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THE IMMUNOSUPPRESSIVE EFFECT OF FUSARIUM MYCOTOXIN AS A FUNCTION OF HLA ANTIGENS

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We examined the blastogenic response to phytohaemaglutinin (PHA) in HLA-B8, DR3 positive and negative subjects in the presence or absence of the immunosuppressive Fusarium mycotoxin. HLA-B8, DR3 haplotype was associated with a depression of the response to mitogen in the absence of the mycotoxin, whereas in the presence of deoxynivabenol we could not detect significant differences among individuals either possessing or lacking this haplotype.

Keywords: deoxynivalenol, immunosuppressive effect, blast transformation, HLA antigens

Introduction

The association between certain human-leukocyte histocompatibility (HLA) antigens and an increased risk of specific diseases has been known for several years [1, 2, 3]. The HLA-B8, DR3 haplotype is over-represented in several autoimmune diseases, implying that genes, which predispose to these disorders are linked to this haplotype. In patients affected by these diseases, as well as in healthy HLA-B8, DR3 individuals, various dysfunctions reflecting an impairment of T-cell activation have been found [4]. One of the most striking immunological abnormalities observed in subjects with this haplotype is an impaired lympho-proliferative response to sub-optimal doses of phytohaemaglutinin (PHA) and concanavalin A (ConA) [5–10].

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This study presents the results of *in vitro* lymphocyte responsiveness to mitogen of individuals possessing HLA-B8 and DR3 antigens in the presence of Fusarium mycotoxin compared with responses in a group of individuals lacking both of these antigens.

Fusarium mycotoxins, which have been proved to exhibit immunosuppressive and carcinogenic properties may appear in domestic food products [11, 12]. Our previous study showed that there are differences in the immunosuppressing effect of the most frequently occurring deoxynivalenol among healthy individuals [13]. Therefore, our further investigations have been focussed to elucidate the genetic effects that may underlie the reactions. The concentrations applied in our experiments were similar to those found in normal human peripheral blood system (100 ng/ml) [13].

The purpose of the present investigation was to study the immunosuppressive effect of deoxynivalenol on the mitogen induced blast transformation as a function of HLA-B8, DR3 haplotype.

Materials and methods

Subjects

Fifteen healthy blood donors were serotyped for HLA specificities. HLA-A, B typing was carried out by the standard NIH microlymphocytotoxicity test [14]. The HLA-DR types were analysed by using the polymerase chain reaction (PCR) amplification method with sequence-specific primers (SSP) based on the procedure of Zetterquist and Olerup [15]. None of these subjects had a history of prolonged disease and none were ill or taking any drug at the time of the study.

Chemicals

The Fusarium mycotoxin, deoxynivalenol (all obtained from Sigma) was dissolved in Met-OH and applied in final concentrations of 100 ng/ml. All other chemicals were of analytical grade and were supplied by Merck (Darmstadt, Germany), Pharmacia (Uppsala, Sweden), Sigma-Aldrich (Budapest, Hungary).

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 2mM L-glutamine prior to the isolation of lymphocytes by Ficoll-Uromiro gradient centrifugation [16].

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

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 deoxynivalenol were incubated in the presence or absence of mitogen: PHA 0.5 μg/ml 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 mitogen) [17].

Statistical analysis

Values given as mean±SD were examined in HLA-B8, DR3-positive and -negative subjects using the Student *t*-test.

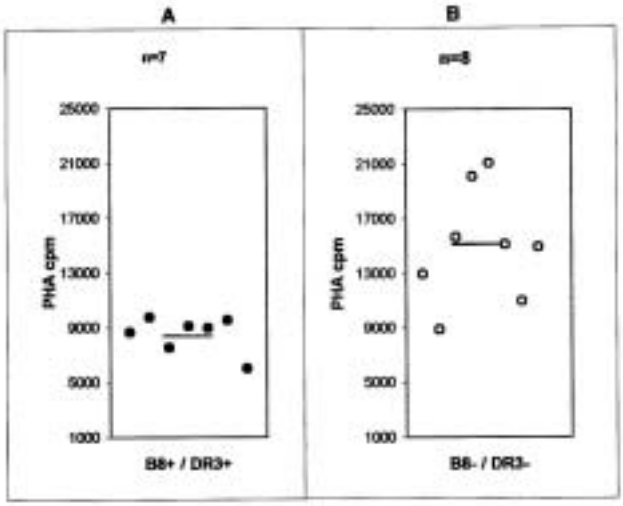
Results

In this study we investigated the immunosuppressive effect of deoxynivalenol which is a secondary metabolite of the Fusarium mycotoxin on mononuclear cells of healthy blood donors typed for HLA antigens.

The lymphocyte blastogenic responses to PHA in the two groups are shown in Figures 1 and 2. The first group consisted of 7 normal subjects with HLA-B8, DR3, whereas the second group consisted of 8 subjects without HLA-B8, DR3. Individuals carrying the HLA-B8, DR3 haplotype (Figure 1/A) had significantly lower lymphocyte blastogenic responses than did the individuals not carrying this haplotype (Figure 1/B). Mean values were 8.518±1.319; and 14.949±4.151 ($p<0.005$), respectively.

In the presence of deoxynivalenol we could not detect a significant difference in response to mitogen in HLA-B8, DR3-positive and -negative individuals (Figure 2/C) as compared to HLA-B8, DR3-negative ones (Figure 2/D). Mean values were 2,912±1,097 *vs.* 3,388±2,550, respectively ($p=0.65579$).

Comparing the mitogen induced blast transformation of HLA-B8, DR3 positive (Figure 1/A and Figure 2/C) and negative groups (Figure 1/B and Figure 2/D), we could detect a significant ($p<0.005$) inhibitory effect of deoxynivalenol.



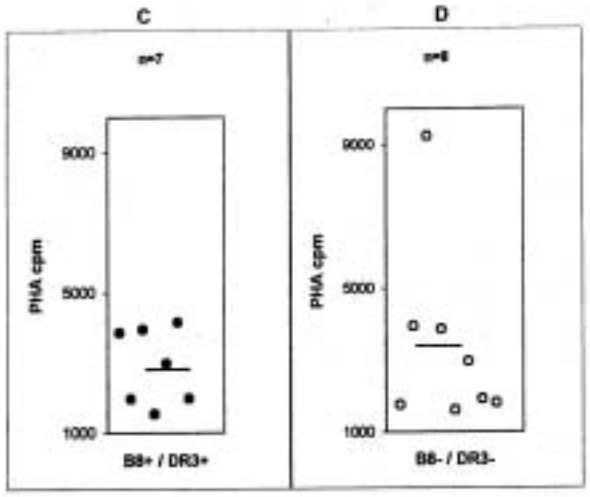
A response to PHA, HLA-B8, DR3 positive subjects; mean=8.518±1.319.
B response to PHA, HLA-B8, DR3 negative subjects; mean=14.949±4.151, $p<0.005$.
Bars indicate means of values.

Fig. 1. Lymphocytes responses of clinically normal subjects with (•) or without (o) HLA-B8, DR3

Otherwise as the previous investigation indicated [13] we could detect the inhibiting effect of deoxynivanelol on mitogen induced blast transformation in the HLA-B8, DR3-positive (A *vs.* C) and negative (B *vs.* D) group as well, $p<0.005$.

Discussion

Several reports have shown that HLA-B8, DR3 positive subjects may display some changes in immune parameters when compared with HLA-B8, DR3 negative ones, and are prone to develop several immunological diseases [1–4]. HLA-linked immune-response genes, susceptibility to infectious agents and the action of other genes in the HLA region are involved in immune functions. Several previous studies as well as our own results suggest that a gene associated with HLA-B8, DR3 may act through an effect on the immune system that is detectable in clinically normal individuals [18].



C response to PHA , HLA-B8, DR3 positive subjects; mean=2.912± 1.097.
D response to PHA, HLA-B8, DR3 negative subjects; mean=3.388±2.550.
p= not significant.
Bars indicate means of values.

Fig. 2. Lymphocyte responses of clinically normal subjects with (•) or without (o) HLA-B8, DR3 in the presence of deoxynivanelol

In the present study we have analysed the proliferative response to PHA in HLA-typed healthy subjects in the presence and absence of deoxynivalenol, which is the most frequently found immunosuppressive Fusarium mycotoxin in domestic food products. In the direct cytotoxicity tests deoxynivalenol exhibited the least toxic effect among the Fusarium mycotoxins in our previous study [13].

In our previous studies the inhibitory effect of deoxynivalenol on mitogen induced blast transformation showed significant differences among subjects. Therefore, we tested the immunosuppressive effect of deoxynivalenol on lympho-proliferative responses to small dose PHA as a function of HLA antigens. Our results are in accordance with a previous report that blastogenic responses to PHA are reduced in individuals having the HLA-B8, DR3 antigens [5].

The difference was significant in proliferative response to PHA between groups of subjects carrying and not carrying the HLA-B8, DR3 haplotype in the absence of deoxynivalenol. However, it was not possible to demonstrate a significant difference between the HLA-B8, DR3-positive and -negative groups in the presence of deoxynivalenol. Mycotoxins probably act through different receptors which are not related with the studied haplotypes.

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