ACTION OF A MYCOTOXIN (Diacetoxyscirpenol) ON THE IMMUNE RESPONSE OF THE MOUSE - INTERACTION WITH AN IMMUNOMODULATOR (OM-89)

C. Bottex, A. Martin and R. Fontanges

Department of Microbiology, Army Healthservice Research Center, 108, Boulevard Pinel, F - 69275 Lyons, France

ABSTRACT

The action of diacetoxyscirpenol (DAS) - a mycotoxin that belongs to the family of trichothecenes - on the immune system of the mouse was investigated. Two experimental models were used: 1) bacterial infection with Salmonella typhimurium and 2) the PFC (plaque-forming cells) test on the splenic lymphocytes of the mouse. The results obtained showed that these were dependent on the chronological order of the administration of DAS. When the toxin was administered after a bacterial infection or antigenic stimulation, it was observed an increase in the mortality rate and a very significant decrease in the antibody response. By contrast, when this mycotoxin was administered before the bacterial infection or antigenic stimulation, the results obtained were close to those from the controls. The importance of the administration of an immunomodulator of bacterial origin OM-89 before the immunodepression with DAS was shown.
INTRODUCTION

The trichothecenes are a group of toxic metabolites produced by several species of Fusarium that may contaminate the crops of cereals and fodder in a persistent manner (1, 22). Those substances with sesquiterpenoid structure have been recognized as being responsible for serious poisoning (alimentary toxic aleucia or ATA) in man, cattle and poultry (2). They are reported to act principally by inhibiting DNA and protein biosynthesis (8,10,15,16,18,21). The cells of the immune system with high mitogenic power are probably the preferred targets of mycotoxins (14,19,20); these toxins behave like immunodepressant agents.

In the present study, we first investigated the effects of a trichothecene - DAS - on the mortality rate of mice infected with Salmonella typhimurium in order to determine the degree of immunodepression. We then studied the effects of this mycotoxin on the immune system by using the PFC (plaque-forming cells) test. We also used the PFC test to investigate the effects of an immunomodulator of bacterial origin (OM-89) to counteract the immunodepression of DAS.

MATERIALS AND METHODS

- Batches of ten Swiss mice (OF1) of both sexes weighing 20 to 25 g and six weeks old, raised in a closed colony at our laboratory were used.

- Crystallized 25 g diacetoxyscirpenol (DAS) from SIGMA, Missouri - 63174, USA), dissolved extemporaneously in 2.5 ml of dimethyl sulfoxide (DMSO) was used. The concentration was monitored using a gas chromatograph, type FRACTOVAP 2900, with a flame ionization detector connected to an integrator, type SP 4100.
OM-89 lyophilizate compound (60 mg equivalent to a human oral dose, Laboratoires OM S.A., Meyrin/Geneva, Switzerland) is an E. coli extract obtained after chemical fractionation of the biomass and extraction through clarification followed by purification.

- For experimental infection, a strain of Salmonella typhimurium (STM) catalogued by the Pasteur Institute under N° 6185/768.84 was used. The mice were infected intraperitoneally at the rate of 15 ± 5 germs/0.2 ml saline/mouse.

STUDY OF THE EFFECTS OF DAS ON THE MORTALITY RATE OF MICE INFECTED WITH STM

- DAS administration before experimental infection
  Batches of ten mice were given intraperitoneally subacute doses of DAS: 5.3 mg/kg and 2.7 mg/kg ten and nine days respectively before bacterial infection for the first batch and four and three days respectively prior to bacterial infection for the second batch. The mortality rate of the animals was monitored every day for 21 days. The results obtained were analyzed using the non-parametric tests of Fisher and of Mann and Whitney. A X^2-test was run at the end of this trial to compare the survival rate of the different batches of animals.

- DAS administration after experimental infection
  Batches of ten mice were given DAS intraperitoneally respectively at 5.3 mg/kg and 2.7 mg/kg three and 24 hours respectively after the experimental infection for the first batch and four and five days respectively after the experimental infection for the second batch. The mortality rate was monitored for 21 days.
STUDY OF THE INFLUENCE OF DAS ON THE ANTI-SRBC ANTIBODY RESPONSE USING THE DIRECT TECHNIQUE OF HEMOLYSIS PLAQUES (PFC)

- **PFC test**

  The number of cells forming hemolysis plaques in a suspension of splenic cells was determined using the direct technique of hemolysis plaques developed by Cunningham and Szenberg (5). The mice were immunized beforehand intraperitoneally with 0.5 ml of a 5% suspension of sheep red blood cells per mouse (SRBC 50%, Bio Mérieux, F-69200 Charbonnières-les-Bains, France). The search for anti-SRBC antibodies was made four days later. The number of PFC per spleen represented the mean of the PFC response of a batch of ten mice. Statistical analysis of the differences between control animals and treated animals was performed using the Student's t-test. The values for which probability was higher than 0.05 were considered non-significant.

- **Study of the PFC response to DAS administration before antigenic stimulation**

  In the first series of experiments, batches of ten mice were given DAS intraperitoneally in two injections of 0.2 ml each: first 5.3 mg/kg in 0.2 ml, then 2.7 mg/kg in 0.2 ml on the following day. Antigenic stimulation was carried out by injecting SRBC five days after the last DAS injection. The PFC test was performed four days later.

- **Study of the PFC response to DAS administration after antigenic stimulation**

  In the second series of experiments, batches of ten mice were stimulated by an intraperitoneal injection of 5% SRBC. On the following day, the animals were given intraperitoneally 5.3 mg/kg in 0.2 ml. A day later, they were given 2.7 mg/kg of DAS.
under the same conditions. The direct PFC response was evaluated four days after the antigenic stimulation by SRBC.

IMMUNOMODULATOR

OM-89, an E. coli extract, obtained after chemical fractionation of the biomass and extraction through clarification followed by purification (OM Laboratories, 1217 Meyrin/Geneva, Switzerland). OM-89 was used dissolved in water and then administered by gavage to certain batches of mice using a probe every day for five days in succession at the dose of 12 mg/0.2 ml. After a 10-day pause, the mice were immunized with SRBC. Twenty-four and 48 hours later, the mice were given intraperitoneally 5.3 mg/kg and 2.7 mg/kg of DAS respectively in a volume of 0.2 ml. The PFC test was run four days later. As control we used OM-89 treatment alone without DAS. An other control was made with LPS.

RESULTS

STUDY OF THE EFFECTS OF DAS ON THE MORTALITY RATE OF MICE INFECTED WITH STM

DAS administration before experimental infection

When DAS was administered intraperitoneally to the mice at 5.3 mg/kg and 2.7 mg/kg ten and nine days respectively prior to the experimental infection with Salmonella typhimurium, we found that the mortality rate was lower in the animals receiving DAS than in the control animals (Figure 1). It was also found that the mortality rate of the mice receiving the same dose of DAS four and three days before the trial was lower than that of the control animals.
Influence of DAS given ip, at 5.3 mg/kg on day-10 (D-10) and 2.7 mg/kg on day-9 (D-9) or 5.3 mg/kg on day-4 (D-4) and 2.7 mg/kg on day-3 (D-3) on the survival rate of mice infected with Salmonella typhimurium on day 0.

Statistical analysis with Mann-Whitney test:
- Significant for DAS on D-10, D-9 (p < 0.05)
- Not significant for DAS on D-4, D-3 in comparison with the controls.

Statistical analysis with \( \chi^2 \)-test:
- Not significant for DAS on D-10, D-9
- Significant for DAS on D-4, D-3 (p < 0.05).

Statistical analysis using the non-parametric test of Mann and Whitney showed that the mortality rate of the animals during the experiment exhibited a significant difference (p < 0.05) between the controls and the treated mice for DAS administered on D-10, D-9, but a not significant one for DAS administered on D-4, D-3. At the end of the trial, by contrast, the mortality rate of the mice treated with DAS four and three days prior to the infection with
STM was significantly lower than that of the control animals ($p < 0.05$, $X^2$-test).

**DAS administration after experimental infection**

When DAS was administered to the mice three hours (5.3 mg/kg) and 24 hours (2.7 mg/kg) after the experimental infection, we found that deaths occurred on the second day already, while among the control deaths did not appear until the 5th day (Figure 2).

Statistical analysis, using Mann and Whitney test, showed, moreover, that the increase in the mortality rate among the animals receiving DAS on D 0 and D+1 was highly significant ($p < 0.001$), and significant among the animals receiving DAS on D+4 and D+5 ($p < 0.05$). At the end of the trial, the mortality rate showed no significant difference between the control animals and the treated ones ($X^2$-test). When DAS was administered intraperitoneally to the mice at doses of 5.3 mg/kg and 2.7 mg/kg four and five days respectively after the infection with STM, the death rate sharply increased significantly ($p < 0.05$) by day 6 compared with the control animals. At the end of the trial, however, no significant difference in the survival rate was noted between the control animals and the treated ones ($X^2$-test).

**STUDY OF THE INFLUENCE OF DAS ON THE ANTI-SRBC ANTIBODY RESPONSE USING THE PFC TEST**

- **Study of the PFC response to DAS administration prior to antigenic stimulation**
  
  When DAS was given intraperitoneally to the mice at 5.3 mg/kg and 2.7 mg/kg, six and five days respectively before the immunization with SRBC, it was observed that the direct anti-SRBC PFC response, expressed per spleen, was better than
Figure 2
Influence of DAS given ip, at 5.3 mg/kg on day 0 (D 0) and 2.7 mg/kg on day+1 (D+1) or at 5.3 mg/kg on day+4 (D+4) and 2.7 mg/kg on day+5 (D+5) on the survival rate of mice infected with Salmonella typhimurium on day 0.

Statistical analysis with Mann-Whitney test:
- Highly significant for DAS on D 0, D+1 (p<0.001)
- Significant for DAS on D+4, D+5 (p<0.05).

Statistical analysis with $\chi^2$-test:
- Not significant either for DAS on D 0, D+1
- or DAS on D+4, D+5.

that of the control animals. But statistical analysis using the Student's t-test showed that this stimulation was not significant (Table I).

- Study of the PFC response to DAS administration after antigenic stimulation

When DAS was administered to the mice under the same conditions but one and two days respectively after antigenic
Table I

<table>
<thead>
<tr>
<th>Batch of animals (10 animals/batch)</th>
<th>PFC/spleen $x 10^4$ (mean value $\pm$ SD)</th>
<th>% suppression (-) or stimulation (+) versus controls</th>
<th>p value Student's t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>52 $\pm$ 13</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>DAS D-6/D-5</td>
<td>82 $\pm$ 24</td>
<td>+ 58</td>
<td>ns</td>
</tr>
<tr>
<td>Control</td>
<td>89 $\pm$ 17</td>
<td>-</td>
<td>ns</td>
</tr>
<tr>
<td>DAS D+1/D+2</td>
<td>5 $\pm$ 1</td>
<td>- 94</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Anti-SRBC PFC response in mice following DAS administration, at subacute doses of 5.3 mg/kg and 2.7 mg/kg, ip, six and five days (DAS D-6/D-5) prior to antigenic stimulation or one and two days (DAS D+1/D+2) after antigenic stimulation with SRBC.

stimulation by SRBC, it was found that the PFC response diminished by 94% compared with that of the control animals (Table I). The Student's t-test showed that this inhibition was highly significant ($p < 0.001$).

STUDY OF THE ABILITY OF AN IMMUNOMODULATOR TO COMPENSATE FOR DAS-INDUCED IMMUNODEPRESSION

OM-89 was orally administered to certain batches of mice for five consecutive days. After a 10-day pause, both the control and treated animals were immunized with SRBC intraperitoneally.

One and two days after antigenic stimulation, the animals treated beforehand with OM-89 were given DAS at 5.3 mg/kg and 2.7 mg/kg. Other batches of mice not treated beforehand with OM-89 were given DAS under the same conditions. Control mice were given neither DAS nor OM-89.
Under these conditions, it was found that the anti-SRBC PFC response sharply decreased in the DAS-treated animals compared with the control animals (Table II). The Student's t-test showed that this inhibition was significant ($p<0.05$).

In the animals treated with OM-89 beforehand, we found that the PFC response was always weaker than that of the control animals. Statistical analysis showed, however, that the PFC response of the treated animals was not significantly different from that of the control animals (Table II).

When the PFC response of the animals treated with DAS alone was compared with that of those treated with OM-89 beforehand, it was found that the latter group's PFC response was stronger. However, statistical analysis using the Student's t-test showed that this difference was not significant. PFC response with OM-89 alone showed a significant increase ($p<0.05$), and LPS (used as a control) administered orally showed no significant increase in PFC response (data not shown).

DISCUSSION

The results of our first series of experiments on the mortality rate of the animals seemed contradictory depending on whether DAS was administered before or after experimental infections with Salmonella typhimurium. When DAS was administered ten or four days before the bacterial infection, a certain "protective" effect was observed. When DAS was administered four days before the infection with STM, this effect proved statistically significant. Those results are in contradiction with those obtained by Boonchuvit (4) and Otokawa (13) but relative to T2 toxin. In fact, they had observed that the administration of this mycotoxin significantly reduced resistance to bacterial or viral infection.
Table II

<table>
<thead>
<tr>
<th>Batch of animals</th>
<th>PFC/spleen x 10^2 (mean value ± SD)</th>
<th>% suppression (-) or stimulation (+) versus controls</th>
<th>p value</th>
<th>Student's t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>110 ± 32</td>
<td>-</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DAS D+1/D+2</td>
<td>59 ± 11</td>
<td>- 46</td>
<td>&lt;0.05</td>
<td></td>
</tr>
<tr>
<td>OM-89 + DAS D+1/D+2</td>
<td>80 ± 24</td>
<td>- 27</td>
<td>ns</td>
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</table>

Effects of OM-89 administered orally, every day, for five days (12 mg/mouse) on an induced immunodepression by ip administration of 5.3 mg/kg and 2.7 mg/kg DAS one and two days (D+1/D+2) following antigenic stimulation with SRBC, as assessed by PFC test.

Our results suggest that DAS is rapidly catabolized. According to Bauer et al. (3), DAS catabolizes within 48 hours after administered to the pig. One may think that when infected with bacteria the unimpaired immunocompetent cells preserve their functional abilities. When DAS was administered immediately after infection or a few days later, however, the mortality rate sharply increased. In this case, our results were comparable with those obtained by Fromentin (7) in mice infected with Candida albicans. The combined administration of DAS and yeast very significantly increased germ proliferation in the kidneys of mice. Using T2 toxin, Kanai (9) also found an increase in the mortality rate of mice infected experimentally with mycobacteria. By its toxic effects, DAS may therefore block the immune system's defense mechanisms already depressed by the bacterial invasion.

In our second series of experiments, we investigated the influence of DAS on the anti-SRBC antibody response assessed using the PFC test. Our results showed that DAS did not affect the anti-SRBC antibody response when administered prior to antigenic
stimulation. The B-lymphocytes were therefore capable of favorably responding to the antigenic stimulation five days after administration of a large dose of DAS. Inversely, when DAS was administered after the antigenic stimulation, we found a very strong inhibition of the antibody response capabilities. Rosenstein (14) showed that an intraperitoneal administration of DAS several days prior to blood sampling caused the level of hemagglutinating antibody to fall in a dose-dependent manner.

It therefore seems that the intraperitoneally administration of mycotoxin (diacetoxyscirpenol) blocks the immune system. If no further immunodepression becomes superadded in the acute phase, it is probable that the action of the toxin on the body will not be lethal. If a DAS attacks in addition to an infection, the immune system will be blocked and cause a high mortality rate.

In view of those results, we wanted to investigate to what extent an immunostimulating treatment could compensate for DAS-induced immunodepression of the mouse's immune system. We found that the mice treated first with an immunomodulator (OM-89) and then with DAS exhibited a less depressed antibody response than the mice receiving DAS alone. OM-89 thus improved or compensated for immunodepression. It has been shown that immunomodulators may have an overall effect on immunity by acting directly on the lymphocytes, the phagocytes or the complement system or indirectly on the immunoregulatory mechanisms (6,11,12,17).

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