Immunobiological Effects of Fumonisin B1 in Experimental Subchronic Mycotoxicoses in Rats

M. G. Theumer,1 A. G. López,2 D. T. Masih,1 S. N. Chulze,3 and H. R. Rubinstein1*

Micológía, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba,1 Instituto de Ciencia y Tecnología de los Alimentos, ICTA, Facultad de Ciencias Exactas Físicas y Naturales, Universidad Nacional de Córdoba, Córdoba,2 and Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto, Río Cuarto,3 Argentina

Received 20 March 2001/Returned for modification 8 June 2001/Accepted 13 September 2001

Fumonisins are produced by toxicogenic strains of the genus Fusarium and are synthesized mainly in media where there are nitrogen-concentration conditions (37). These mycotoxins have a chemical structure similar to that of ceramides, and it has been shown that they interfere in the lipid metabolism of the cell (30, 40). After isolation and characterization of fumonisin B1 (FB1) and FB2 from cultures of Fusarium verticillioides (Gibberella fujikuroi mating population A), is a potent toxin that can be found in fungus-contaminated corn and corn-based food products. We have investigated the immunobiological effects of subchronic dietary exposure to FB1 in male Wistar rats. Animals were fed with diets containing 0 (control) or 100 ppm of FB1 for 12 weeks. The total FB1 intake on day 90 was 810 mg/kg of body weight. Food consumption, body weight, and body weight gain on day 90 were reduced in animals exposed to FB1. Histopathologic changes consisted of histiocytic perivascular infiltrate and an increased number of Kupffer cells in the liver, necrosis and apoptosis of tubular epithelial cells in the kidney, and increased mitotic figures and lymphocytic infiltrate in the small intestine. Serum enzyme alkaline phosphatase was significantly elevated in rats fed FB1, while triglyceride levels decreased compared to controls. Treatment with FB1 in vivo or in vitro did not have a significant effect on mitogen-induced proliferation of spleen mononuclear cells. However, increased levels of interleukin-4 (IL-4) and decreased levels of IL-10 were released by these cells in culture compared to controls. FB1 in vivo or in vitro decreased the hydrogen peroxide (H2O2) released by peritoneal macrophages, while no changes in levels of superoxide anion produced by total peritoneal cells were detected. The results from the present work demonstrate that subchronic FB1 intake could affect the small intestine and alter the interleukin profile and some main functions of macrophages in antitumor activity.

* Corresponding author. Mailing address: Micología, Departamento de Bioquímica Clínica, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Ciudad Universitaria, Córdoba, Argentina. Phone: 054-351-4334164. Fax: 054-351-4334187. E-mail: hectorrr @bioclin.fcq.unc.edu.ar.
induce NO₂ production by rat splenic macrophages and to stimulate T-cell proliferation (11).

Other mycotoxins produced by Fusarium, such as vomitoxin (deoxynivalenol), are able to override interleukin secretion in CD4⁺ cell cultures, at the same time and in addition to the cell proliferation inhibition (1). Vomitoxin in an experimental macrophage model in vitro also appears to interfere with the associated functions of activated macrophages regulating H₂O₂ production, depending on the dosage used (20).

Furthermore, a series of microscopic alterations in target organs, such as liver and kidney, has been described. It has been observed that F344 female and male rats that consumed between 0 and 484 ppm of FB₁ for 28 days showed apoptosis in liver hyperplasia of the bile ducts and apoptosis of tubular epithelial cells of the kidney (38). According to Bondy et al. (4), the kidney was one of the organs most affected by fumonisin toxicity in male Sprague-Dawley rats, in which they were able to observe necrosis of tubular epithelial cells of the inner cortex, cytoplasmatic basophilia, and atrophy of tubular epithelial cells.

Diet in animals and humans can be contaminated with low levels of fumonisins, producing chronic mycotoxicoses that can alter immunologic mechanisms. The experimental models used to date to study mycotoxin effects on laboratory animals are based mainly on the production of acute mycotoxicoses; however, the alterations at the immunologic level have been studied in only a few cases.

The main objective of this study was to evaluate the immunologic effects caused in rats by an FB₁ administration similar to that occurring in nature.

MATERIALS AND METHODS

Animals. Male Wistar inbred rats, 6 to 8 weeks old, were housed in age-matched pairs in stainless-steel cages. The cages were kept in environmentally controlled rooms with a 12-h light-dark cycle. Animals were housed and cared for in the animal resource facilities of the Department of Clinical Biochemistry, Faculty of Chemical Sciences, National University of Córdoba, in accordance with institutional guidelines.

Preparation of fumonisin extracts. FB₁ was produced using maize as a substrate in a shake-flask system (5). A culture of F. verticillioides (4), the kidney was one of the organs most affected by fumonisin consumption. The food consumption per day was calculated from the difference between the weights of the portions given and uneaten. The body weight was determined on the 30th, 60th, and 90th days of feeding as the weight difference in comparison to the weight in the previous month. The total fumonisin consumption on days 30, 60, and 90 for the group given FB₁ was calculated by taking into account the food consumption and the toxin concentration in the food. The results are expressed in relation to body weight.

Examination of tissues. Specimens of lungs, spleen, liver, kidney, and small intestine were obtained on the 90th day of feeding. For examination by light microscopy, tissues were fixed in 10% neutral buffered formalin (pH 7.2). Paraffin sections (4 μm) of tissues were stained with hematoxylin and eosin. Photoradiographs were taken with a Zeiss Axioptot microscope attached to a Zeiss Axioscop 2, equipped with a Zeiss MC2 digital camera (Zeiss, Jena, Germany) and analyzed using a computer software package (Image J, National Institutes of Health, Bethesda, Md.).

SMCs. Spleen mononuclear cell (SMC) suspensions were prepared by the method described by Kisaki et al. (23). Briefly, spleens were removed aseptically from miniced, and minced through stainless-steel mesh to obtain single-cell suspensions. The cells were washed with RPMI 1640 medium (Sigma) and resuspended in sterile RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Gibco), gentamicin (50 μg/ml), and β-mercaptoethanol (5 × 10⁻⁵ M). Splenic cell suspensions were prepared aseptically and adjusted to 10⁶ cells/ml.

Peritoneal cells. Peritoneal cells were obtained by sterile washing with Krebs-Ringer phosphate dextrose buffer (pH 7.0) containing gentamicin (30 mg/liter) and heparin (20 U/ml). Cells were washed twice, resuspended in culture medium, counted, and diluted. Resident cells were obtained from rats fed with control diet and from rats fed the FB₁ diet.

Mitogenic responses of SMCs. Cell suspensions (50 μl; 6 × 10⁵ cells/ml; 3 × 10⁵ cells) were dispensed into each well of 96-well culture plates containing 100 μl of medium culture medium (RPMI 1640). Concanavalin A (Con A) (type IV; Sigma) and lipopolysaccharide (LPS) (055:B5; Sigma) were added at optimal final concentrations of 10 and 40 μg/ml, respectively. The viability of cells was assessed by the trypan blue (0.1%) exclusion test, for in vitro assays, FB₁ was added at an optimal final concentration of 10 μM. The cultures were incubated with the mitogens at 37°C in an atmosphere containing 5% CO₂ and were labeled during the last 18 h of 96-h cultures with a 1 μCi of [H]thymidine (Comisión Nacional de Energía Atómica). These cells were harvested 18 h thereafter on a glass fiber filter using an automated cell harvester (Skatron; Molecular Devices, Sunnyvale, Calif.). Incorporation of tritiated thymidine into cell DNA was measured in triplicate using a beta liquid scintillation counter.

Cytokine measurement. SMC suspensions were collected in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (Gibco), gentamicin (50 μg/ml), β-mercaptoethanol (5 × 10⁻⁵ M), and Con A (type IV; Sigma), at an optimal final concentration of 10 μg/ml. Supernatants from cultures were collected after 24 h for determination of interleukin-2 (IL-2) and after 72 h for determination of IL-4 and IL-10 and were frozen at −70°C until analyzed.

FB₁ quantification. Samples (100 μl) obtained from the extracts were diluted with acetonitrile (100 μl). Before the quantification assays, the samples were diluted 1/50 with acetonitrile-water (1:1). The quantification of the diluted extracts was performed by the methodology proposed by Shephard et al. (36). Briefly, an aliquot (50 μl) of this solution was derivatized with 200 μl of o-phthalaldehyde. This solution was obtained by adding 5 ml of 0.1 M sodium tetraborate and 50 μl of 2-mercaptoethanol to 1 ml of methanol containing 40 mg of o-phthalaldehyde. The derivatized samples were analyzed with a high-pressure liquid chromatograph (Hewlett-Packard) equipped with a fluorescence detector. The wavelengths used for excitation and emission were 335 and 440 nm, respectively. An analytical reverse-phase C₁₈ column (150 by 4.6 mm [internal diameter]; 5-μm particle size) was connected to a C₁₈ precolumn (20 by 4.6 mm; 5-μm particle size) with a guard column (20 by 4.6 mm; 5-μm particle size). The mobile phase was methanol-0.1 M NaH₂PO₄ (75:25), the pH was set at 3.35 ± 0.2 with ortho-phosphoric acid, and a flow rate of 1.5 ml/min was used. The quantification of FB₁ was carried out by comparing the peak areas obtained for rats fed FB₁ with those corresponding to standards of 10.5, 5.25, and 2.625 μg of FB₁ per ml (Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg, Republic of South Africa).

Diet. (i) Control diet. The control diet was prepared by adding 435 ml of aqueous extract of maize without inoculation of F. verticillioides to a solution of agar (Difco) (15 g) in 453 ml of distilled water. This mixture was warmed until the agar mixture was dissolved and then cooled to 50°C. Next, 1,000 g of a balanced rat-mouse food (Cargill S.A. C.I. Saladillo, Buenos Aires, Argentina), finely ground and free of mycotoxins, was continuously shaken until a homogeneous mixture was obtained. Pieces of approximately 20 g each were molded, and after solidification they were stored at −18°C until they were used. The final concentration of FB₁ in the food was <0.3 ppm.

(ii) Diet with FB₁. The diet with FB₁ was prepared as the control diet, was, using fumonisin extract as described above. The final FB₁ concentration in the food was 100 ppm.

Experimental model. Two groups of rats were used. One (control) (n = 6) was fed a control diet, and the other (n = 6) was fed a diet with FB₁. Animals were housed in pairs in different cages and fed for 90 days. The food ration was replaced daily, and the weights of food portions given and left uneaten after 24 h were determined. Animals were weighed on the 30th, 60th, and 90th days of being fed. After this period, blood samples were obtained by intracardiac puncture and the animals were killed by cervical dislocation.

Determination of food consumption, body weight, body weight gain, and fumonisin consumption. The food consumption per day was calculated from the difference between the weights of the portions given and uneaten. The body weight was determined on a scale (Ohaus, Florham Park, N.J.) with a precision of 0.05 g. The body weight gain of each animal was determined on the 30th, 60th, and 90th days of feeding as the weight difference in comparison to the weight in the previous month. The total fumonisin consumption on days 30, 60, and 90 for the group given FB₁ was calculated by taking into account the food consumption and the toxin concentration in the food. The results are expressed in relation to body weight.

Examination of tissues. Specimens of lungs, spleen, liver, kidney, and small intestine were obtained on the 90th day of feeding. For examination by light microscopy, tissues were fixed in 10% neutral buffered formalin (pH 7.2). Paraffin sections (4 μm) of tissues were stained with hematoxylin and eosin. Photoradiographs were taken with a Zeiss Axioptot microscope attached to a Zeiss Axioscop 2, equipped with a Zeiss MC2 digital camera (Zeiss, Jena, Germany) and analyzed using a computer software package (Image J, National Institutes of Health, Bethesda, Md.).
Interleukins were measured using an sandwich enzyme-linked immunosorbent assay protocol (35). Briefly, a purified fraction of anti-IL-2, anti-IL-4, and anti-IL-10 antiserum (PharMingen) was used as capture antibody in conjunction with the biotinylated anti-IL-2, IL-4, and IL-10 monoclonal antibody. Dilutions of recombinant rat IL-2, IL-4, and IL-10 were used as standards. After being washed four times with phosphate-buffered saline—Tween 20, the plates were reacted with horseradish peroxidase-streptavidin (Sigma) and o-phenylenediamine was added. After 5 to 20 min, the reaction was stopped with 25 μl of sulfuric acid (1:9, vol/vol). The reactions were read in a microplate reader (Bio-Rad), and results are expressed as nanograms per milliliter.

Detection of H$_2$O$_2$ released by adherent cells. The phenol red oxidation microassay was used. Briefly, cells (8 × 10^5/ml) were placed in 96-well plates and left to stand for 2 h at 37°C in 5% CO$_2$. The medium was then repleted with 250 μl of PRS buffer [NaCl (140 mM), dextrose (5.5 mM), phenol red (200 μM), and peroxidase [Sigma] [EC 1.11.1.7] (8.5 U/ml) in phosphate-buffered saline, pH 7.0). For the in vitro assays, FB1 was added at an optimal final concentration of 10 μM (7.2 μg/ml). Wells were treated with phorbol 12-myristate 13-acetate (PMA) (100 ng/ml) and incubated for 45 min at 37°C in 5% CO$_2$. The reaction was stopped with 10 μl of 1 N NaOH, and the reactive wells were read in a microplate reader (Bio-Rad) with a 595-nm filter. Results are expressed as nanomoles of H$_2$O$_2$ released by 10$^6$ cells in 30 min.

Detection of O$_2^-$ released by resident peritoneal cells. Superoxide anion was quantitatively determined by nitroblue tetrazolium reduction. Peritoneal cells (4 × 10$^6$) were incubated in the dark for 30 min at 37°C with 5% CO$_2$ in the presence of nitroblue tetrazolium (0.1%) with or without PMA at an optimal final concentration of 100 μM. The reaction was stopped with 0.4 ml of 0.1 N HCl. Cells were centrifuged, and insoluble formazan was extracted twice with 1 ml of 1,4-dioxane. Optical densities in supernatants were determined at 560 nm, and results are expressed as the percentage of optical densities developed in control tubes.

Statistical evaluation. Data from these studies were analyzed by one-way analysis of variance. Results giving P values of ≤0.05 were considered significantly different.

RESULTS

Food consumption, body weight, body weight gain, and fumonisin consumption. Daily observations for 90 days did not indicate detectable alterations in the general state of any of the animals. The average food consumptions until the 30th, 60th, and 90th days were 36.8, 40.2, and 41.5 g, respectively, for control group animals, while the average consumptions for rats fed FB1 at 17 ppm of FB3 for a 3-week period (43). In contrast to what happened in rats, the food consumption was significantly higher (17.4%), in weight and in weight gain in the animals; however, in comparison with controls. On the other hand, in liver samples of FB1-fed rats, a perivascular histiocytic infiltrate (Fig. 1a) and an increased number of Kupffer cells and changes in the normal structure (Fig. 1c) in comparison with control animals (Fig. 1b and d, respectively) were detected. In the kidneys of rats fed FB1, apoptotic bodies and necrotic alterations in tubular epithelial cells (Fig. 1e), an increase in the capsular space (Fig. 1f), and the presence of proteinogenous material in the tubular lumen (lights) were observed. These kidney alterations were not detected in control rats. In addition, the microscopic examination showed an increase in the average number of mitotic cells in the base of the crypt (Fig. 2a) and a major lymphocytic infiltrate (Fig. 2b) in the small intestine in rats fed FB1.

Serum biochemical measurements. The data obtained from the biochemical profile are shown in Table 1. In the sera of animals fed FB1, an increase of ALP activity (P ≤ 0.001) and a decrease of TG levels (P ≤ 0.05) in comparison with control rats were observed. No significant changes in Chol or Ca levels or in ALT, AST, and GGT activities were observed.

Mitogenic responses of SMCs. To examine the effects produced in the immunologic system by the subchronic FB1 intoxication, SMCs from control rats and from those fed FB1 in the basal state and in the presence of ConA and LPS were cultured. No significant differences were observed in [H]thymidine uptake by SMCs in the basal state or in the presence of ConA or LPS between 72 and 96 h of culture. In in vitro assays, there were no important changes in the [H]thymidine uptake when SMCs of normal rats were cultured with FB1 at 10 μM (7.21 μg/ml) in comparison to the basal proliferation or when they were cultured with FB1 at 10 μM (7.21 μg/ml) plus ConA at 10 μg/ml, in contrast to SMCs cultured with ConA at 10 μg/ml (data not shown).

Cytokine measurement. After 72 h of culture, supernatants of SMCs from animals fed FB1 had significantly higher concentrations of IL-4 (P ≤ 0.01) and lower concentrations of IL-10 (P ≤ 0.01) than controls (Fig. 3). There were no alterations in IL-2 levels produced by cells from FB1-fed rats in comparison with controls (data not shown). In in vitro assays, there were no changes in the levels of IL-2, IL-4, and IL-10 produced by SMCs of normal rats in the presence of FB1 (10 μM) with respect to controls.

H$_2$O$_2$ and O$_2^-$ released by resident peritoneal cells. The levels of H$_2$O$_2$, produced by adherent peritoneal cells and the levels of anion superoxide produced by total peritoneal cells in the basal state and in the presence of PMA were quantified. The levels of H$_2$O$_2$ found are shown in Fig. 4. The peritoneal cells of animals fed FB1 produced significantly lower levels of H$_2$O$_2$ (P ≤ 0.01) than controls stimulated with PMA, while there were no differences in H$_2$O$_2$ levels produced in the basal state (Fig. 4A). In in vitro assays, adherent peritoneal cells from normal animals produced significantly lower concentrations of H$_2$O$_2$ (P ≤ 0.01) in the presence of FB1 (10 μM) and when stimulated by PMA than controls (Fig. 4B). There were no changes in anion superoxide production in the basal state or in the presence of PMA (data not shown).

DISCUSSION

In this study we have detected immunobiological alterations produced by ingestion of FB1 in a model of experimental subchronic mycotoxicosis in rats. In this model, the total ingestion was 303 mg of FB1 during 90 days, producing significant decreases in food consumption (17.4%), body weight (17.9%), and body weight gain (125.7%). Under similar experimental conditions in a murine model, our group observed that with a total ingestion of 7.37 mg of FB1 there were also losses in weight and in weight gain in the animals; however, in contrast to what happened in rats, the food consumption was higher (5). On the other hand, Voss et al. have reported a decrease in food consumption, body weight, and body weight gain in a model of rats that consumed 228 ppm of FB1 plus 58 ppm of FB2 plus 17 ppm of FB3 for a 3-week period (43). These observations suggest that FB1 can modify these param-
FIG. 1. Hematoxylin-and-eosin-stained sections of rat organs exposed to a diet containing 100 ppm of FB1 or a diet without FB1 (control) for 90 days. In the livers of animals fed FB1, a perivascular histiocytic infiltrate (a) with respect to control rats (b) and an increased number of Kupffer cells (c) compared to those of control rats (d) were the main alterations found. Histological findings in the kidney included apoptosis (arrows) and necrosis (arrowheads) of tubular epithelial cells (e), and increased capsular space (f) was also found. Magnifications, ×200 (a and b), ×100 (c, d, and f), and ×400 (e).
eters in different ways, depending on the animal model and the experimental scheme used.

In histopathologic examination alterations similar to those described by other authors were found, indicating that the liver and kidney are the principal target organs for FB1 action in rats. Furthermore, the lymphocytic infiltrate and increased average number of mitotic cells found by the crypt base in the intestine were present in all samples of animals fed FB1. Despite the fact that the small intestine is not one of the organs most affected by this mycotoxin, it is exposed to the same FB1 concentrations via oral administration. FB1 is able to have toxic activity on the intestinal cell by interference in the lipid metabolism (13), causing alteration of the cellular cycle (29) and increasing the cell number in different phases of mitosis (Fig. 2a). These findings would be related to a major susceptibility to infections by pathogens that enter via the oral route (39).

The level of serum TGs is influenced by fats introduced in the diet and endogenous synthesis in the liver and intestine (2). In this work, a decrease in the TG concentration in animals fed FB1 was observed (Table 1). Bondy et al. (4) have reported a similar finding in a study of acute toxicity in rats in which the food consumption and therefore the fats in the diet were diminished for only a short time. On the other hand, Enongene et al. (13) have reported alterations in lipid metabolism in the epithelial cells of the small intestine and hepatocytes. These results indicate that FB1 could be a cause of the decreased levels of TGs in serum in our model, interfering in the biosynthesis of endogenous TGs.

Among the parameters studied in the biochemical profile, an increase of ALP activity was found. Although the liver is the major source of this enzyme, in some cases in which the intestinal metabolism is stimulated, the intestinal isoenzyme could represent an important factor (21). A similar effect is obtained due to cellular alterations in the proximal convoluted tubules of the kidney, which may contribute to the total serum ALP activity (21). These results are related to the histopathologic findings in the kidney (Fig. 1e).

The failure to observe changes in SMC proliferation in animals fed FB1 (in the basal state or stimulated), as well as in the proliferation of normal SMCs exposed in vitro to FB1, is due to the mycotoxin concentration used. These results are related to the observations of Tryphonas et al. (39) that the daily ingestion of 25 mg of FB1/kg of body weight/day for 14 days did not produce changes in the proliferative response of rat lymphocytes. Charoenpornsook et al. (6), using bovine peripheral blood mononuclear cells, have observed a 50% decrease of proliferation in the presence of ConA when the cells

![FIG. 2. Hematoxylin-and-eosin-stained sections of rat organs exposed to a diet containing 100 ppm of FB1 for 90 days. In the small intestine, an increased number of mitotic cells (arrows) (a) and lymphocytic infiltrate (b) were present. Magnification, ×400 (a) and ×100 (b).](image)

**TABLE 1. Serum parameters for rats (n = 6) on day 90**

| Rats   | Mean (SEM) level                  |
|--------|----------------------------------|
|        | Chol (mg/dl) | TGs (mg/dl) | Ca (mg/dl) | AST (kat/liter) | ALT (kat/liter) | GGT (kat/liter) | ALP (kat/liter) |
| Control | 73.50 (3.22) | 157.50 (15.82) | 11.37 (0.13) | 2.10 (0.19) | 0.65 (0.02) | 0.09 (0.02) | 2.64 (0.13) |
| FB1     | 71.67 (6.44) | 103.00 (15.62) | 11.37 (0.08) | 2.33 (0.19) | 0.92 (0.11) | 0.09 (0.01) | 4.53 (0.53) |

*One katal catalyzes 1 mol of product/s under defined conditions.*

*Rats fed control diet for 90 days.*

*Rats fed diet with FB1 for 90 days.*

\(P \leq 0.05.\)

\(P \leq 0.001.\)
were exposed to 35 μg of FB1 per ml. Taking into account the pharmacokinetic data reported by Martinez-Larranaga et al. (27), in our experimental model the major FB1 concentrations that could arise in blood would be 5 to 10 μg/ml. Even if there are differences among species, higher FB1 concentrations in rats than the one used in this work (7.21 μg/ml) would be needed to produce alterations in the normal blastomytogenic response of lymphocytes.

Little is known about the function of interleukins in a mycotoxicosis produced by fumonisins. In our work, higher concentrations of IL-4 and lower concentrations of IL-10 in supernatants of SMCs in rats fed with FB1 were found with respect to controls (Fig. 3). This increase of IL-4 could be stimulated by the presence of FB1 and/or the accumulation of sphingoid bases (sphingosine) in the intracellular space by means of an unknown mechanism (28). Therefore, the ingestion of FB1 during a subchronic period could produce a break in the balance of Th1 and Th2 subsets. In models of chronic FB1 intoxication, the main expression of some interleukins could be related to the evasion of tumor cells from immunologic surveillance (24). Furthermore, it was determined that among the functions of IL-10, this cytokine could act as a costimulator for the growth of mature thymocytes. It also functions as a cytotoxic-T-cell differentiation factor, promoting a higher number of IL-2-activated cytotoxic-T-lymphocyte precursors to proliferate and differentiate into cytotoxic effector cells (7). It has also been suggested that IL-10 is an essential immunoregulator of the intestinal tract and that the generalized bowel inflammation in IL-10-deficient animals is due to uncontrolled immune responses stimulated by enteric antigens (23). The decrease of IL-10 found in animals fed FB1 could contribute to the alterations observed in the small intestine. On the other hand, the absence of modifications in the IL-2 levels in these animals in comparison with controls would be related to the results obtained on the proliferation of SMCs in the presence of ConA.

The presence of some Th2 profile cytokines could have also been modulating the macrophage function (32). The hydrogen peroxide and anion superoxide produced by these cells have an important role in the host defense against tumors and microorganisms. In our experimental model, peritoneal macrophages exposed in vivo and in vitro to FB1 produced less hydrogen peroxide (Fig. 4); however, alterations in the production of anion superoxide were not found. These results suggest that FB1 can have immunosuppressive effects on some...

---

**Fig. 3.** Interleukins released by SMCs obtained from rats fed a control diet or a diet with FB1 (Problem). Cytokine levels in supernatants of cells cultured for 72 h after recovery from spleens were determined by enzyme-linked immunosorbent assay. Error bars indicate standard errors. *, P ≤ 0.01.

**Fig. 4.** H₂O₂ released by adherent peritoneal cells. Macrophages were incubated with or without PMA as a stimulant. Results are expressed as mean (standard error) nanomoles of H₂O₂ released by 10⁶ cells in 30 min. (A) In vivo exposure to FB1 (n = 6 rats). Control, rats fed control diet for 90 days; Problem, rats fed diet with FB1 for 90 days. (B) In vitro exposure to FB1. For in vitro assays a pool of peritoneal cells from four normal rats was used. Cells were incubated with (Problem) or without (Control) 10 μM FB1. *, P ≤ 0.05; **, P ≤ 0.01.
of the macrophage immunologic mechanisms, diminishing their cytotoxic capacity, which would be related to a lower antitumor activity.

The results obtained in this work indicated that FBS has the liver and kidney as principal target organs for subchronic toxicity in rats. Further, in this model the small intestine is clearly affected. With the doses used, FBS is able to produce a modification of the excretion of interleukins, acting on macrophage function. A more extensive study on the accumulation of sphingoid bases in the immune cell system and its functionality would be able to clarify the mechanisms acting in the pathogenesis of this intoxication.

ACKNOWLEDGMENT

This work was supported by Agencia Nacional de Ciencia y Tecnología grant FONCYT-PICT 09-03688.

REFERENCES

1. Azcona-Olivera, J. I., L. Y. Louyang, R. E. Warner, J. E. Linz, and J. J. Pestka. 1995. Effects of vomitoxin (deoxynivalenol) and cycloheximide on IL-2, 4, 5, 6 secretion and RNA levels in murine CD4+ cells. Food Chem. Toxicol. 33:443–447.
2. Bachrach, P. S., R. E. Levy, and B. M. Rifkind. 1993. Lípidos y dislipopro- teínemias, p. 195–221. In J. B. Henry (ed.), Diagnóstico y tratamiento clínicos por el laboratorio. 19th ed. W. B. Saunders Co., Philadelphia, Pa.
3. Blackwell, B. A., O. E. Edwards, A. Frucher, J. W. ApSimon, and J. D. Miller. 1996. NMR structural studies of fumonisin B1 and related compounds from Fusarium moniliforme. Adv. Exp. Med. Biol. 392:75–91.
4. Bondy, G. M., B. Mueller, R. S. Fernie, J. D. Miller, C. Armstrong, S. L. Hengstler, J. G., B. Van de Burg, P. Steinberg, and F. Oesch. 1999. Inter- actions of sphingolipid biosynthesis on T lymphocyte activation. Biochemistry (Moscow). 63:102–110.
5. Merrill, A. H., E. M. Schmelz, D. L. Dillehay, S. Spiegel, J. A. Shyamian, J. J. Schroeder, R. T. Riley, K. A. Voss, and E. Wang. 1997. Sphingolipids—the enzymatic lipid class biosynthesis, physiology, and pathophysiology. Toxicol. Appl. Pharmacol. 142:208–225.
6. Merli, A. H., E. Wang, T. R. Vales, E. R. Smith, J. J. Schroeder, D. S. Menalldino, C. Alexander, H. M. Crane, J. Xia, D. C. Lattisa, F. I. Meredith, and R. T. Riley. 1996. Fumonisin toxicity and sphingolipid biosynthesis. Adv. Exp. Med. Biol. 392:297–306.
7. Moon, E. Y., D. K. Rhee, and S. Pyo. 2009. Inhibition of various functions in murine peritoneal macrophages by aflatoxin B1 exposure in vivo. Int. J. Immu- nopharmacol. 21:57–65.
8. Moustafa, M., Jr., and L. M. Soares. 2000. Fumonisins B1 and B2 in Brazilian corn-based food products. Food Addit. Contam. 17:875–879.
9. Marasas, W. F. C., K. Jaskiewicz, F. S. Senter, and D. J. Schalkwyk. 1988. Fusarium moniliforme contamination of maize in oesophageal cancer areas of south Africa. S. Afr. Med. J. 71:28–53.