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What is This?
Light Microscopic and Ultrastructural Pathology of Seminiferous Tubules of Rats Given Multiple Doses of *Pasteurella multocida* Group D Protein Toxin*

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**ABSTRACT**

Male Holtzman rats were given subcutaneous doses of a purified *Pasteurella multocida* group D heat-labile toxin on alternate days for up to 22 days. Rats were necropsied at 18 days or 36 days (14 days after last dose of toxin) or when moribund, and testicles were taken for histologic and ultrastructural examination. Other selected tissues, including liver and spleen, were taken for histologic examination. Histologically, testicular and splenic lesions occurred more consistently and at much smaller doses when compared with lesions in other target organs such as liver. Testicular and splenic lesions were present in all rats (6/6) given 0.8 μg/kg toxin and were seen in some rats (1/6) given as little as 0.2 μg/kg toxin. Only 3/6 rats given 0.8 μg/kg toxin had hepatic lesions; no hepatic lesions were seen at doses of 0.2 μg/kg. Testicles from toxin-treated rats were smaller and weighed less than controls. Seminiferous tubules were moderately dilated and lined by polygonal sertoli cells. The normal spermatogenic maturation sequence and mature spermatids were absent, and many tubules contained multinucleate spermatocytes. Severely affected tubules were necrotic and mineralized. Ultrastructurally, there was necrosis of adluminal spermatocytes, multinucleate cell formation, and spaces between Sertoli cell plasma membranes. Testicular lesions were similar to those described for vitamin D-deficient rats, vitamin A-deficient rats, vasectomized rats, and rats given intravenous tumor necrosis factor; however, rats given lethal doses of toxin did not have elevated levels of TNFα activity.

**Keywords.** Testicle; tumor necrosis factor α; atrophic rhinitis; spermatogenesis; multinucleate spermatocytes; spermatocyte; Sertoli cell

**INTRODUCTION**

The protein toxin purified from toxigenic strains of *P. multocida* plays a central role in the development of atrophic rhinitis in swine. The toxin is a glycoprotein (MW 142,000) that, in vitro, is mitogenic to fibroblasts and causes an increase in the levels of inositol phosphate (14). Experimentally, single doses of small amounts of the toxin cause turbinate atrophy (6, 19). Larger, single doses of toxin, however, cause hepatic necrosis and death in pigs, rats, dogs, and turkeys (3, 4). Other lesions, such as irregular growth plates of bones and intestinal villous tip necrosis, also occur during acute toxicity in pigs and rats, but testicular lesions have not been described (3, 4). In preliminary studies, we have found that rats given multiple, small doses of toxin have degenerate seminiferous tubules.

Degeneration of spermatogenic cells and/or Sertoli cells of the seminiferous tubules can be caused by vitamins A, D, or E deficiency, tumor necrosis factor (TNF), hypophysectomy, vasectomy, inanition, diabetes mellitus, and early zinc or manganese deficiency (8, 11, 12, 15, 20). Other general reactions of the rat testicle to injury include: 1) altered testosterone secretion by interstitial cells (endotoxin, TNF, interleukin 1-β [IL-1β]); 2) infiltrates of inflammatory cells (IL-1β, luteinizing hormone [LH] or human chorionic gonadotropin [HCG], autoimmune, and infectious diseases); and 3) vascular alterations (5, 7, 17, 20). In order to better understand...
TABLE I. - Average weights of rats given varying doses of Pasteurella multocida group D heat-labile toxin.

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Toxin dose (µg/kg)</th>
<th>Average weight (g) Day 0</th>
<th>Day 18</th>
<th>Day 36</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
<td>259</td>
<td>255</td>
<td>-*</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>257</td>
<td>280</td>
<td>239</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>258</td>
<td>322</td>
<td>386</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>259</td>
<td>336</td>
<td>419</td>
</tr>
<tr>
<td>5</td>
<td>Diluent</td>
<td>253</td>
<td>373</td>
<td>457</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>202</td>
<td>-</td>
<td>ND*</td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>203</td>
<td>180</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>Diluent</td>
<td>202</td>
<td>348</td>
<td>ND</td>
</tr>
</tbody>
</table>

* - No surviving rats.
* Control group.
* Not done.

stand the in vivo effects and mechanisms of action of the P. multocida toxin, this study was designed to: 1) determine the effects of multiple, small doses of purified toxin on spermatogenic and Sertoli cells of the seminiferous tubules of rats and 2) compare the incidence of testicular lesions with those in other target organs such as spleen and liver.

MATERIALS AND METHODS

Preparation of D-Toxin

Toxin from P. multocida non-encapsulated strain P-4533 was purified as described previously (19). Briefly, bacteria were grown on horse blood agar and lysed with ethylenediaminetetraacetic acid (EDTA), lysozyme, Triton X-100, phenylmethylsulfonyl fluoride, and pepstatin A. The sonicate was centrifuged, and crude toxin was precipitated with ammonium sulfate. The precipitate was resuspended in 1-ml of phenylmethylsulfonyl fluoride, centrifuged, and the supernatant was applied to a 2.6 x 19 cm DEAE-sepharose (Pharmacia, Piscataway, NJ) column. The appropriate peak was concentrated and applied to a 4.4 x 85 cm Sephacryl S-200 (Pharmacia) column. The appropriate (second) peak was concentrated and used as purified toxin.

Experimental Design

Thirty male Holtzman rats were distributed into 5 groups of 6 rats each. Rats were selected to achieve a similar distribution of weights within groups 1-5 and an average weight of 258 g/rat/group (Table I). Rats were injected subcutaneously in the dorsal thoracic area on alternate days 0-22 of the experiment, with either toxin or sterile phosphate-buffered saline (controls), according to the dosages in Table I. One-half of the rats from each group were killed on day 18 using carbon dioxide, and the remaining rats were killed on day 36. Rats appearing moribund or lethargic were killed. Testis from rats killed on day 36, 14 days after the last injection of toxin, were used to evaluate possible regenerative/recovery changes.

Histopathology

All rats were necropsied, including those killed before scheduled necropsies. Sections of liver, lung, trachea, kidney, spleen, thymus, cerebrum, cerebellum, heart, adrenal gland, small and large intestine, cecum, pancreas, left proximal humerus, and testis were fixed by immersion in 10% neutral buffered formalin. To monitor for bacteremia or bacterial hepatitis (both of which may lead to hematoegenous spread of endotoxin [lipopolysaccharide]), liver samples from 7 rats were aseptically removed and cultured on appropriate media for aerobic and anaerobic organisms. Bone sections were decalcified with EDTA decalcifying solution (Scientific Products, McGaw Park, IL). Tissues were routinely processed, embedded in paraffin, cut at 4-6 µm, stained with H&E, and examined by light microscopy. Testicular lesions were scored on a scale from 1-4 using the following scale: 1 = mild cellular rounding and dissociation of spermatogenic cells in up to 50% of tubules; 2 = cellular rounding and dissociation in >50% tubules, decreased spermatids; 3 = marked cellular dissociation and cell rounding with giant syncytial cell formation, few spermatids, necrosis of seminiferous tube cells in occasional (one tubule/section) tubules; and 4 = severe loss of spermatogenic cells with giant cell formation, necrosis and mineralization of multifocal tubules (greater than one/section) and no spermatids.

Testicular Ultrastructure and Histopathology of Selected Tissues

In a similar protocol, 21 male Holtzman rats were divided into 3 groups; average weight of each group was 202 g. The rats were given subcutaneous injections of toxin on alternate days for 18 days and necropsied. One group (number 6) received 0.8 µg/kg/injection, a second group (number 7) received 0.4 µg/kg, and a third group (number 8) received phosphate-buffered saline as control. At the end of 18 days, rats were killed with carbon dioxide. One cubic millimeter portions of left testicle were fixed in 2.5% glutaraldehyde, rinsed in cacodylate buffer and processed through a series of graded ethanols and propylene oxide, and then the tissues were embedded in epoxy resin (epon). Thin sections were examined on a Philips electron microscope. The right testicle of each rat was weighed, fixed in neutral-buffered formalin, and examined with a light microscope. Other tissues taken for light microscopy included spleen, liver, and thymus.

TNFa Assay

Twenty-five male Holtzman rats with an average weight of 185 g were divided into 5 groups and received: 0.1 ml (1.0 µg/g body weight) purified LPS
TABLE I.—Lesion scores and weights of testicles from rats given purified type D toxin.

<table>
<thead>
<tr>
<th>Group number</th>
<th>Dose of toxin (µg/kg)</th>
<th>Mean score ± standard error</th>
<th>Average testicle weights (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.8</td>
<td>3.3 ± 0.37</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>0.4</td>
<td>1.5 ± 0.28</td>
<td>ND</td>
</tr>
<tr>
<td>3</td>
<td>0.2</td>
<td>0.5 ± 0.27</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>0.1</td>
<td>0 ± 0</td>
<td>ND</td>
</tr>
<tr>
<td>5 (control)</td>
<td>PBS</td>
<td>0 ± 0</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>4.0 ± 0</td>
<td>0.44</td>
</tr>
<tr>
<td>7</td>
<td>0.4</td>
<td>4.0 ± 0</td>
<td>0.49</td>
</tr>
<tr>
<td>8 (control)</td>
<td>PBS</td>
<td>0 ± 0</td>
<td>1.49</td>
</tr>
</tbody>
</table>

* Lesions were subjectively scored by using pre-determined criteria defined in Materials and Methods.
° ND = Not done.
PBS = Phosphate-buffered saline.
° Testicular weight based on moribund rats killed at 15 days of age.

(lipopolysaccharide) from Escherichia coli 055:B5 in nonpyrogenic, sterile saline (Baxter Healthcare Corporation, Deerfield, IL) (group 1); 0.1 ml (1.0 µg/g body weight) purified LPS from P. multocida X-73B in nonpyrogenic saline (group 2); 0.1 ml of 0.8 µg/kg purified P. multocida toxin in nonpyrogenic saline (group 3); 0.1 ml of nonpyrogenic saline (group 4); and no injections (group 5). One rat from each group was bled at 1, 2, 3, 4, and 20 hr.

Serum from each rat was assayed for tumor necrosis factor α (TNF) activity using TNF-sensitive WEHI 164 clone 13 cells according to previously described methods with a few modifications (17). Briefly, serum samples and standards were diluted in culture media (RPMI 1640 with HEPES) and 100 µl was added to wells of 96-well microtiter plates followed by 100 µl of 3 x 10⁵ WEHI cells/ml. Plates were incubated at 39°C, 5% CO₂ for 20 hr then centrifuged at 800 x g for 5 min and decanted. Dimethylthiazol diphenyltetrazolium bromide (MTT), 100 µl of 1 mg/ml, was added to wells, and plates were incubated at 37°C, 5% CO₂ for 1 hr. The plates were centrifuged, decanted and dried in a 40°C oven. Isopropanol, 100 µl, was added to wells and plates were read at OD560-OD690. Percent lysis = 100 x OD sample/OD media alone. TNF activity is proportional to percent lysis. Activity in samples was compared with rmTNF (Genzyme, Boston, MA) standards. One unit of TNF activity was equivalent to 25 pg rmTNF.

RESULTS

Rats given toxin, including those that did not have histologic lesions, gained weight at a much slower rate than controls, despite a subjective interest in food (Table I). The testicles from rats given toxin were notably smaller (groups 1–4, 6, and 7) and weighed less (groups 6 and 7) than testicles from control rats in group 8 (Table II). No growth occurred on aerobic and anaerobic plates inoculated with homogenized liver tissues.

Histopathology and Electron Microscopy of the Testes

Lesions which were generally restricted to groups of rats given the highest doses of toxin (groups 1, 2, 6 and 7), were seen at scheduled necropsies, as well as in moribund rats that were killed (Table III). There were no regenerative changes in seminiferous tubules of rats necropsied on day 36 (14 days of recovery time).

Seminiferous tubules had multifocal lesions that varied in degree of severity (Table III). Markedly affected tubules were often distended by sloughed cells, lacked an organized layer of mature spermatids, and contained large (approximately 25 µm in diameter), round cells with up to 8 small nuclei (Fig. 1). Other tubules were mildly dilated and spermatoctyes were necrotic. These tubules were lined by irregular, often cuboidal to flattened, Sertoli cells and were distended by necrotic cell debris (Fig. 2). Some tubules were partially mineralized, and there was mild peritubular edema. No prominent inflammatory cell infiltrates or vascular lesions were seen in any sections of testicle. Testicular lesions were classified according to severity, and the highest dos-

<table>
<thead>
<tr>
<th>Group</th>
<th>Death</th>
<th>Testicular degeneration</th>
<th>Splenic sheath degeneration</th>
<th>Liver necrosis</th>
<th>Nodular regeneration</th>
<th>Thymic necrosis</th>
<th>Osteoblast necrosis</th>
<th>Salivary duct necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/6</td>
<td>6/6</td>
<td>6/6</td>
<td>3/6</td>
<td>0</td>
<td>3.6</td>
<td>2/6</td>
<td>2/6</td>
</tr>
<tr>
<td>2</td>
<td>1/6</td>
<td>5/6</td>
<td>5/6</td>
<td>3/6</td>
<td>1/6</td>
<td>2/6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>1/6</td>
<td>1/6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>7/7</td>
<td>7/7</td>
<td>7/7</td>
<td>2/7</td>
<td>0</td>
<td>6/7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>7</td>
<td>3/7</td>
<td>7/7</td>
<td>7/7</td>
<td>5/7</td>
<td>0</td>
<td>3/7</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

a Ratios indicate numbers of rats with lesions/total number of rats per group.
b Control.
c ND = Not done.
es of toxin were associated with the most severe lesions (Table II). Epididymi generally lacked lesions, although epididymal tubules of severely affected testicles were dilated and empty.

Histologically, all toxin-treated rats in groups 6 and 7 developed severe testicular lesions (score = 4). Ultrastructurally, Sertoli cells of these rats were cuboidal to elongate, lacked cytoplasmic extensions, and contained one to several small to large (0.25–1 μm in diameter) round, homogeneously stained, cytoplasmic lipid inclusions. Some cells also contained autophagosomes and myelin figures. The smooth endoplasmic reticulum was markedly dilated and often contained a flocculent, osmiophilic substance. There were numerous glycogen rosettes in the cytoplasm and microtubules were sparse. Mitochondria of Sertoli cells were elongate and some were curvilinear with bilobed ends that partially surrounded some portions of cytoplasm. Between the plasma membranes of adjacent Sertoli cells, there were oval to elongate dilated and empty spaces. These spaces were separated by small extensions of cytoplasm joined centrally by tight junctions (Fig. 3). Similar intercellular spaces were also present between adjacent Sertoli and spermatogonial cells; however, tight junctions between these cells were absent. The Sertoli cell basement membrane was highly convoluted, and reduplicated in some areas. There was increased space (up to 250 nm) between the basement membrane and the subjacent myoid cell. This space often contained numerous collagen fibrils.

Secondary and tertiary spermatids were necrotic or rounded, individualized, and free in the tubular lumen (Fig. 4). The rounded cells had a markedly dilated cytocavitary network and an expanded, lucent cytoplasmic area. Some tubules contained multinucleate giant cells. These cells had abundant cytoplasm, dilated smooth and rough endoplasmic reticulum, Golgi apparatuses, and numerous perinuclear elongate and curvilinear mitochondria with lobed ends (Fig. 5). Some mitochondria formed small rings that encircled small areas of cytoplasm similar to those in Sertoli cells. These cells had one to several oval to round (0.5–1 μm) nuclei which peripheralized to crescent-like intranuclear material, and some contained mitochondridial elements. The lumen of seminiferous tubules contained numerous variably-sized (100–3,000 nm) circular membrane-bound vesicles and small (10 nm) osmiophilic flocculent densities of cell debris (Fig. 3).

Myoid cells were contracted and angular with plasma membranes segmentally accentuated by subplasmalemma osmiophilic densities (Fig. 4). Some cells were markedly contracted, which resulted in an irregular angular shape with several elongated cytoplasmic extensions. The cytoplasm was dark, and mitochondria remained within the central portion of the cell in a linear arrangement. The basement membrane subjacent to myoid cells was similar to Sertoli cell basement membrane; convoluted, reduplicated, separated by a short distance (300–600 nm) from the myoid cell, and this space contained numerous collagen fibrils.

Other Tissues

All rats in groups 1, 6, 7; 5 of 6 rats in group 2; and 1 rat in group 3 had lesions in both testicle and spleen. Rats in groups 1, 2, 3, 6 and 7 had decreased widths of splenic marginal zones, and marginal zones in several rats in groups 6 and 7 were replaced by numerous fibroblasts (Figs. 6 and 7). There was an inverse relationship between toxin dose and width
of marginal zones; that is, rats given higher doses of toxin generally had thinner marginal zones. The decreased width of splenic marginal zone macrophages affected all ensheathed arteries in individual rats to a similar degree. The thickness of the marginal zone was determined by counting the number of cell layers surrounding five ensheathed arteries from each spleen. In several rats that died of acute
FIG. 5.—Electron micrograph, rat testicle from group 7. A multinucleate cell which has several nuclei with peripheralized densities. One of these (arrow) contains several mitochondria. The nuclei are surrounded by numerous mitochondria which are often curved or form small circular structures with a central cytoplasmic area. The cytoplasm also contains numerous dilated vesicular spaces. The outer portion of cytoplasm is devoid of mitochondria. ×2,800.

toxicity, there was marked necrosis of macrophages in the marginal zone.

In livers from rats in groups 1, 2, 6, and 7, there were multifocal areas of hepatocellular necrosis and one liver from a rat in group 2 had multiple areas of nodular regeneration. Livers were similar to those described by Cheville et al (4) in toxin-treated rats and were characterized by multifocal areas of coagulative necrosis, moderate infiltrates of neutrophils and macrophages, and mild infiltrates of lymphocytes, and plasma cells. These areas also contained moderate numbers of fibroblasts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TNF activity (U/ml)</th>
<th>1 hr</th>
<th>2 hr</th>
<th>3 hr</th>
<th>4 hr</th>
<th>20 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS, <em>E. coli</em></td>
<td>7.0</td>
<td>6,000</td>
<td>18.2</td>
<td>6.2</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>LPS, <em>P. multocida</em></td>
<td>1.2</td>
<td>6.6</td>
<td>1.2</td>
<td>3.2</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td><em>P. multocida</em> toxin</td>
<td>t*</td>
<td>1.0</td>
<td>t</td>
<td>t</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Nonpyrogenic NaCl</td>
<td>t</td>
<td>0.4</td>
<td>t</td>
<td>t</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>No treatment</td>
<td>t</td>
<td>1.0</td>
<td>t</td>
<td>t</td>
<td>t</td>
<td></td>
</tr>
</tbody>
</table>

* t = trace amounts of TNF activity.

Thymuses from rats in groups 1, 2, 6, and 7 had necrosis of the outer cortex and were characterized by marked lymphocytolysis with large macrophages containing ingested cell debris. Necrosis of osteoblasts lining trabeculae of the primary spongiosa was seen in two rats in group 1 that died spontaneously.

**TNFα Assay**

Serum from rats given LPS from either *E. coli* or *P. multocida* had increased levels of TNF activity (Table IV). The highest levels of TNF activity were present at 1, 2, 3, and 4 hr. TNF activity was increased only slightly in 2 rats given toxin, in 1 rat given nonpyrogenic saline, and in 2 rats that did not receive injections. At 20 hr, the remaining toxin-treated rat was depressed, reluctant to move, and had a reddish-yellow nasal discharge; rats from other groups had no obvious clinical signs.

**DISCUSSION**

Although multiple small doses of purified *P. multocida* toxin resulted in seminiferous tubule degeneration, inflammatory cells were not associated with the initiation or progression of testicular damage. It
may be that: 1) the damage is caused by elevated levels of cytokines (TNF) induced by the toxin; 2) the lesions developed due to hormonal alterations; 3) the lesions are secondary to testicular ischemia; 4) the lesions are related to dietary alterations; or 5) the toxin has a direct effect on spermatogenic cells.

Some investigators have suggested that the *P. multocida* toxin may induce release of TNF or IL-1β. Rats given intravenous TNF develop testicular degeneration similar to the changes seen in this study (11). Although increases in the serum TNFα were not seen in rats given lethal doses of toxin, the toxin may induce testicular interstitial macrophages (a resident macrophage of the testicle) to produce TNF locally without an increase in serum levels. Increases in local production of TNF without corresponding elevations in serum concentrations occurs in alveolar macrophages of monkeys that are infected with simian immunodeficiency virus (SIV) (10).

In contrast to TNF, testicular damage in rats caused by IL-1β and altered levels of luteinizing hormone (LH) and human chorionic gonadotrophin (HCG) are associated with infiltrates of neutrophils (1, 2). Since inflammatory infiltrates were not seen in this study, it is unlikely that the toxin either mimicked or induced release of IL-1β or altered the levels of LH and HCG. Ischemia was also probably not the reason for testicular lesions, since intravascular fibrinous thrombi, vasculitis, and endothelial degeneration were not seen. Although rats appeared to retain an appetite, the lesions may have been exacerbated, or caused by inanition. Inadequate food intake may result in an insufficient supply of nutrients (zinc, manganese, vitamins A, D, and E) to the testis or possibly alter the levels of certain hormones, such as testosterone.

The testis does have a high level of vitamin D receptors, and non-inflammatory seminiferous tubule degeneration has been reported in vitamin D-deficient rats (13). It is not known whether the toxin influences this receptor, but high levels of vitamin D receptor are present in the trabeculae of the porcine turbinate, which is the most obvious site of toxin action in development of atrophic rhinitis. It is unlikely that the toxin, a glycoprotein that increases intracellular levels of inositol phosphate, would bind vitamin D receptor (which functions like a steroid receptor [intracytoplasmic binding]) (14). The toxin may instead bind a membrane component that is highly expressed in vitamin D-responsive tissues.

The mol. wt. of the toxin (142,000) would exclude it from passage across the blood-testis barrier (allows passage of molecules with M.W. < 10,000) under normal conditions; however, dilated spaces between Sertoli cells seen ultrastructurally may allow toxin to directly contact adluminal cells. Dye studies are needed to determine if there is an alteration in the integrity of the blood-testis barrier. Alterations in Sertoli cell function would also affect spermatocyte development, and may result in adluminal spermatocyte necrosis. Ultrastructural lesions present in Sertoli cells (dilated endoplasmic reticulum, increased autophagosomes, and myelin figures) are most likely indicative of decreased function, but whether these lesions are caused by the toxin or by the absence of developing spermatocytes could not be answered in this study. Sertoli and spermatogonial cells had direct exposure to toxin since these cells are not protected by the blood-testis barrier. Spermatogonial cells remained relatively unaffected and may lack receptors for the toxin or may not be affected by altered levels of as yet undetermined...
indigenous molecules, such as IL-1, IL-6, or hormones.

It is possible the toxin affects only those cells undergoing replication. With some cell lines (vero and endothelial cells) in vitro, the toxin has little effect on cells after 24 hr of incubation but has a profound effect (decreased growth and cell rounding) if added to the cell medium when cells are subcultured and undergo rapid replication. Coincidentally, organs with rapidly dividing cells (i.e., spleen, thymus, and testicle) were affected by toxin in the current study.

The effect of toxin on the testicle is probably not limited to the rat, since recently we have been able to produce similar testicular lesions in dogs given intravenous toxin at weekly intervals (data not shown).

The necrosis of adluminal spermatocytes seen ultrastructurally, along with the formation of giant multinucleated cells and sparing of spermatogonia, are similar to the lesions seen in TNF-treated rats, vitamin D-deficient rats, vitamin A-deficient rats, and vasectomized rats (4). In addition, vitamin E deficiency also results in spermatocyte necrosis and giant cell formation, and the giant cells of vitamin E-deficient rats have characteristic “crescent-like” nuclei (19). Multinucleate giant cells are commonly seen in degenerate seminiferous tubules of many species. Those present in this study had peripheralized to crescent-like intranuclear material and also certain cytoplasmic morphologic features (i.e., curvilinear mitochondria) that resembled Sertoli cells.

The occurrence of consistent severe lesions in testicles from groups 6 and 7 was likely due to the younger age of these rats when given their first dose of toxin. These rats also weighed less at the beginning of the experiment, with an average of 202 g for rats in groups 6 and 7 and 258 g for groups 1–5.

The decreased width of splenic marginal zones seen in some rats may be due to necrosis or degeneration of macrophages in this area followed by impaired ability to repopulate lost cells. This is supported by the finding of acute necrosis of marginal zone macrophages in several rats that were killed before scheduled necropsies. Cytokines such as HB-EGF may be released by degenerate and dying macrophages, resulting in splenic fibrosis (9). In our laboratory, we have applied toxin to mouse macrophage cell lines and found that cells round up and develop cytoplasmic vacuoles whereas control cells become elongate and lack such vacuoles (unpublished observations). Similar changes in the splenic marginal zone have also been seen in female rats given multiple doses of recombinant human cachectin (16).

In a previous study in our laboratory, severe hepatic lesions have been described in rats given a single dose of purified toxin (19). The lesions were suggested to be caused by direct hepatotoxicity and/or activation of hepatic macrophages to release toxic levels of IL-1 and TNF. Similar hepatic lesions were seen in some rats in this study; however, the more consistent lesions were testicular degeneration and decreased width of splenic sheaths. Moreover, testicular and splenic lesions were present in some rats in the absence of hepatic lesions. These organs may be more sensitive to multiple injections of low doses of toxin than is the liver.

REFERENCES


