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dermonecrotic toxin-challenged rats by toxoid-
induced antibody

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(Accepted 3 September 1992)

ABSTRACT


Two different doses of glutaraldehyde-treated Pasteurella multocida dermonecrotic toxin (PMDT) were used to immunize rats. Rats developed serum IgG antibodies specific for native PMDT, and IgG titers increased with dose and number of toxoid immunizations. Survival rates in both active immunization and passive serum neutralization experiments were dependent on dose of toxoid vaccination and serum levels of anti-PMDT IgG. Vaccination with toxoid prevented weight loss but not leukocytosis and increased complement titers in toxin-challenged rats. Toxoid, itself, induced minimal leukocytosis but no alterations in complement titers or weight gain.

INTRODUCTION

Pasteurella multocida dermonecrotic toxin (PMDT) plays a critical role in swine atrophic rhinitis (Chanter et al., 1986; Dominick and Rimler, 1986). As such, many commercial atrophic rhinitis vaccines currently in use or development incorporate inactivated toxin.

Rats are an excellent model for evaluating both potential atrophic rhinitis vaccines, and the localized and systemic effects of PMDT (Cheville and Rimler, 1989; Thurston et al., 1989, 1991, 1992). Systemic changes in rats induced by PMDT are similar to those seen in swine. In both animals PMDT directly or indirectly causes weight loss, hepatic necrosis, leukocytosis, elevated complement titers, and at high doses, death (Ackermann et al., 1992; Cheville and Rimler, 1989; Thurston et al., 1989, 1991).

Several atrophic rhinitis vaccine studies emphasize the importance of in-
corporating inactivated PMDT. Foged et al., (1989) demonstrated that vaccination of pregnant gilts with purified inactivated PMDT resulted in significantly decreased incidence of progressive atrophic rhinitis in offspring intranasally inoculated with *Bordetella bronchiseptica* and toxigenic *P. multocida*. Similarly, vaccination of pregnant gilts with a recombinant nontoxic derivative of PMDT protected offspring from intranasal inoculations of *B. bronchiseptica* and toxigenic *P. multocida* (Nielsen et al., 1991). In both studies the ability of the vaccines to control the disease was associated with colostral antibody transfer to piglets. Rats vaccinated with inactivated PMDT were protected from lethal challenge with native PMDT (Thurston et al., 1991).

In this study we used the rat model to investigate dose-dependence of toxoid vaccination, protective value of toxoid-induced antibody, and to determine whether toxoid, like native PMDT, induces leukocytosis, elevated complement titers, and weight loss.

**MATERIALS AND METHODS**

Toxin and toxoid. Toxin from *P. multocida* strain P-4533 was purified as described previously (Cheville and Rimler, 1989). Preparation of glutaraldehyde-inactivated PMDT has been described in detail (Thurston et al., 1991).

Rats. Virus-free, outbred, male white rats (Holtzman Laboratory Animals, Madison WI) were housed individually or in pairs on ground corn cob bedding with unlimited access to food and fresh water. Rats were weighed daily Monday through Friday, and divided into groups matched for average weight and distribution of weight.

Bleeding, vaccination and challenge schedule. (See Treatment in Table 1 for an outline of the following experiments). Rats were anesthetized with CO₂ and bled by cardiac puncture prior to immunization on days 0 and 14, and again on day 21. Serum from individual rats was collected and stored at −80°C. For passive neutralization studies, individual rat serum from day 21 was collected and equal volumes pooled according to group.

On days 0 and 14, group B received 10 μg of toxoid, group C 100 μg of toxoid, and group A, phosphate buffered saline (PBS) (all s.c.). Groups A, B, and C were challenged with 1 μg purified toxin/kg body weight (i.p.) on day 22.

For the passive neutralization experiment on day 22, toxin was preincubated 30 min at 37°C with pooled sera (collected on day 21) before each inoculation. Group D received 1 μg purified toxin/kg body weight in 0.3 ml pooled group A serum, group E received 1 μg purified toxin/kg body weight
in 0.3 ml pooled group B serum, and group F received 1 μg purified toxin/kg body weight in 0.3 ml pooled group C serum (all i.p.).

Experiments were terminated on day 27 when survival rats were bled under CO₂ anesthesia and euthanized with CO₂.

Hematology. Total leukocyte numbers were determined by electronic counting (Nova Celltrak, Waltham, MA) as previously described (Thurston et al., 1991).

Serum complement. Complement titers were assessed using sheep erythrocytes sensitized with rabbit anti-sheep hemolysin as previously described (Thurston et al., 1991). Complement titers were expressed as the log₁₀ of the 50% hemolytic endpoint.

ELISA. For detection of anti-PMDT IgG, 96-well Biodyne nylon membrane plates (Pall BioSupport Corp, East Hills, NY) were used. All incubations were at room temperature. Each membrane well was coated with 2.0 μg of purified PMDT in 0.05 M Tris, 0.1 M NaCl, 0.002 M EDTA buffer, pH 8.3 for 1 h. Wells were emptied and blocked for 1 h with 200 μl of filtered 1% casein in 0.05 M Tris, 0.15 M NaCl buffer, pH 7.5. Wells were then emptied and washed with PBS. Dilutions of individual rat antisera in blocking solution were applied for 1 h, and the wells emptied and washed three times with blocking solution. One hundred microliters of a 1:1000 dilution of anti-rat IgG peroxidase (Kirkegaard and Perry Laboratories, Gaithersburg, MD) in blocking solution was added to each well and incubated for 1 h, and the wells emptied and washed three times in PBS. Antibody was detected colorimetrically by the addition of 200 μl 4-chloro-1-naphthol H₂O₂ substrate solution (Kirkegaard and Perry Laboratories). No reaction was observed in the absence of rat antiserum. Anti-PMDT IgM was not detected by a similar ELISA using anti-rat IgM peroxidase.

RESULTS

Groups of rats are identified and experimental events summarized in Table 1.

Vaccination with toxoid protected rats from challenge with lethal doses of PMDT (Table 1). Dose of toxoid was related to efficacy, with higher doses yielding greater protection. No rats vaccinated with PBS (group A) survived challenge, 50% of rats vaccinated with 10 μg toxoid (group B) survived, and all rats vaccinated with 100 μg toxoid (group C) survived.

In passive neutralization experiments, exposure of group F to lethal PMDT doses plus antisera from 100 μg toxoid-vaccinated rats afforded complete
<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>A (n=7)</th>
<th>B (n=8)</th>
<th>C (n=9)</th>
<th>D (n=5)</th>
<th>E (n=5)</th>
<th>F (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td></td>
<td>0 PBS</td>
<td>10 µg Toxoid</td>
<td>100 µg Toxoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14 PBS</td>
<td>10 µg Toxoid</td>
<td>100 µg Toxoid</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>22 Toxin</td>
<td>Toxin</td>
<td>Toxin</td>
<td>Toxin + Pool</td>
<td>Toxin + Pool</td>
<td>Toxin + Pool</td>
</tr>
<tr>
<td></td>
<td>A Serum</td>
<td>b</td>
<td>B Serum</td>
<td>C Serum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Deaths/Group</td>
<td>27</td>
<td>7/7</td>
<td>4/8</td>
<td>0/9</td>
<td>5/5</td>
<td>5/5</td>
<td>0/5</td>
</tr>
<tr>
<td>Anti-toxin IgG/Group</td>
<td>0</td>
<td>0/7</td>
<td>0/8</td>
<td>0/6c</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>0/7</td>
<td>1/8</td>
<td>3/6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>21</td>
<td>0/7</td>
<td>3/8</td>
<td>6/6</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

aA-F challenged with 1 µg purified toxin/kg body weight
bPooled serum used in D-F collected on day 21
cDue to experimental error, 3/9 rats in group C were not bled on day 0
TABLE 2

Mean values of white blood cells (WBC), serum complement (C') titers and body weights in all rats from days 0-21, and in rats surviving toxin-challenge (day 27)

<table>
<thead>
<tr>
<th>Day</th>
<th>Group</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td>PBS</td>
<td>10 µg Toxoid</td>
<td>100 µg Toxoid</td>
<td>–</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>PBS</td>
<td>10 µg Toxoid</td>
<td>100 µg Toxoid</td>
<td>–</td>
</tr>
<tr>
<td>22</td>
<td></td>
<td>Toxin</td>
<td>Toxin</td>
<td>Toxin</td>
<td>Toxin + Day 21 Pool C Serum</td>
</tr>
<tr>
<td></td>
<td>WBC x 10^3/mm^3</td>
<td>9</td>
<td>8</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>10</td>
<td>12</td>
<td>13</td>
<td>ND</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>11</td>
<td>13</td>
<td>13</td>
<td>ND</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>–</td>
<td>18</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>C' Log_{10} Titer</td>
<td>21</td>
<td>1.8</td>
<td>1.8</td>
<td>1.8</td>
<td>ND</td>
</tr>
<tr>
<td>27</td>
<td></td>
<td>–</td>
<td>1.9</td>
<td>2.0</td>
<td>2.0</td>
</tr>
<tr>
<td>Weight</td>
<td>0</td>
<td>168</td>
<td>170</td>
<td>169</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td></td>
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<td>229</td>
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<td>14</td>
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<td>288</td>
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<td>21</td>
<td></td>
<td>340</td>
<td>349</td>
<td>343</td>
<td>325</td>
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<tr>
<td>27</td>
<td></td>
<td>–</td>
<td>369</td>
<td>358</td>
<td>357</td>
</tr>
</tbody>
</table>

* A–F challenged with 1 µg purified toxin/kg body weight

b Lab standard, 32 normal rats \( \bar{x} = 12 \) S.E. = 0.7 (Thurston et al., 1991)

No Data

Lab standard, 32 normal rats \( \bar{x} = 1.7 \) S.E. = 0.01 (Thurston et al., 1991)

Protection (Table 1). Serum from rats immunized twice with 10 µg toxoid or PBS did not neutralize PMDT in groups E and D, respectively.

Survival rates paralleled anti-PMDT IgG response (Table 1). Less than half of the rats vaccinated with 10 µg toxoid (group B) produced detectable anti-PMDT IgG by day 21 (geometric mean titer = 4 on day 21). All rats vaccinated with 100 µg PMDT (group C) produced relatively high titers of anti-PMDT IgG by day 21 (geometric mean titer = 4 on day 14, and 141 on day 21). Lower anti-PMDT IgG titers in group B corresponded with 50% mortality in active immunization experiments, and 100% mortality in passive neutralization experiments. In contrast, antiserum from group C (100 µg toxoid-vaccinated) completely neutralized the lethal effects of PMDT in active immunization and passive neutralization experiments.

In contrast to native PMDT (Cheville and Rimler, 1989; Thurston et al., 1989, 1991), inoculation with toxoid caused no alteration in complement titers or weight gain relative to PBS-vaccinated rats (Table 2, days 0–21). Total white blood cell (WBC) counts for groups B (10 µg toxoid-treated) and C (100 µg toxoid-treated) on day 21 were similar, and their mean slightly higher than PBS-vaccinated controls (group A) (diff = 2.1 ± 0.9; \( P < 0.05; t \) test).
After toxin challenge on day 22, surviving rats in groups B and C gained weight through day 27 in a similar manner, although at a reduced rate compared to pre-challenge rates (approx. 2.5 g/day compared to approx. 8 g/day) (Table 2). After toxin exposure, rats in group F gained an average of 5 g daily through day 27. Complement titers in groups B, C, and F on day 27 were slightly but consistently elevated compared to day 21 prechallenge levels, and laboratory standard values (Table 2). Total WBC numbers in groups B, C, and F were markedly higher on day 27 relative to day 21 prechallenge levels, and laboratory standard values (Table 2). Only one of 18 surviving rats in groups B, C, and F exhibited a reduction in total WBC counts after toxin challenge.

DISCUSSION

Immunization with inactivated PMDT or nontoxic PMDT derivatives has been shown to protect against swine atrophic rhinitis when animals are challenged with the two organisms commonly associated with the disease, *B. bronchiseptica* and toxigenic *P. multocida* (Foged et al., 1989; Nielsen et al., 1991). These studies suggest that antitoxin immunity alone may prevent progressive atrophic rhinitis. As PMDT is located intracellularly (Nakai, et al., 1985), it is not clear how anti-PMDT antibody protects against challenge with organisms lacking toxin surface antigen. It has been suggested that PMDT directly or indirectly enhances colonization (Foged et al., 1989), a process that would be inhibited in the presence of anti-PMDT antibodies.

In this study, a glutaraldehyde-treated purified PMDT vaccine provided dose-dependent protection of rats. Efficacy of the toxoid vaccine paralleled serum levels of anti-PMDT IgG. Similarly, Foged et al. (1989) noted toxoid-dose dependent anti-PMDT antibody titers in swine serum.

In unprotected rats and swine, PMDT induces weight loss, leukocytosis, elevated complement titers, and at high doses, death (Ackermann et al., 1992; Cheville and Rimler, 1989; Thurston et al., 1989, 1991). In this study we found that the biological activity of glutaraldehyde-treated PMDT in rats were markedly different from native PMDT. Only one of these effects, leukocytosis, was observed with toxoid treatment, and WBC counts were only slightly elevated relative to controls. Although total WBC counts, complement titers, and weight gains were similar in 10 and 100 μg toxoid-treated rats, survival rates after toxin challenge were quite different. As described above and verified with passive neutralization experiments, this is likely due to higher anti-PMDT IgG titers in 100 μg toxoid-treated rats.

Vaccination with toxoid did not prevent two common systemic effects of PMDT challenge. At five days post-challenge, surviving rats had dramatic increases in total WBC counts, and slightly elevated complement titers, relative to pre-challenge levels. Thus, although the epitope(s) of PMDT directly
or indirectly responsible for lethality was neutralized, epitopes responsible for at least two other biological effects were not.

ACKNOWLEDGEMENT

We thank Ms. Kim Driftmier for excellent technical assistance.

REFERENCES


