Immunity induced in rats vaccinated with toxoid prepared from heat-labile toxin produced by Pasteurella multocida serogroup D

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ABSTRACT

Rats were vaccinated with a toxoid (D-toxoid) prepared from purified heat-labile toxin (D-toxin) produced by Pasteurella multocida serogroup D. Vaccination of rats with D-toxoid prevented death and other effects of D-toxin (hepatic necrosis, development of elevated leukocyte counts, lymphopenia, neutrophilia, and elevated complement titers) that occurred in phosphate buffered saline (PBS)-vaccinated control rats.

INTRODUCTION
The heat-labile toxin (D-toxin) produced by certain isolates of Pasteurella multocida serogroup D is an important factor in the pathogenesis of atrophic rhinitis in swine (Chanter and Rutter, 1989). In addition to localized effects on nasal turbinates, D-toxin is dermonecrotic, and capable of causing systemic effects such as weight loss, hepatic necrosis, lymphopenia, increased serum complement titers, and death (Dominick and Rimler, 1986, 1988; Elling et al., 1988; Cheville et al., 1988; Cheville and Rimler, 1989; Rimler and Rhoades, 1989; Thurston et al., 1989; Williams et al., 1990). Elling et al. (1986), Rüschoff et al. (1987), and Cheville and Rimler (1989) demonstrated that systemic changes induced in rats by D-toxin are similar to those observed in pigs given D-toxin. As a consequence of these studies and reports of prevention of turbinate atrophy by immunization with toxoid prepared from toxigenic strains of P. multocida (Foged, 1988; Foged et al., 1989), we immunized rats with a toxoid (D-toxoid) prepared from purified D-toxin to determine if immunized rats were protected against effects of D-toxin, namely
death, hepatic necrosis, depressed weight gain, alterations in leukocyte numbers, and increased complement activity.

MATERIALS AND METHODS

Preparation of D-toxoid

Toxin from *P. multocida* strain P-4533 was purified as described previously (Cheville and Rimler, 1989). For toxoid preparation, 8 mg of toxin was mixed with 72 mg of bovine serum albumin fraction V in 3.2 ml of 0.2 M acetate buffer, pH 5.0. The mixture was stirred at room temperature, and 0.32 ml of 2.5% aqueous glutaraldehyde was added. The mixture was allowed to polymerize for 3 h and then was ground with saline in a Ten-Broeck tissue grinder. The ground suspension was washed twice with saline by centrifugation and adjusted to the desired concentration in phosphate buffered saline (PBS) containing 0.01% sodium azide. Concentration of the toxoid was estimated to be approximately 280 µg per ml.

Rats

Fifty-eight male Holtzman rats (Holtzman Laboratory Animals, Madison, WI), were housed individually on ground corn cob bedding with unlimited access to food and fresh water, and were weighed daily. Average rat weight on day 0 was 118 g. They were divided into two groups: one group received 0.25 ml of D-toxoid subcutaneously on day 0 and day 10, and the other (control) group received 0.25 ml of PBS on the same days. On day 24, each group of rats was subdivided into four challenge groups of six rats and were given subcutaneous injections of either 0.1, 0.2, 0.4, or 0.8 µg D-toxin/kg of the average rat weight on day 24 (320 g). The experiment was terminated on day 27 when surviving rats and the remaining ten untreated, unchallenged rats were anesthetized with CO₂ and blood samples were taken by cardiac puncture. Rats were killed with CO₂ and tissues were collected for histopathological study.

Serum complement

Serum collected on day 27 of the experiment was tested for complement activity as previously described using sheep erythrocytes sensitized with rabbit anti-sheep hemolysin. Complement titers were expressed on the log_{10} of the 50% hemolytic endpoint (Garvey et al., 1977). An aliquot of pooled normal rat serum was included as a standard in each set of complement titrations.

Hematology

At the time blood samples were taken from the rats, smears were made for differential leukocyte counts, and blood was placed in tubes containing EDTA for determination of total leukocyte counts (WBC) by electronic counting (Nova Celltrak, Waltham, MA).


**Histopathology**

Samples from left and right liver lobes of rats surviving until day 27 were fixed in neutral buffered formalin and processed routinely. Sections were stained with hemolysin and eosin.

**RESULTS**

No deaths from D-toxin occurred in any D-toxoid-vaccinated rats, and no microscopic changes typical of individual cell necrosis were observed in any of these rats (Table 1).

Mild to marked necrosis of individual hepatocytes was seen in surviving (control) rats. Hepatocellular necrosis was most severe in portal areas but also present, to a lesser degree, throughout lobules (Fig. 1). The necrotizing process was generalized throughout liver sections and lesions were greatest in rats from groups challenged with 0.4 and 0.8 µg/kg of D-toxin.

Rats gained weight uniformly from day 0 until day 24 when all D-toxoid and PBS rats were given subcutaneous injections of D-toxin. After injection of D-toxin, rats vaccinated with D-toxoid gained an average weight of 16 g from day 24 until day 27. Surviving control rats lost an average of 11 g weight from day 24 to day 27.

Total leukocyte counts, percent of neutrophils, lymphocytes, and serum complement titers of D-toxoid and surviving control rats did not show any obvious dose-related responses to challenge with the four concentrations of D-toxin (Fig. 2) for either vaccine group by regression analysis (F-tests). Therefore, overall means and standard errors were computed for the two groups, for the ten normal rats in the current study, and for 32 normal rats from an unpublished study which served as a laboratory standard (Table 2). Rats vaccinated with D-toxoid and challenged with D-toxin had identical values to those of normal rats, which differed markedly from control rats chal-

<table>
<thead>
<tr>
<th>D-toxin (µg/kg of body weight)</th>
<th>Day 27</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Death per group</td>
</tr>
<tr>
<td></td>
<td>D-toxoid</td>
</tr>
<tr>
<td>0.1</td>
<td>0/6</td>
</tr>
<tr>
<td>0.2</td>
<td>0/5*</td>
</tr>
<tr>
<td>0.4</td>
<td>0/6</td>
</tr>
<tr>
<td>0.8</td>
<td>0/6</td>
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</tbody>
</table>

*One death occurred unrelated to D-toxin.
Fig. 1. Section of hepatic portal area from a rat vaccinated with PBS and challenged with 0.4 μg of D-toxin. There is necrosis of individual hepatocytes (arrows). Necrotic hepatocytes have pyknotic nuclei, dark staining cytoplasm, and irregular borders. Hematoxylin and cosin stain. 400×.

Fig. 2. Mean values of hematology (a, b, c) and of complement titers (d) in rats vaccinated with either D-toxoid or PBS and challenged with 0.1, 0.2, 0.4, or 0.8 μg/kg of D-toxin.
### TABLE 2

Mean values of hematology and serum complement titers for standard and normal rats and all D-toxoid- and PBS-vaccinated rats surviving on day 27

<table>
<thead>
<tr>
<th>Variable</th>
<th>Day 27</th>
<th>Standard ((n=32))</th>
<th>Normal ((n=10))</th>
<th>D-toxoid-vaccinated ((n=23))</th>
<th>PBS-vaccinated ((n=11))</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC×10³/mm³</td>
<td>Mean 12</td>
<td>16 a</td>
<td>16 a</td>
<td>30 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.E. 0.7</td>
<td>1.2</td>
<td>0.9</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td>% neutrophils</td>
<td>Mean 11</td>
<td>11</td>
<td>11</td>
<td>22 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.E. 1.1</td>
<td>1.7</td>
<td>1.4</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>% lymphocytes</td>
<td>Mean 88</td>
<td>87</td>
<td>87</td>
<td>75 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.E. 1.1</td>
<td>2.0</td>
<td>1.0</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>C log₁₀ titer</td>
<td>Mean 1.74</td>
<td>ND c</td>
<td>1.74</td>
<td>1.92 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>S.E. 0.01</td>
<td>ND</td>
<td>0.014</td>
<td>0.033</td>
<td></td>
</tr>
</tbody>
</table>

*Different from the standard *P* < 0.05.  
**Different from D-toxoid *P* < 0.001.  
'ND' = not done.

Lenged with D-toxin, and the differences were all statistically significant (*t*-tests; *P* < 0.01). The average total leukocyte count of the lab standard rats was somewhat lower than for normal and D-toxoid-vaccinated rats, and the difference was statistically significant (*P* < 0.05). Percent of neutrophils and lymphocytes for lab standard rats, normal, and D-toxoid-vaccinated rats were virtually identical, and differences were statistically nonsignificant.

### DISCUSSION

Rats vaccinated with D-toxoid were protected against the effects of D-toxin, including death and hepatocellular necrosis. They also had weight gain, hematologic values, and serum complement titers within limits we have observed for normal Holtzman rats. D-toxin caused an increase in total leukocyte counts of control rats. Shifts in numbers and proportions of lymphocytes and neutrophils were prevented by vaccination of rats with toxoid prepared from D-toxin. Williams et al. (1990) observed lymphopenia in pigs 24 h after they were given D-toxin (designated in their study as turbinate atrophy toxin). They also demonstrated D-toxin-stimulated activity of peripheral blood lymphocytes in vitro, but did not lyse lymphocytes or other cells even though D-toxin in vivo causes hepatic necrosis (Cheville et al., 1988) and dermonecrosis (Rimler and Rhodes, 1989) in pigs and rats (Cheville and Rimler, 1989). The hematologic changes described by Williams et al. (1990) and hematological data in this study indicate a need for further investigation of the effects of D-toxin on numbers and function of peripheral blood lymphocytes and neutrophils in relationship to the as yet unresolved mode of action of D-
toxin. The results suggest the rat model used in the study of alteration caused by D-toxin (Rüschhoff et al., 1987) will be useful in testing of vaccines designed to immunize against atrophic rhinitis, particularly vaccines purported to contain toxoid prepared from the heat-labile protein toxin of *P. multocida* serogroup D.

**ACKNOWLEDGEMENTS**

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**REFERENCES**


