1 (FB_1) is a mycotoxin produced by *Fusarium verticillioides,* a fungus that commonly contaminates maize. In the present study, we investigated the effects of FB_1 on swine peripheral blood mononuclear cells (PBMC) by measuring cell proliferation, cell cycle progression and interleukin (IL)-2 production. Forty-eight hours after treatment *in vitro*, FB_1 induced a decrease of PBMC proliferation as measured by cell counting and dehydrogenase enzyme activity. This effect was observed starting with 10 μM FB_1. The effect of FB_1 on cell cycle progression was analyzed by flow cytometry. Incubation of PBMC with FB_1 increased the percentage of cells blocked in G0/G1 phase of the cell cycle. Treatment with 200 μM FB_1 induces a high blockade of the cell cycle, with 92.4% of cells in G0/G1 phase. This blockade was observed in all lymphocyte subsets tested ($CD2^+$, $CD4^+$, $CD8^+$ and Ig^+) as evidenced by dual staining for DNA and membrane surface molecules. A significant decrease of IL2 production was also observed in the supernatants of ConA stimulated PBMC treated with 100 or 200 μM FB_1. In conclusion, these data suggest that FB_1 may affect immune functions by inhibiting lymphocyte proliferation and IL2 production, suggesting a possible role of FB_1 exposure during infectious disease and cancer.

Keywords: Cell cycle progression / Fumonisin / Lymphocyte / Proliferation / Swine

Received: March 30, 2007; revised: June 12, 2007; accepted: June 13, 2007

1 Introduction

Mycotoxins are fungal secondary metabolites, produced by many species of *Aspergillus*, *Fusarium*, *Penicillium*, *Claviceps* and *Alternaria* [1] and known to be associated with different human and animal diseases. Among the most common mycotoxins, distributed worldwide, are aflatoxins, ochratoxin, trichothecenes, zearalenone and fumonisins. The toxicological symptoms caused by ingestion of mycotoxins range from acute mortality, to retarded growth and altered reproduction. Consumption of fungal toxins may also result in impaired immunity and decreased resistance to infectious diseases [2]. The global occurrence of mycotoxin is considered an important risk factor for both human and animal health as, according to some reports, up to 25% of the world crop production is contaminated [3].

The fumonisins are a group of mycotoxins produced by a limited number of *Fusarium* species, of which *F. verticillioides* and *F. proliferatum* are the most frequent worldwide [4]. Fumonisin B_1 (FB_1) is the most abundant toxin of this family in naturally contaminated foods and feeds [5]. Recent surveys have raised concerns about the extent of FB_1 contamination and its implication for food safety [6, 7]. Studies published in 2007, showed that in Portugal fumonisins were found in 22% of cereals samples at concentrations between 113 and 2026 ppb [8] while in Spain up to 13.3% of the conventional corn samples contained fumonisins B_1 and B_2 at mean concentrations of 43 and 22 ppb [9]. For human exposure, the potential danger is mostly but not

Correspondence: Dr. Isabelle Oswald, Laboratoire de Pharmacologie Toxicologie, INRA, 180 Chemin de Tournefeuille, 31931 Toulouse Cedex 9, France
E-mail: ioswald@toulouse.inra.fr
Fax: +33-5-6128-5310

Abbreviations: ConA, concanavalin A; **FB_1**, fumonisin B_1; **IL**, interleukin; **PBMC**, peripheral blood mononuclear cells; **PI**, propidium iodide; **PWM**, pokeweed mitogen

* Daniela E. Marin and Marie-Estelle Gouze equally contributed to this work.

exclusively from homegrown corn in underdeveloped countries as Iran [10 – 12].

Ingestion of high doses of FB_1 is hepatotoxic in all species studied and induces different species-specific effects in domestic and laboratory animals including pulmonary edema and cardiovascular changes in the pig, leukoencephalomalacia in horses and nephrotoxicity in rats, rabbits and lambs [13, 14]. Ingestion of this toxin is also a possible risk factor associated with human esophageal cancers [6] and neural tube defects [15]. Ingestion of low doses of FB_1 increases intestinal and pulmonary infections [16, 17] and alters immune responses in many species including rodents and pigs [18 – 20]. Considering that pigs are high consumers of corn and are thus particularly exposed to fumonisins [21, 22], we used swine peripheral blood mononuclear cells to determine the effect of FB_1. Moreover, pig is also considered as a good model for extrapolation to humans [23, 24].

The mechanisms of toxicity for FB_1 are complex and may involve several molecular sites [25]. The primary biochemical effect of FB_1 is to inhibit the ceramide synthase enzyme, leading to the cellular accumulation of sphingoid bases (sphinganine and/or sphingosine) and to the depletion of ceramide and complex sphingolipids [26, 27]. Sphingoid bases are known to mediate several key biological processes such as cell proliferation or DNA replication [27]. Moreover, cell cycle control, including cell growth and proliferation as well as cell cycle arrest and apoptosis, plays a central role in cellular immune responses [28]. In this context, FB_1 has been shown to alter some lymphocyte functions and to reduce specific antibody production after vaccination with *Mycoplasma agalactiae* [29, 30], to alter cytotoxic functions [31] and to decrease proliferation [32, 33]. However, the underlying mechanism has been poorly described. The aim of the present study was to confirm the effects of FB_1 on swine lymphocyte proliferation, to characterize at which phase of the cell cycle progression the lymphocytes were blocked and to compare the susceptibility of the different lymphocyte subset to FB_1.

2 Materials and methods

2.1 Fumonisin B_1

Fumonisin B_1 (FB_1) (>98% pure by NMR, and HPLC) extracted and purified according to the method of Cawood [34] was obtained from PROMEC/MRC (Tygerberg, South Africa). The toxin was dissolved in water and further diluted in complete cell culture medium.

2.2 Isolation and culture of porcine peripheral mononuclear cells

Blood from six healthy crossbreed pigs available in the laboratory was collected from the jugular vein into heparinized Vacutainer tubes (Becton Dickinson, Plymouth, UK). Following gradient centrifugation over Ficoll-Hypaque (density 1.077, Eurobio, Les Ulis, France), peripheral blood mononuclear cells (PBMC) were isolated, washed twice in PBS and resuspended in RPMI-1640 (Eurobio) supplemented with 10% fetal calf serum (Sigma), 2 mM L-glutamine, 100 U/mL penicillin and 50 μg/mL streptomycin (Eurobio). Cells were counted and viability assessed using trypan blue exclusion method counting 50 to 100 cells (Eurobio). PBMC were then cultivated with different concentrations of fumonisin B_1.

2.3 Measurement of proliferation

PBMC, cultured at a density of 1×10^6 cells/well in 96-well flat-bottom tissue plates (Falcon, Franklin Lakes, NJ), were stimulated with 10 μg/mL concanavalin A (ConA) (Sigma). PBMC were incubated for 48 h at 37°C and 5% CO_2, with concentrations of FB_1 of 0, 10, 50, 100 or 200 μM. Stimulated and unstimulated cells without FB_1 were used as controls. Cell metabolic activity, as an indicator of cell proliferation, was determined, in three independent experiments, by an MTS test using a cellTiter 96 aqueous non-radioactive test (Promega, Charbonniere, France). Absorbance of the soluble formazan produced was measured at 492 nm after 4 h of incubation with MTS solution.

2.4 DNA staining and analysis of the cell cycle

PBMC (1×10^6 cells/mL) were stimulated with ConA or pokeweed mitogen (PWM) (Sigma), at final concentration of 10 μg/mL, and cultured in presence of 100 μM FB_1. After 48 h, cells were harvested by centrifugation (10 min at $500 \times g$), washed in 2 mL of PBS and fixed with 1% Paraformaldehyde (Sigma) in PBS, 20 min at room temperature. Cells were then permeabilized 5 min at room temperature with 0.5% Triton X-100 (Sigma), treated with 5 U RNase A, and stained with 20 μg/mL propidium iodide (PI) (Sigma) for 30 min at room temperature. Cell cycle analysis was done by flow cytometry using a Coulter XL 4C (Becton Dickinson Immunocytometry Systems, San Jose, CA, USA). Ten thousand cells were analyzed and the percentage of cells in each stage of the cell cycle was estimated using CellQuest software (Becton Dickinson).

2.5 Staining for cell surface antigen

PBMC cells treated and stimulated, as already described for cell cycle analysis, were harvested, fixed with 1% paraformaldehyde in PBS (pH = 7.4) and incubated 30 min with NH_4Cl (50 mM). Following saturation treatment in 3% bovine serum albumin (BSA) for 1 h, PBMC were labeled with primary specific mAb (VMRD, Pullman, USA) directed against CD2 (clone MSA 4), CD4 (clone 74-12-4), CD8 (clone 76-2-11) or CD25 (clone PGBL25A). Mouse

anti-porcine IgG (light chain) (clone K139 3E1, Serotec, Oxford, U.K) was used as isotype control.

After 1 h of incubation at 37°C, cells were washed in PBS and labeled with goat FITC secondary anti murine antibody (Sigma) for 30 min at 37°C. For dual staining of surface antigen and DNA, cells were then permeabilized as described previously. Cells were then analyzed by flow cytometry analysis as described previously.

2.6 Determination of interleukin-2 by ELISA

PBMC, cultured at a density of 5×10^6 cells/well and stimulated with 10 μg/mL ConA, were incubated with the different doses of FB_1. Twenty-hours later, culture supernatants were then collected and analyzed for cytokine content by ELISA. Briefly, a purified fraction of anti-swine interleukin (IL)-2 (clone A150D3F1, Biosource Europe, CliniSciences, Montrouge, France) was used as capture antibody in conjunction with biotinylated anti-swine IL2 mAb (clone A150D8H10, Biosource). Streptavidin-HRP (Biosource) and TMB (Fermentas, MD, USA) were used for detection. Absorbance was read at 450 nm using an ELISA plate reader (Spectra Thermo, Tecan, NC, USA). Recombinant swine IL2 was used as standard and results were expressed as picograms of cytokine/mL.

2.7 Statistical analysis

Student's *t*-tests were used to analyze the differences in term of lymphocyte proliferation, cell percentages in G0/G1 phase of the cell cycle, and synthesis of IL2 synthesis between control cells and cells treated with various concentration of FB_1. The p values < 0.05 were considered significant.

3 Results

3.1 FB_1 inhibits lymphocyte proliferation

Porcine lymphocyte proliferation after ConA stimulation and FB_1 treatment was evaluated. We first determined the metabolic activity of the cells by measuring the evaluation of dehydrogenase enzyme activity (Fig. 1). As expected, ConA stimulation significantly increased dehydrogenase metabolic activity, reflecting an increased PBMC proliferation. Addition of increasing concentrations of FB_1 in ConA stimulated cultures inhibited dehydrogenase activity. The effect of FB_1 was already observed with the lowest concentration used (10 μM, $p < 0.05$) and was dose dependent.

In order to verify that the decreased metabolic activity measured by the MTS assay was associated with a decreased cell number, trypan blue exclusion experiments were performed. In three independent experiment, we observed that the cell number increases from 1.75 ± 0.09×10^6 cells/mL in control PBMC to 2.44 ± 0.09×10^6 cells/mL in ConA-stimulated cells and was back to 1.56 ± 0.06×10^6 cells/mL in ConA-stimulated cells treated for 48 h with 200 μM FB_1 ($p < 0.001$, comparison between ConA- and ConA + FB_1-treated cells). This decrease cell number was not associated with an increased cell death.

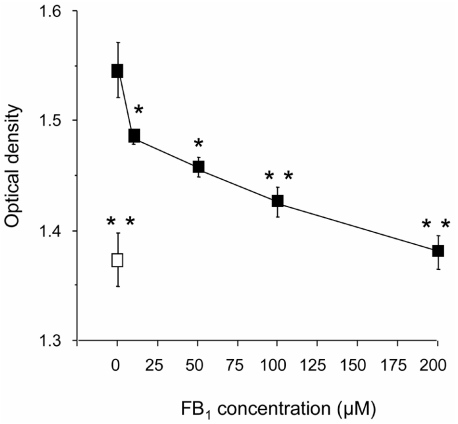

Figure 1. Effect of FB_1 on peripheral blood monocyte cell proliferation. PBMC stimulated with ConA were incubated for 48 hours with concentrations of FB_1 of 0, 10, 50, 100 and 200 μM/well. Cell proliferation was determined through a cell metabolic activity test. Absorbance of soluble formazan produced was measured at 492nm using an ELISA plate reader. Data are mean ± standard errors of three independent experiments. Blank square represent optical density average value for unstimulated cells. * Indicates differences between FB_1-treated cells and ConA-stimulated cells.

3.2 FB_1 blocks the cell cycle of PBMC in G0/G1 phase

In order to better understand the effect of FB_1 on lymphocyte proliferation, the percentage of cells within the different phases of the cell cycle was determined by flow cytome-

Table 1. Effect of FB_1 on G0/G1 phase of the cell cycle[a)]

Cell treatment		Percentage of cell in G0/G1 phase of the cell cycle
Con A stimulation (10 μg/mL)	FB1 treatment (μM)	
–	0	95.8 ± 0.5
+	0	83.2 ± 0.7[b]
+	1	86.3 ± 0.3[c]
+	5	87.6 ± 0.3
+	10	86.9 ± 0.7[c]
+	20	87.6 ± 0.5[c]
+	50	89.0 ± 0.2[d]
+	100	90.5 ± 0.5[d]
+	150	90.2 ± 1.3[d]
+	200	92.5 ± 0.3[e]

a) Data are mean ± standard errors of three independent experiments. Means with different letters in the same column are significantly different ($p < 0.05$).

Table 2. Effect of FB_1 on the different lymphocyte subsets

Lymphocyte population Cell surface maker	Cell treatment			Statistical analysis[c]	
	Control	Mitogen[b]	Mitogen + FB1	Mitogen effect	FB_1 effect
T lymphocyte					
$CD2^+$	94.3 ± 1.8[a]	80.2 ± 4.6	88.5 ± 0.6	**	*
$CD4^+$	94.9 ± 1.8	78.0 ± 4.8	87.3 ± 2.0	**	*
$CD8^+$	96.2 ± 0.6	70.9 ± 2.7	81.4 ± 1.3	**	*
B lymphocyte					
Ig^+	97.5 ± 2.6	82.2 ± 1.4	86.9 ± 0.6	**	*

a) Data are mean ± standard errors of three independent experiments.
b) ConA (10 μg/mL) was used to stimulate T lymphocytes and Pokeweed mitogen (10 μg/mL) was used to stimulate B lymphocytes.
c) Student's *t*-tests were performed to determine the effect of mitogen (difference between control cell and mitogen-treated cells) and the effect of 100 μM FB_1 (difference between mitogen-treated cells and cells treated with mitogen + FB_1): * $p < 0.05$, ** $p < 0.01$.

try analysis. For this purpose, PBMC were stimulated with ConA, incubated for 48 h with increasing concentrations of FB_1 (0 to 200 μM) and their DNA contents were analyzed after PI staining. In ConA-stimulated cultures, 83.1 ± 0.4% cells were in G0/G1 phase compared to 95.8 ± 0.28% in control cells (Table 1). Treatment with FB_1 blocked the cell cycle progression of ConA stimulated PBMC in the G0/G1 phase in a dose-dependent manner. The highest FB_1 concentration induces a partial blockade of the cell cycle. Indeed, the percentage of cell in G0/G1 phase in ConA stimulated PBMC treated with 200 μM almost reach the one of unstimulated cells, 92.4 ± 0.5% and 95.8 ± 0.3%, respectively.

The ability of FB_1 to alter the cell cycle progression of different lymphocyte subsets was then investigated. PBMC were stimulated with T or B cell mitogen (Con A or PWM, respectively) and cultured for 48 h in the presence of 100 μM FB_1. Lymphocytes were stained for surface molecules ($CD2^+$, $CD4^+$, $CD8^+$ and IgG^+) with specific antibody and for DNA content with PI.

As shown in the Table 2, in each lymphocyte population considered, FB_1 treatment induced a cell cycle arrest in G0/G1 phase. FB_1 inhibited the mitogenic response of the cells by 59.6, 51.4, 41.7 and 38.1% for $CD2^+$, $CD4^+$, $CD8^+$ and surface IgG^+ cell, respectively.

3.3 Effects of FB_1 on IL2 synthesis

Because of its implication in lymphocyte proliferation, the effect of FB_1 on IL2 production was investigated. As expected, IL2 production was significantly increased by Con A stimulation (3.8 ± 0.1 pg/mL versus 1025.1 ± 17.8 pg/mL, $p < 0.0001$). As shown in Fig. 2, high concentrations of FB_1 significantly decrease IL2 production. For example, concentration of 100, 150 and 200 μM FB_1 reduced IL2 synthesis by 19.3, 30.8 and 35.7%, respectively ($p = 0.017$, 0.007 and 0.011, respectively). This modulation

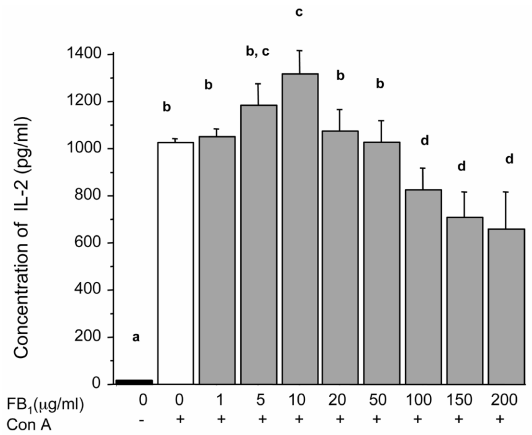

Figure 2. Effect of FB_1 on IL2 synthesis in porcine PBMC cultures. PBMC stimulated with 10 μg/mL ConA, were incubated with 0 – 200 μM FB_1 for 24 h. Culture supernatants were then collected and analyzed for IL2 content using an ELISA assay. Recombinant swine IL2 were used as standards and results were expressed in pg/mL. Data are mean ± standard errors of three independent experiments. a, b, c, d indicate statistically significant differences for IL2 synthesis between treatments ($p < 0.05$).

of IL2 synthesis was not associated with changes in the surface expression of the IL2 receptor (CD25). Indeed, the percentages of PBMC expressing CD25 on their surface were 17.3, 19.6, and 17.3% in untreated cells, ConA-stimulated cells and ConA stimulated cells treated with 100 μM FB_1, respectively.

4 Discussion

In the present *in vitro* study, we demonstrated that FB_1 blocks lymphocyte proliferation in the G0/G1 phase of the cell cycle. This effect was observed with a very low dose of

FB_1 (1 μM) and was dose dependent (Table 1). A cell cycle arrest has already been described in epithelial cell lines [18, 35, 36], but this is the first report describing a blockade of the cell cycle by FB_1 in primary cells such as lymphocytes.

This arrest in cell cycle of porcine PBMC is associated with a dose-dependent anti-proliferative effect (Fig. 1). Inhibition of lymphocyte proliferation by FB_1 has also been observed in other species. In turkey, ingestion of FB_1-contaminated feed or *in vitro* exposure of PBMC to FB_1 inhibits lymphocyte proliferation [37]. In mice, subcutaneous injection of FB_1 caused a decrease in splenocyte proliferation [38]. Similarly, in human, inhibitory effect of FB_1 was observed in T lymphocytes from patients with systemic lupus erythematosus [39] as well as on a erythroleukemia cell line [40].

In vivo or *in vitro* treatment with FB_1, leads to repression of certain protein kinase C isoforms, alteration in the expression of genes associated with the cell cycle, cell-cycle arrest and programmed cell death [41 – 44]. These alterations were associated with a repression of the factors that promote G1 progression and S-phase entry such as CDK2, CDK3 and cyclin E and a induction of two CDK inhibitors, Kip1 and Kip2 [36]. Also, it was shown that the activation of activated protein kinase B by FB_1 leads to increased survival, inhibition of glycogen synthase kinase 3beta activity and post-translational stabilization of cyclin D1, all events responsible for disruption of the cell cycle G(1)/S restriction point in hepatocytes [45]. The mechanisms of toxicity for fumonisins are complex and may involve several molecular sites. The primary biochemical effect of fumonisin is to inhibit ceramide synthase. This enzyme is involved in sphingoid base turnover and it is a target for cell polarity-stimulating signaling cascades. The alteration of cell polarity may provide a mechanism for the cytotoxic effects of fumonisin B_1 [46]. Moreover, inhibition of the ceramide synthase by FB_1 has been shown to lead to the accumulation of sphingoid bases and sphingoid base metabolites, and to the depletion of more complex sphingolipids [47]. Alterations of sphingolipid metabolism contribute to the increased cell death and cytolethality of FB_1 observed in murine microglial cells and murine cultured astrocytes [48]. Alteration of sphingolipid metabolism result also in an increase of sphingosine (sphinganine) 1-phosphates in the organs of the exposed animals [49 – 51]. Sphingosine 1-phosphate is a bioactive lipid that participates in the regulation of numerous cell processes, such as cell proliferation, differentiation, migration [52] and the antiapoptotic, promitotic activities of this molecule [53, 54] could be responsible for some fumonisin related diseases.

In order to see if the arrest in G0/G1 phase of the cell cycle was restricted to a particular subset of lymphocytes, dual staining for DNA and membrane surface molecules were performed (Table 2). We demonstrated that FB_1 treatment induced an arrest of the cell cycle in G0/G1 phase in both the $CD4^+$ and the $CD8^+$ subset of T lymphocytes and as well as in B lymphocytes. Cells blocked in the phase G0/G1 have a finite proliferative potential and could not follow a normal cell cycle. Our results are consistent with a 36% and a 57% decrease in the number of T lymphocytes and B lymphocytes, respectively, observed in mice injected subcutaneously with FB_1 [38].

Transition from G0 to G1 is marked by transcriptional activation of IL2 and IL2 receptor genes [55]. IL2, also called T cell growth factor, is a pleiotropic cytokine produced by activated T lymphocytes that modulates the function of B and T lymphocytes. In our study, concentrations of 100 to 200 μM FB_1 induced a significantly decrease of IL2 production; this effect was not observed with the low doses (Fig. 2). Treatment of murine primary splenic T cell and T cell hybridomas with 100 μM FB_1 also decreased TCR induced IL2 production [56]. By contrast, treatment of the human Jurkat T cell with 80 μM FB_1 does not modify IL2 mRNA level upon mitogenic stimulation [57].

In conclusion, FB_1 inhibits the proliferation of the different lymphocyte subsets through a blockade of their cell cycle in G0/G1 phase and a decrease in IL2 synthesis. Since, lymphocyte proliferation is critical for the development of an immune response, our results may explain the increased susceptibility to infectious diseases [17, 18] and the decreased response to vaccination of pigs exposed to fumonisin [29, 30]. Considering that high concentrations of FB_1 may be present in animal feeds and human food preparations [7 – 9, 58], this might have clinical relevance against infectious disease.

Drs. D. Marin and I. Taranu were supported by fellowships from a ECO-NET project granted by the "Ministère de l'Éducation Nationale, de la Recherche et de la Technologie" (Paris, France) and by INRA. This work was supported in part by funds from the region Midi-Pyrénées, France (DAER-Rech/99008345). The authors thank Marie Tossollini for her help with the IL2 analysis.

5 References

[1] Bennett, J. W., Klich, M., Mycotoxins, *Clin. Microbiol. Rev.* 2003, *16*, 497 – 516.

[2] Oswald, I. P., Marin, D. E., Bouhet, S., Pinton, P., *et al*., Immunotoxicological risk of mycotoxins for domestic animals, *Food Addit. Contam*. 2005, *22*, 354 – 360.

[3] Fink-Gremmels, J., Mycotoxins: their implications for human and animal health, *Vet. Q.* 1999, *5*, 115 – 120.

[4] Marin, S., Magan, N., Ramos, A. J., Sanchis, V., Fumonisin-producing strains of Fusarium: a review of their ecophysiology, *J. Food Prot.* 2004, *5*, 1792 – 1805.

[5] Shephard, G. S., Marasas, W. F., Leggott, N. L., Yazdanpanah, H. *et al*., Natural occurrence of fumonisins in corn from Iran, *J. Agric. Food Chem*. 2000, *48*, 1860 – 1864.

[6] International Programme on Chemical Safety (IPCS), Fumonisin B1, *Environ. Health Criteria*. 2000, *219*, 43 – 50.

[7] Shephard, G. S., van der Westhuizen, L., Thiel, P. G., Gelderblom, W. C., *et al*., Disruption of sphingolipid metabolism in non-human primates consuming diets of fumonisin-containing *Fusarium moniliforme* culture material, *Toxicon*. 1996, *34*, 527 – 534.

[8] Silva, L. J., Lino, C. M., Pena, A., Molto, J. C., Occurrence of fumonisins B1 and B2 in Portuguese maize and maize-based foods intended for human consumption, *Food Addit Contam*. 2007, *24*, 381 – 390.

[9] Arino, A., Juan, T., Estopanan, G., Gonzalez-Cabo, J. F., Natural occurrence of Fusarium species, fumonisin production by toxigenic strains, and concentrations of fumonisins B1, and B2 in conventional and organic maize grown in Spain, *J. Food Prot.* 2007, *70*, 151 – 156.

[10] Yazdanpanah, H., Shephard, G. S., Marasas, W. F., van der Westhuizen, L., *et al*., Human dietary exposure to fumonisin B1 from Iranian maize harvested during 1998 – 2000. *Mycopathologia*. 2006, *161*, 395 – 401.

[11] Sun, G., Wang, S., Hu, X., Su, J., *et al*., Fumonisin B1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China, *Food Addit Contam*. 2007, *24*, 181 – 185.

[12] Chelule, P. K., Gqaleni, N., Dutton, M. F., Chuturgoon, A. A., Exposure of rural and urban populations in KwaZulu Natal, South Africa, to fumonisin B(1) in maize, *Environ Health Perspect*. 2001, *109*, 253 – 256.

[13] Bolger, M., Coker, R. D., Dinovi, M., Gaylor, D. *et al*., Fumonisins. In: Safety evaluation of certain mycotoxins in food, *Food and Agriculture Organization of the United Nations. World Health Organization Food Additives*. 2001, *47*, 103 – 279.

[14] Haschek, W. M., Gumprecht, L. A., Smith, G., Tumbleson, M. E., *et al*., Fumonisin toxicosis in swine: an overview of porcine pulmonary edema and current perspectives, *Environ. Health Perspect*. 2001, *109*, 251 – 257.

[15] Missmer, S. A., Suarez, L., Felkner, M., Wang, E., *et al*., Exposure to fumonisins and the occurrence of neural tube defects along the Texas-Mexico border, *Environ. Health Perspect*. 2006, *114*, 237 – 241.

[16] Oswald, I. P., Desautels, C., Laffitte, J., Fournout, S., *et al*., Mycotoxin fumonisin B1 increases intestinal colonization by pathogenic *Escherichia coli* in pigs, *Appl. Environ. Microbiol*. 2003, *69,* 5870 – 5874.

[17] Halloy, D. J., Gustin, P. G., Bouhet, S., Oswald, I. P., Oral exposure to culture material extract containing fumonisins predisposes swine to the development of pneumonitis caused by *Pasteurella multocida*, *Toxicology* 2005, *213*, 34 – 44.

[18] Bouhet, S., Hourcade, E., Loiseau, N., Fikry, A., *et al*., The mycotoxin fumonisin B1 alters the proliferation and the barrier function of porcine intestinal epithelial cells, *Toxicol. Sci*. 2004, *77*, 165 – 171.

[19] Sharma, R. P., He, Q., Johnson, V. J., Suzuki, H., Mice lacking both TNFalpha receptors show increased constitutive expression of IFN-gamma: a possible reason for lack of protection from fumonisin B1 hepatotoxicity, *Cytokine* 2006, *34*, 260 – 270.

[20] Dombrink-Kurtzman, M. A., Gomez-Flores, R., Weber, R. J., Activation of rat splenic macrophage and lymphocyte functions by fumonisin B1, *Immunopharmacology* 2000, *49*, 401 – 409.

[21] Colvin, B. M., Harrison, L. R., Fumonisin-induced pulmonary edema and hydrothorax in swine, *Mycopathologia* 1992, *117*, 79 – 82.

[22] Haschek, W. M., Motelin, G., Ness, D. K., Harlin, K. S., *et al*., Characterization of fumonisin toxicity in orally and intravenously dosed swine, *Mycopathologia*. 1992, *117*, 83 – 96.

[23] Almond, G. H., Research application using pigs, *Vet. Clinics North America: Food Anim. Practice*. 1996, *12*, 707 – 716.

[24] Rothkotter, H. J., Sowa, E., Pabst, R., The pig as a model of developmental immunology, *Hum. Exp. Toxicol*. 2002, *2*, 533 – 536.

[25] Riley, R. T., Voss, K. A., Norred, W. P., Sharma, R. P., *et al.*, Fumonisins: mechanism of mycotoxicity, *Rev. Med. Vet*. 1998, *149*, 617 – 626.

[26] Merrill, A. H. Jr, Sullards, M. C., Wang, E., Voss, K. A., *et al*., Sphingolipid metabolism: roles in signal transduction and disruption by fumonisins, *Environ. Health Perspect*. 2001, *2*, 283 – 289.

[27] Spiegel, S., Merrill, A. H. Jr., Sphingolipid metabolism and cell growth regulation, *FASEB J.* 1996, *10*, 1388 – 1397.

[28] Holcombe, H., Mellman, I., Janeway, C. A., Bottomly, K., The immunosuppressive agent 15-deoxyspergualin functions by inhibiting cell cycle progression and cytokine production following naive T cell activation, *J. Immunol.* 2002, *169*, 4982 – 4989.

[29] Taranu, I., Marin, D. E., Bouhet, S., Pascale, F., *et al*., Mycotoxin fumonisin B1 alters the cytokine profile and decreases the vaccinal antibody titer in pigs, *Toxicol. Sci.* 2005, *84*, 301 – 307.

[30] Marin, D. E., Taranu, I., Pascale, F., Lionide, A., *et al*., Sex-related differences in the immune response of weanling piglets exposed to low doses of fumonisin extract, *Br. J. Nutr.* 2006, *95*, 1185 – 1192.

[31] Qureshi, M. A., Hagler, W. M. Jr., Effect of fumonisin-B1 exposure on chicken macrophage functions *in vitro*, *Poult. Sci.* 1992, *71*, 104 – 112.

[32] Dombrink-Kurtzman, M. A., Fumonisin and beauvericin induce apoptosis in turkey peripheral blood lymphocytes, *Mycopathologia* 2003, *156*, 357 – 364.

[33] Charoenpornsook, K., Fitzpatrick, J. L., Smith, J. E., The effects of four mycotoxins on the mitogen stimulated proliferation of bovine peripheral blood mononuclear cells *in vitro*, *Mycopathologia* 1998, *143*, 105 – 111.

[34] Cawood, M. E., Gelderblom, W. C., Alberts, J. F., Snyman, S. D., Interaction of 14C-labelled fumonisin B mycotoxins with primary rat hepatocyte cultures, *Food Chem Toxicol*. 1994, *32*, 627 – 632.

[35] Wang, W., Jones, C., Ciacci-Zanella, J., Holt, T., *et al*., Fumonisins and *Alternaria alternata* lycopersici toxins: sphinganine analog mycotoxins induce apoptosis in monkey kidney cells, *Proc. Natl. Acad. Sci. USA* 1996, *93*, 3461 – 3465.

[36] Ciacci-Zanella, J. R., Merrill, A. H. Jr., Wang, E., Jones, C., Characterization of cell-cycle arrest by fumonisin B1 in CV-1 cells, *Food Chem. Toxicol*. 1998, *36*, 791 – 804.

[37] Dombrink-Kurtzman, M. A., Bennett, G. A., Richard, J. L., An optimized MTT bioassay for determination of cytotoxicity of fumonisins in turkey lymphocytes, *J. AOAC Int.* 1994, *77*, 512 – 516.

[38] Johnson, V. J., Sharma, R. P., Gender-dependent immunosuppression following subacute exposure to fumonisin B1, *Int. Immunopharmacol*. 2001, *1*, 2023 – 2034.

[39] Sakata, K., Sakata, A., Vela-Roch, N., Espinosa, R., Fas (CD95)-transduced signal preferentially stimulates lupus peripheral T lymphocytes, *Eur. J. Immunol*. 1998, *28*, 2648 – 2660.

[40] Minervini, F., Fornelli, F., Flynn, K. M., Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B1 in a human erythroleukemia cell line, *Toxicol. in Vitro* 2004, *18*, 21 – 28.

[41] Huang, C., Dickman, M., Henderson, G., Jones, C., Repression of protein kinase C and stimulation of cyclic AMP response elements by fumonisin, a fungal encoded toxin which is a carcinogen, *Cancer Res.* 1995, *55*, 1655 – 1659.

[42] Bondy, G. S., Barker, M. G., Lombaert, G. A., Armstrong, C. L. *et al*., A comparison of clinical, histopathological and cell-cycle markers in rats receiving the fungal toxins fumonisin B1 or fumonisin B2 by intraperitoneal injection, *Food Chem Toxicol*. 2000, *38*, 873 – 886.

[43] Lemmer, E. R., Vessey, C. J., Gelderblom, W. C., Shephard, E. G. *et al*., Fumonisin B1-induced hepatocellular and cholangiocellular tumors in male Fischer 344 rats: potentiating effects of 2-acetylaminofluorene on oval cell proliferation and neoplastic development in a discontinued feeding study, *Carcinogenesis* 2004, *25*, 1257 – 1264.

[44] Gelderblom, W. C., Galendo, D., Abel, S., Swanevelder, S., *et al*., Cancer initiation by fumonisin B(1) in rat liver-role of cell proliferation, *Cancer Lett*. 2001, *28*, 127 – 137.

[45] Ramljak, D., Calvert, R. J., Wiesenfeld, P. W., Diwan, B. A., *et al*., A potential mechanism for fumonisin B(1)-mediated hepatocarcinogenesis: cyclin D1 stabilization associated with activation of Akt and inhibition of GSK-3beta activity, *Carcinogenesis* 2000, *21*, 1537 – 1546.

[46] Marasas, W. F., Fumonisins: their implications for human and animal health, *Nat. Toxins* 1995, *3*, 193 – 198.

[47] Wang, E., Norred, W. P., Bacon, C. W., Riley, R. T., *et al*. Inhibition of sphingolipid biosynthesis by fumonisins. Implications for diseases associated with *Fusarium moniliforme*, *J. Biol. Chem*. 1991, *266*, 14486 – 14490.

[48] Osuchowski, M. F., Sharma, R. P., Fumonisin B1 induces necrotic cell death in BV-2 cells and murine cultured astrocytes and is antiproliferative in BV-2 cells while N2A cells and primary cortical neurons are resistant, *Neurotoxicology* 2005, *26*, 981 – 992.

[49] Riley, R. T., Voss, K. A., Differential sensitivity of rat kidney and liver to fumonisin toxicity: organ-specific differences in toxin accumulation and sphingoid base metabolism, *Toxicol Sci*. 2006, *92*, 335 – 345.

[50] Hsiao, S. H., Constable, P. D., Smith, G. W., Haschek, W. M., Effects of exogenous sphinganine, sphingosine, and sphingosine-1-phosphate on relaxation and contraction of porcine thoracic aortic and pulmonary arterial rings, *Toxicol Sci*. 2005, *86*, 194 – 199.

[51] Tran, S. T., Tardieu, D., Auvergne, A., Bailly, J. D., *et al*., Serum sphinganine and the sphinganine to sphingosine ratio as a biomarker of dietary fumonisins during chronic exposure in ducks, *Chem. Biol. Interact*. 2006, *10*, 41 – 50.

[52] Saba, J. D., Lysophospholipids in development: miles apart and edging, *J. Cell Biochem*. 2004, *92*, 967 – 992.

[53] Riley, R. T., Enongene, E., Voss, K. A., Norred, W. P., *et al*., Sphingolipid perturbations as mechanisms for fumonisin carcinogenesis. *Environ Health Perspect*. 2001, *2*, 301 – 308.

[54] Merrill, A. H. Jr., Schmelz, E. M., Dillehay, D. L., Spiegel, S., *et al*., Sphingolipids-the enigmatic lipid class: biochemistry, physiology, and pathophysiology, *Toxicol. Appl. Pharmacol*. 1997, *142*, 208 – 225.

[55] Crabtree, G. R., Contingent genetic regulatory events in T lymphocyte activation. *Science* 1989, *243*, 355 – 361.

[56] Tonnetti, L., Veri, M. C., Bonvini, E., D'Adamio, L., A role for neutral sphingomyelinase-mediated ceramide production in T cell receptor-induced apoptosis and mitogen-activated protein kinase-mediated signal transduction, *J. Exp. Med.* 1999, *189*, 1581 – 1589.

[57] Luongo, D., Severino, L., Bergamo, P., De Luna, R., *et al*., Interactive effects of fumonisin B1 and alpha-zearalenol on proliferation and cytokine expression in Jurkat T cells, *Toxicol. In Vitro* 2006, *20*, 1403 – 1410.

[58] Murphy, P. A., Hendrich, S., Hopmans, E. C., Hauck, C. C., *et al*., Effect of processing on fumonisin content of corn, *Adv. Exp. Med. Biol*. 1996, *392*, 323 – 334.

