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Effects of mycotoxins on human immune functions in vitro

L. Berek [a,*], I.B. Petri [a], Á. Mesterházy [b], Joseph Téren [c], Joseph Molnár [d]

[a] *Blood Transfusion Center, Albert Szent-Gyorgyi Medical University, Pecsi u. 4/B, H-6720 Szeged, Hungary*
[b] *Cereal Research Non-Profit Company, Also-Kikoto sor 9, H-6726, Szeged, Hungary*
[c] *Animal Health and Food Control Station, Becsi krt. 5, H-6722 Szeged, Hungary*
[d] *Faculty of Medicine, Institute of Microbiology, Dom ter 10-11, H6720, Szeged, Hungary*

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Abstract

Immunosuppressive and carcinogenic *Fusarium* mycotoxins may appear in domestic food products. Therefore, the immunological effects of *Fusarium* mycotoxins were tested on human peripheral blood mononuclear cells from different blood donors. In the present study we investigated deoxynivalenol (DON), 3-acetyldeoxynivalenol, fusarenon-X, T-2 toxin, zearalenone, α-zearalenol, β-zearalenol and nivalenol for their effects on T and B cells in a proliferation assay, antibody-dependent cellular cytotoxicity (ADCC) and natural killer (NK) cell activity on human peripheral blood mononuclear cells. The concentrations applied in our experiments were similar to those which can be found in normal human peripheral blood system (0.2–1800 ng/ml). Among the eight mycotoxins tested, T-2 toxin, fusarenon X, nivalenol and deoxynivalenol exerted the highest immunosuppressing effect on human peripheral blood mononuclear cells in vitro. Mycotoxin-induced immunosupression was manifested as depressed T or B lymphocyte activity. Furthermore, by virtue of inhibition of NK cell activity, the protection against tumor development may also be attenuated.

Keywords: *Fusarium* mycotoxins; Immunomodulating effect; Blast transformation; ADCC reaction; NK cell activity

1. Introduction

Fusarium mycotoxins are the major group of secondary metabolites, produced by several species including *Fusarium graminearum culmorum* and *F. sporotrichioides*. *Fusarium* mycotoxins occur worldwide on corn, wheat and other cereal grains intended for human and animal consumptions (Bullerman, 1996). Some of them have been proven to exhibit nephrotoxic, immunosuppressive, teratogenic and carcinogenic properties in animals (Corrier, 1991). Although the molecular basis for many of the specific immunosuppressive effects of mycotoxins are presently unclear, inhibition of DNA, RNA and protein synthesis via a variety of different mechanisms appears to be directly or indirectly responsible for the immunosuppressive action of many mycotoxins. The immunotoxic effect of the majority of *Fusarium* toxins has been demonstrated on animals; however, there is not enough information in the case of humans. This lack of information and the reported severe cases of human toxicosis (Ehling et al., 1997) prompted us to investigate how these toxins influence the human immune system. The purpose of the present investigation was to study the immunomodulating effects of eight *Fusarium* mycotoxins, especially the most frequently found toxins, deoxynivalenol, T-2 toxin and nivalenol, on human peripheral blood mononuclear cells. The criteria we used were lymphocyte blast transformation activity, antibody-dependent cellular cytotoxicity (ADCC), and in particular natural killer (NK) cell activity.

2. Material and methods

2.1. Chemicals

The mycotoxins (all obtained from Sigma) were dissolved in Met-OH and applied in final concentrations of

Abbreviations: ADCC, antibody-dependent cellular cytotoxicity; Con A, concanavalin A; DON, deoxynivalenol; FCS, fetal calf serum; NK, natural killer; PBM, peripheral blood mononuclear; PHA, phytohaemaglutinin; RBC, red blood cells;

* Corresponding author. Tel.: +36-6245-5115; fax: +36-6254-4572.

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10, 50, 100, 1000 and 5000 ng/ml, respectively. All other chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany), Pharmacia (Uppsala, Sweden), Sigma-Aldrich Kft. Budapest, Hungary).

2.2. Isolation of mononuclear cells

Human peripheral blood mononuclear (PBM) cells were isolated from healthy blood donors by Ficoll-Uromiro gradient centrifugation. The cells were washed and resuspended in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/ml penicillin, 100 μg/ml streptomycin and 2 mM L-glutamine. To obtain monocyte-free (PBM-Mo^-) samples the freshly drawn heparinized blood samples were treated with carbonyl iron powder at 37°C for 5 min, prior to the isolation of lymphocytes by Ficoll-Uromiro gradient centrifugation.

Viability of the cells in the presence of mycotoxins were determined by trypan blue exclusion after 4 h of incubation.

2.3. Lymphocyte blast transformation (T lymphocyte proliferation assays)

Mononuclear cells were routinely cultured in flat-bottomed Greiner microtiter plates at 2×10^5 cells (0.2 ml/well). The triplicate cultures containing the mycotoxins were incubated in the presence or absence of mitogens: PHA 1:200 diluted stock solution, Con A 5 μg/ml, respectively, in a CO_2 incubator for 72 h. For the determination of lymphocyte DNA synthesis, 0.5 μCi [^{3}H]thymidine was added to all cultures for the last 5 h and the cells were collected on GFC filter paper. The amount of radioactivity incorporated was determined in a liquid scintillation counter. The results are expressed as the difference in cpm between the incorporated activity of transformed cells and control cells (without mitogens) (Petri et al., 1995).

2.4. Antibody-dependent cellular cytotoxicity (ADCC) test

A triply washed suspension of freshly taken Rh (D, C)-positive O red blood cells (RBC) was used as a target cells. ^{51}Cr labelling was performed in the presence of Na-citrate, by the addition of 100 μCi $Na_2^{51}CrO_4$ (Amersham; sp. act. 7–8 GBq/mg Cr) for 60 min at 37°C. After labelling, the cells were washed three times, and the cell count was adjusted to 10^6/ml. The effector lymphocytes were isolated on a Ficoll-Uromiro powder, and the cells were suspended in RPMI containing 10% FCS. The tests were performed in round-bottomed microtitre plates (Greiner) according to Urbaniek (1976). Each well contained a volume of 200 μl, consisting of 50 μl labeled RBC and effector cell suspensions (from 10^7/lymphocytes) and antiserum. Anti-Rh(D) antisera were added to give a final dilution of 1:20. Human O Rh-positive RBC were used as a target and PBM Mo^- cells as an effector in a 1:10 ratio. The reaction was mediated by RBC-specific anti-D antibodies. The cultures were incubated at 37°C for 16 h and the amount of released ^{51}Cr in each supernatant was determined using a gamma counter. From the average of triplicate release values, the percentage of cytotoxicity was calculated according to the following formula (Petri et al., 1996):

Spontaneous release indicated cultures without anti-D antibody.

$$\text{ADCC activity (\%)} = 100 \times \frac{\text{test}^{51}\text{-Cr release} - \text{spontaneous } ^{51}\text{Cr release}}{\text{maximum } ^{51}\text{-Cr release} - \text{spontaneous } ^{51}\text{Cr release}}$$

The results are expressed in % of inhibition.

$$\text{\% of inhibition} = 1 - \frac{\text{ADCC reaction with mycotoxin}}{\text{ADCC reaction with control}} \times 100$$

2.5. Natural killer cell activity (NK assay) (Molnár et al., 1993)

^{51}Cr-labelled K562 cells (used as the target), and PBM cells (used as the effector) were mixed in a ratio of 1:50. After incubation at 37°C for 4 h, the amount of radioactivity released into culture supernatants was determined. The results are expressed as percentage of inhibition, as described above.

The in vitro immunological effects of the mycotoxins were tested on PBM cells of three different blood donors.

In previous experiments using the ELISA technique (Tápai et al., 1997), we could not detect the presence of mycotoxins in sera of the healthy blood donors.

2.6. Statistical analysis

The mean values obtained on the mononuclear cells of the three different donors, in the case of the three most well-spread mycotoxins are shown together with SD, and the P values were calculated with the Student's t-test.

3. Results

We investigated the effect of mycotoxins at the same concentration as can occur in the normal human peripheral blood (0.1–1800 ng/ml) of donors.

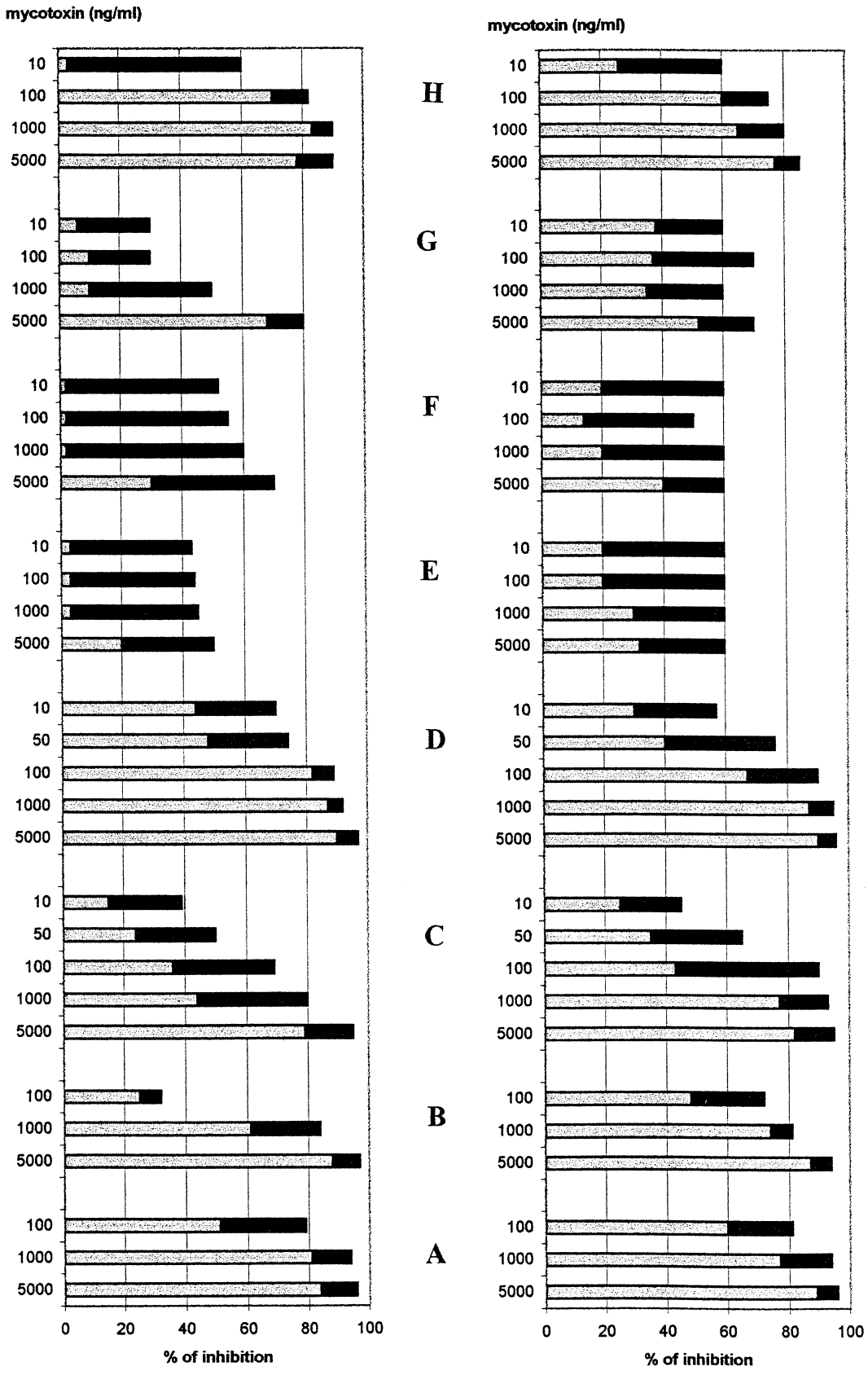

Fig. 1. (A) Deoxynivalenol, (B) 3-acetyldeoxynivalenol, (C) fusarenon X, D: T-2 toxin, (E) zearalenone, (F) α-zearalenol, (G) β-zearalenol, (H) nivalenol. The grey columns illustrate the lowest inhibition values on lymphocytes blast transformation tested in three different donors in the presence of mycotoxins, while the black columns show the highest inhibition values.

Table 1
The most frequently found mycotoxins on mitogen-induced blast transformation in the presence of PHA and ConA[a]

	PHA	ConA
	Mean±S.D.	Mean±S.D.
Control	18 610±7600	12 640±7160
Mycotoxins (ng/ml)		
Deoxynivanelol		
100	5087±2830[b]	3583±1323[b]
1000	1608±1107[b]	2278±1668[c]
5000	1679±939[b]	837±431[b]
T2 toxin		
10	7089±2224[c]	6405±1630[b]
50	5943±1393[b]	4658±2207[b]
100	2280±531[b]	2401±1430[c]
1000	2210±453[b]	948±624[b]
5000	1003±508[b]	857±408[b]
Nivalenol		
10	31 108±31941[c]	2632±759[c]
100	39 962±1191[b]	1415±381[c]
1000	2150±514[b]	1229±336[b]
5000	2509±1072[b]	781±184[b]

[a] The table shows the mean values ±S.D. obtained from three different experiments.
[b] $P < 0.05$.
[c] P = not significant.

Table 2
The most frequently found mycotoxins on ADCC and NK reaction[a]

	ADCC	NK
	Mean±S.D.	Mean±S.D.
Control	35±0	35±5
Mycotoxins (ng/ml)		
Deoxynivanelol		
50	22±5[c]	20±4[c]
100	22±2[c]	15±5[b]
1000	22±3[c]	2±0.75[b]
T2 toxin		
50	29±2[c]	17±4[b]
100	27±2.64[b]	10±1.85[b]
1000	19±4[c]	1.16±0.56[b]
Nivalenol		
10	41±3[c]	4.76±1[c]
50	38±5[c]	4±1.4[c]
100	37±5[c]	4.2±1.6[c]
1000	37±5[c]	2±0.2[b]

[a] The table shows the mean values ±S.D. obtained from three different experiments.
[b] $P < 0.01$.
[c] $P < 0.05$.

The figures describe the smallest and largest values of inhibition exerted by the mycotoxins expressed against percent inhibition in relation to the control culture. T-2 toxin, fusarenon X, 3-acetyldeoxynivalenol and deoxynivalenol inhibited significantly the T and B cell proliferation and this effect was dose dependent. Nivalenol also had a marked inhibitory effect in a high concentration

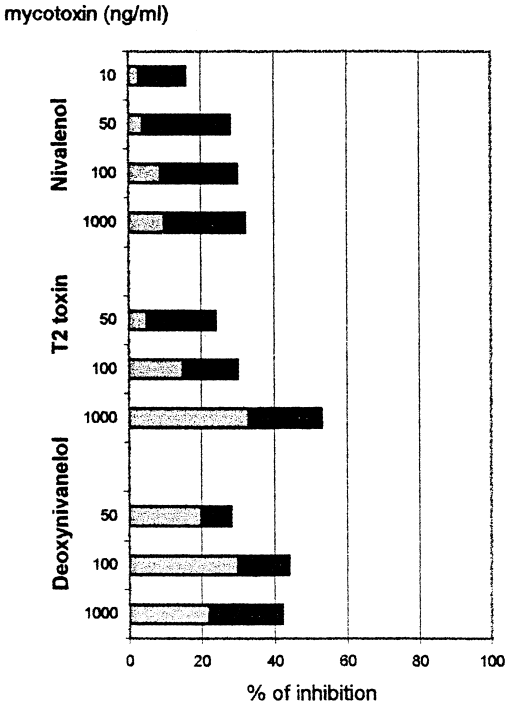

Fig. 2. The grey columns illustrate the lowest inhibition values on lymphocytes blast transformation tested in three different donors in the presence of mycotoxins, while the black columns show the highest inhibition values.

on the leukocyte blast transformation. The other mycotoxins had significantly less influence or none at all (Fig. 1).

Table 1 shows the mean values of inhibition, SD and P values of the Con A- and PHA-induced lymphocyte blast transformations in the case of the most commonly occuring mycotoxins in the diet. The reactions were realised on the mononuclear cells of three differents healthy blood donors.

The effects of mycotoxins on the leucocytes killer activity were tested with the most effective three *Fusarium* toxins: deoxynivalenol, T2 toxin and nivalenol. The inhibitory effects of the toxins on ADCC reaction were also significant, and the effect of T2 toxin was dose dependent (Fig. 2). All the three *Fusarium* toxins demonstrated dose-dependent inhibitory effects on NK cell activity, with significant inhibition at the higher concentrations (Fig. 3).

Table 2 shows the mean values of inhibition, SD and P values in the case of ADCC and NK reactions.

4. Discussion

Consumption of some mycotoxins, at levels that do not cause overt clinical mycotoxicosis, may suppress immune functions and decrease resistance to infectious disease. The sensitivity of the immune system to mycotoxin-induced immunosuppression arises from the

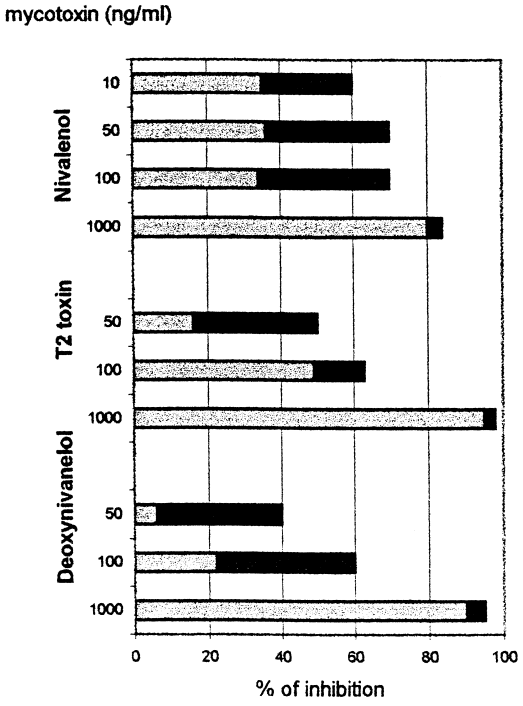

Fig. 3. The grey columns illustrate the lowest inhibition values on lymphocytes blast transformation tested in three different donors in the presence of mycotoxins, while the black columns show the highest inhibition values.

vulnerability of the continually proliferating and differentiating cells that participate in immunomediated activities and regulate the complex communication network between cellular and humoral components (Corrier, 1991).

Previous studies report some immunotoxic effects of T-2 toxin produced mostly by *Fusarium sporotrichioides,* which almost completely suppressed the IL-2 and IL-5 production at 5 ng/ml or more. Thyoma cell line EL4 culture studies indicated that T-2 toxin inhibits cytokine production and all proliferation (Marin et al., 1996). The *Fusarium* toxins also affected the secretion of TNF-α and mitochondrial respiration of bone marrow-derived mononuclear phagocytes; their effects on tumor activity were at best marginal (Keller et al., 1994). Immunotoxicological studies in mice confirm that DON modulates cellular and humoral immune function; it alters mitogen-induced proliferative response of murine lymphocytes (>0.3 mg/kg body weight/day), viability of splenic lymphocytes and host resistance (Bonbi and Pestka, 1991).

It is very important to know about the mycotoxins with carcinogenic effects (Groves et al., 1999) and how they influence immunity in the human system, especially the NK reaction, which is one of the most important immunological functions in tumor surveillance. So far, there is not enough data about the immunosuppressive effects of the *Fusarium* mycotoxins in humans.

In the present study, we investigated the immunosuppressive effect of the eight most important *Fusarium* mycotoxins that may be contaminants of various plant products, including stored cereal grains. The most frequently found toxins in the products were deoxynivalenol, T-2 toxin, nivalenol and zearalenone. We investigated the immunomodulatory effects of the mycotoxins on mononuclear cells of healthy blood donors whose sera were free of mycotoxins. The criteria that we used were the mitogen-induced blast transformation, antibody-dependent cellular cytotoxicity and natural killer cell activity, which are simple and effective in vitro models for evaluating the immunomodulatory capacity of various toxins.

Among the eight mycotoxins we used in our study, T-2 toxin and fusarenon X exerted the most immunosuppressing effect on the tested *in vitro* reactions. The effect showed a dose-dependent response. Nivalenol and deoxynivalenol had a great inhibitory effect on the mitogen induced blast transformation and the NK reaction. Zearalenone, α-zearalenol and β-zearalenol only in a high concentration and a very low scale yielded immunosuppressive effects tested in the blast transformation. The NK reactions play a significant role in immunological defence reactions against malignancy and tumor cells; therefore, we emphasize the inhibitory effect of the mycotoxins on the NK reaction. Thus, we would like to investigate the underlying background differences of the specific NK reactions. Mycotoxin-induced immunosuppression may be manifested as depressed mitogen-induced blast transformation, suppressed NK activity and slightly inhibited antibody-dependent cellular cytotoxic reaction. Our results show the differences between the inhibitory effects of the main *Fusarium* mycotoxins.

Humans consume large amounts of cereals. In epidemic years, the toxin contamination can be very high (Chelkowski, 1998, 1989; Arseniuk and Goal, 1992; Mesterházy, 1997), especially in a population consuming cereal products such as bran-containing bread, breakfast cereals, as well as various rice and wheat products. It is imaginable that many secondary diseases in humans may be connected with the mycotoxin uptake because the mycotoxin levels in grain products are very often many times higher than the value sufficient to inhibit the normal activity of the immune system.

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