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Experimental Epididymitis due to *Chlamydia trachomatis* in Rats

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A new animal model of epididymitis due to *Chlamydia trachomatis* was developed. Adult male Wistar rats were inoculated in the vas deferens with *C. trachomatis* biovar mouse pneumonitis. After infection, *C. trachomatis* was recovered from the epididymides for up to 90 days. At day 30, organisms were also reisolated from the testes. Clinical findings included enlargement of infected epididymides and concurrent atrophy of the ipsilateral testes. Histological lesions in the epididymides consisted of pyogranulomatous inflammation, abscesses, and spermatic granulomas. Infection of the testis by *C. trachomatis* was associated with pyogranulomatous changes. In addition, testicular degeneration, characterized by moderate to severe loss of the germinal epithelium, was noted. Chlamydial antigen was detected within epithelial cells, intratubular macrophages, and macrophages in the stroma of the epididymis by immunoperoxidase staining. This rat model of chlamydial epididymitis appears to clinically and histopathologically mimic the human disease. This model offers the opportunity for further studies on the pathogenesis and sequelae of chlamydial epididymitis.

Chlamydia trachomatis is one of the most common sexually transmitted pathogens and causes a wide spectrum of genital tract infections in humans. Clinical manifestations include cervicitis, endometritis, and salpingitis in women and urethritis and epididymitis in men (10).

Epididymitis caused by *C. trachomatis* most often affects sexually active young men (4, 13). In the United States, it is estimated that 200,000 cases of *C. trachomatis*-caused epididymitis occur each year (12). Clinically, the disease is characterized by unilateral, painful enlargement of the epididymis. Possible complications are abscess formation, testicular infarction, the development of chronic epididymitis, and impairment of fertility (3).

Studies on the pathogenesis of chlamydial epididymitis have been hampered by lack of a satisfactory animal model. To date, animal models using human strains of *C. trachomatis* in nonhuman primates and mice have been reported (9, 11). However, these models differ from the human disease with respect to the pathohistological responses.

The present study was undertaken to determine whether epididymitis in rats induced by the murine biovar of *C. trachomatis* (mouse pneumonitis [MoPn] agent) shows similarities to the human disease. We report on the clinical, microbiological, serological, and histopathological responses after inoculation of rats with the MoPn agent.

MATERIALS AND METHODS

Animals. Adult male Wistar rats (Zentralinstitut für Versuchstierzucht, Hannover, Germany) weighing 250 to 300 g were used. Animals were housed individually in a 12-h-light–12-h-dark cycle. Food and water were provided ad libitum. Several animals were screened by culture of homogenates of lungs and epididymides and serology for naturally occurring chlamydial infections. All animals were culture negative and seronegative.

Organism. The murine biovar of *C. trachomatis* (ATCC VR-123; American Type Culture Collection, Rockville, Md.) was grown in BGM cell monolayers in Eagle minimal essential medium supplemented with 10% fetal calf serum and 1 μg of cycloheximide per ml. Infected monolayers were harvested from culture flasks with a cell scraper and sonicated for 30 s. Cellular debris was removed by centrifugation at 500 × *g* for 10 min at 4°C. The supernatant was concentrated by centrifugation at 30,000 × *g* for 45 min at 4°C. The resulting pellet was suspended in 2-sucrose-phosphate buffer (pH 7.4) with 5% fetal calf serum, aliquoted, and stored at −70°C until use.

Inoculation procedure. All inoculations were performed under ketamine-xylazine anaesthesia and under surgical conditions. Following a scrotal incision, the right testis and epididymis were exteriorized. The inoculum (100 μl; 4×10^7 inclusion-forming units) was instilled into the lumen of the vas deferens by using a 27-gauge needle. Thirty-six animals were inoculated with *C. trachomatis*, and 12 control animals were inoculated with 100 μl of uninfected BGM cell preparations. At 3, 7, 14, 30, and 90 days postinfection (p.i.), groups of six infected animals were sacrificed. Six control animals were killed at 7 and 90 days p.i. At the time of sacrifice, epididymides and testes were examined for gross changes and removed aseptically. Organs were weighed and then sectioned longitudinally. Half were fixed in 10% nonbuffered formalin, and the remaining half were stored at −70°C.

Cultures for *C. trachomatis*. Tissue specimens were thawed and homogenized in Eagle minimal essential medium containing 10% fetal calf serum, 50 μg of gentamicin per ml, and 2.5 μg of amphotericin B per ml. Homogenates were sonicated for 30 s and centrifuged at 500 × *g* for 15 min at 4°C. The supernatant was inoculated onto BGM cell monolayers grown on coverslips (12 mm in diameter) in shell vials. The vials were centrifuged at 3,000 × *g* for 45 min at 4°C. After centrifugation, the inoculum was replaced by fresh medium containing 1 μg of cycloheximide per ml. Cells were incubated for 72 h at 37°C in 5% CO_2. Cells were then fixed with methanol, stained with Giminez, and examined microscopically for inclusions in a blind fashion. Inclusion-negative cultures were passaged a second time and screened as just described.

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TABLE 1. Weight responses of rat epididymides and testes after inoculation of *C. trachomatis* into the right vas deferens

Sample	Organ wt (g) at day p.i.[a]:				
	3	7	14	30	90
Right epididymis[b]	0.68 ±0.10	1.02 ± 0.37	1.21 ± 0.31	0.90 ± 0.28	0.52 ± 0.13
Left epididymis	0.55 ± 0.02	0.51 ± 0.06	0.56 ± 0.03	0.58 ± 0.03	0.63 ± 0.05
Right testis[c]	1.67 ± 0.23	1.02 ± 0.23	0.96 ± 0.37	0.74 ± 0.37	0.71 ± 0.18
Left testis	1.73 ± 0.24	1.56 ± 0.19	1.69 ± 0.10	1.72 ±0.08	1.63 ± 0.10

[a] Data represent mean ± standard deviation for six animals per time point.
[b] $P < 0.0001$ for all values compared with those of left epididymides (ANOVA).
[c] $P < 0.0001$ for all values compared with those of left testes (ANOVA).

Antibody response. Blood samples were collected by cardiac puncture from all animals before the inoculation and at the time of sacrifice. Serum antibody levels were determined by an enzyme-linked immunosorbent fluorescence assay described in detail before (7, 14). Elementary bodies of the MoPn agent were purified by discontinuous gradient centrifugation in Urografin (Urografin 76%; Schering, Berlin, Germany) and used as antigen (5). Control antigen consisted of uninfected BGM cells. Immunoglobulin M (IgM) and IgG antibodies were measured by using affinity-purified alkaline phosphatase-labeled rabbit anti-rat IgM and IgG (Jackson Immuno Research, West Grove, Pa.). Net fluorescence values were calculated by subtracting mean values of sera obtained before the inoculation from mean values of sera collected at the time of sacrifice.

Rabbit anti-*C. trachomatis* serum. New Zealand White rabbits were immunized intradermally with 100 μg of Urografin-purified elementary bodies of the MoPn agent in Freund's incomplete adjuvant at weeks 0, 2, and 6. One week after the last immunization, animals were bled. IgG antibodies were purified by affinity chromatography using protein A-Sepharose (Pharmacia, Freiburg, Germany) (6).

Histopathology and immunocytochemistry. Formalin-fixed tissues were embedded in paraffin. For histological examination, sections were stained with hematoxylin and eosin. To demonstrate *C. trachomatis* antigen, sections were stained immunohistochemically as described previously (2). Briefly, 4- to 6-μm-thick sections were mounted on gelatin-covered slides, dried, deparaffinized, and rehydrated. Following blockade of endogenous peroxidase, sections were sequentially incubated with rabbit anti-*C. trachomatis* antibody, the biotinylated link antibody, and the avidin-biotin-peroxidase complex (Vectastain Laboratories, Burlingame, Calif.).

Statistical analysis. Statistical analysis was performed with the two-way analysis of variance (ANOVA).

RESULTS

Clinical findings. After inoculation of *C. trachomatis* into the right vas deferens, mild to severe significant enlargement of the epididymides and concurrent significant atrophy of the ipsilateral testes were noted ($P < 0.0001$, ANOVA). Epididymal swelling was most prominent at 14 days p.i., when right epididymides were approximately twofold heavier than left epididymides (Table 1). In contrast, no swelling of the epididymis or atrophy of the testis was detected in any control animal. No significant differences in weight between the right and left epididymides and testes were observed in these animals (data not shown).

Cultures for *C. trachomatis*. Results of reisolation of *C. trachomatis* from the epididymides and testes are shown in Table 2. Chlamydiae were reisolated from the right epididymides of all 18 animals examined at 3 to 14 days p.i. At 30 and 90 days p.i., organisms were reisolated from epididymal tissues in four and five animals, respectively. Infection of the right testis was observed in only two animals at 30 days p.i. The largest numbers of viable chlamydiae (>70% of cells per coverslip infected) were recovered from the epididymides at 7 and 14 days p.i. In contrast, epididymides of rats sacrificed at 90 days p.i. yielded only small numbers of organisms (<5% of cells per coverslip infected). Left epididymides and testes of infected animals and the gonads of control animals were culture negative.

Antibody response. Antibodies to *C. trachomatis* in serum were first detected at 7 days p.i., when IgM antibodies were present in five rats and IgG antibodies were measured in three of these animals (Fig. 1). All animals examined at 14 and 30 days p.i. had specific IgM and IgG antibodies. At 90 days p.i., IgG antibodies were detectable in all animals and IgM antibodies were still observed in three animals.

No specific antibodies were found in the sera of control animals or in sera collected before the inoculation.

Histopathology. In the epididymides at 3 days p.i., mild interstitial cellular infiltration characterized by neutrophils and mononuclear cells was observed. Between 7 and 30 days p.i., intra- and intertubular inflammatory changes were most prominent. Lesions were found frequently in the cauda and rarely in the corpus and caput epididymidis. Tubular changes exhibited a broad spectrum of pathological alterations. Some tubules were dilated and engorged with neutrophils, macrophages, and intermingled spermatozoa (Fig. 2). Occasionally, spermatic granulomas with syncytial giant cells at the periphery and abscesses were observed. In other areas, few intratubular macrophages and detached tubular lining cells were present. In addition, moderate to focally severe interstitial inflammation consisting of lymphocytes, plasma cells, mast cells, and few neutrophils was noted. At 90 days p.i., lesions varied between individual animals from mild to moderate and consisted of mononuclear cellular infiltration, interstitial fibrosis, and multifocal pyogranulomatous changes.

TABLE 2. Reisolation of *C. trachomatis* from epididymides and testes after inoculation into the right vasa deferentia of rats

Sample	No. of culture-positive animals at day p.i.[a]:				
	3	7	14	30	90
Right epididymis	6	6	6	4	5
Left epididymis	0	0	0	0	0
Right testis	0	0	0	2	0
Left testis	0	0	0	0	0

[a] Six animals were tested at each time.

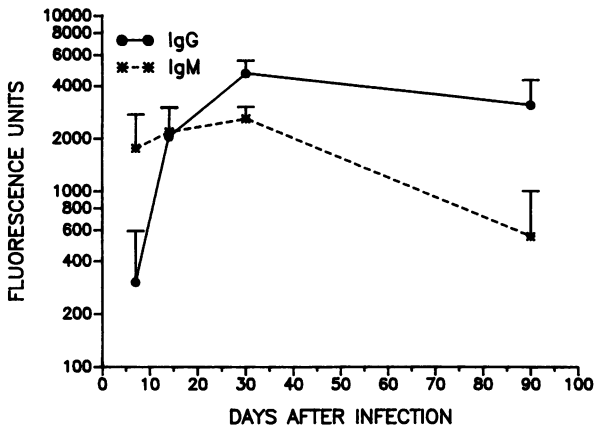

FIG. 1. Serum IgM and IgG antibody responses in rats as determined by an enzyme-linked immunosorbent fluorescence assay. Each point represents the mean ± standard deviation for six animals.

Degenerative changes of the right testes were observed in 21 animals. Testicular lesions were characterized by moderate to severe loss of the germinal epithelium, occurrence of spermatid giant cells, and increased interstitial fibrosis (Fig. 3). These changes were first recognized at 7 days p.i. and were still present on day 90. In addition, pyogranulomatous orchitis was detected in two animals at 30 days p.i.

Histopathological examination of the contralateral epididymides and testes and of the gonads of control animals did not reveal significant microscopic lesions.

Immunocytochemistry. *C. trachomatis* antigen was demonstrated predominantly in the cauda and rarely in the corpus or caput epididymidis. Positive immunoreaction occurred in cytoplasmic inclusions within the epididymal epithelium, in intratubular macrophages, and rarely in interstitial macrophages (Fig. 4). In the tubular lumen, fine granular immunopositive material, presumably representing individual organisms or antigenic debris, was frequently observed. Chlamydial antigen was detected in 21 of 27 culture-positive epididymides. At 3 days p.i., one rat was immunopositive. At 7 and 14 days p.i., all 12 animals were positive, whereas at 30 days p.i., five rats stained positive for chlamydial antigen, and at 90 days p.i., three rats stained positive.

In the testes, chlamydial antigen was seen in the two animals with culture-positive orchitis. Antigen distribution was restricted to the lesions, and antigen was found in the germinal epithelium and in the lumina of seminiferous tubules.

DISCUSSION

In the animal model described here, epididymitis was induced by inoculation of the murine biovar of *C. trachomatis* into the vasa deferentia of rats. Clinically, animals responded to the infection with marked enlargement of epididymides and concurrent atrophy of ipsilateral testes. Infection was confirmed by reisolation of chlamydiae, immunohistochemical demonstration of chlamydial antigen in tissues, and detection of a specific serological response.

Histopathological examination of infected epididymides revealed findings similar to those described in humans (8). Lesions were prominent in the cauda epididymidis and included distension of the epididymal duct, infiltration and destruction of the lining epithelium, abscess formation, and occurrence of spermatic granulomas. These changes may lead to an occlusion of the epididymal duct. In addition to inflammatory lesions in the epididymides, degenerative changes in the ipsilateral testes, characterized by decreased

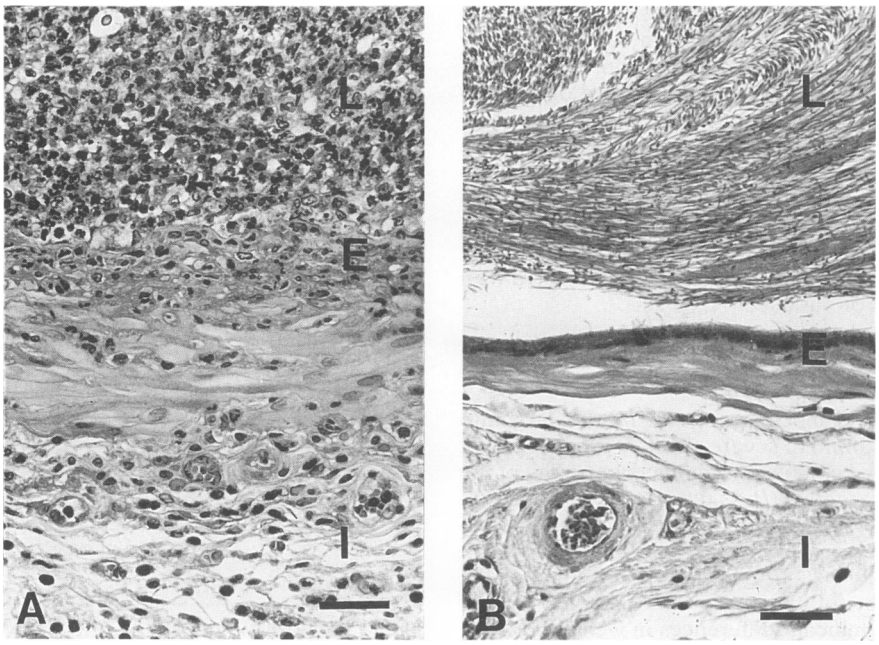

FIG. 2. (A) Photomicrograph of rat epididymis showing intratubular (L) abscessing and mild interstitial mononuclear cellular infiltration at 7 days after inoculation of *C. trachomatis* into the vas deferens. (B) Photomicrograph of the epididymis from a sham-inoculated control animal showing normal morphology. Tissue was removed at 7 days postinoculation. L, lumen of the epididymal duct; E, epididymal epithelium; I, interstitium. Hematoxylin and eosin stain. Bars, 40 μm.

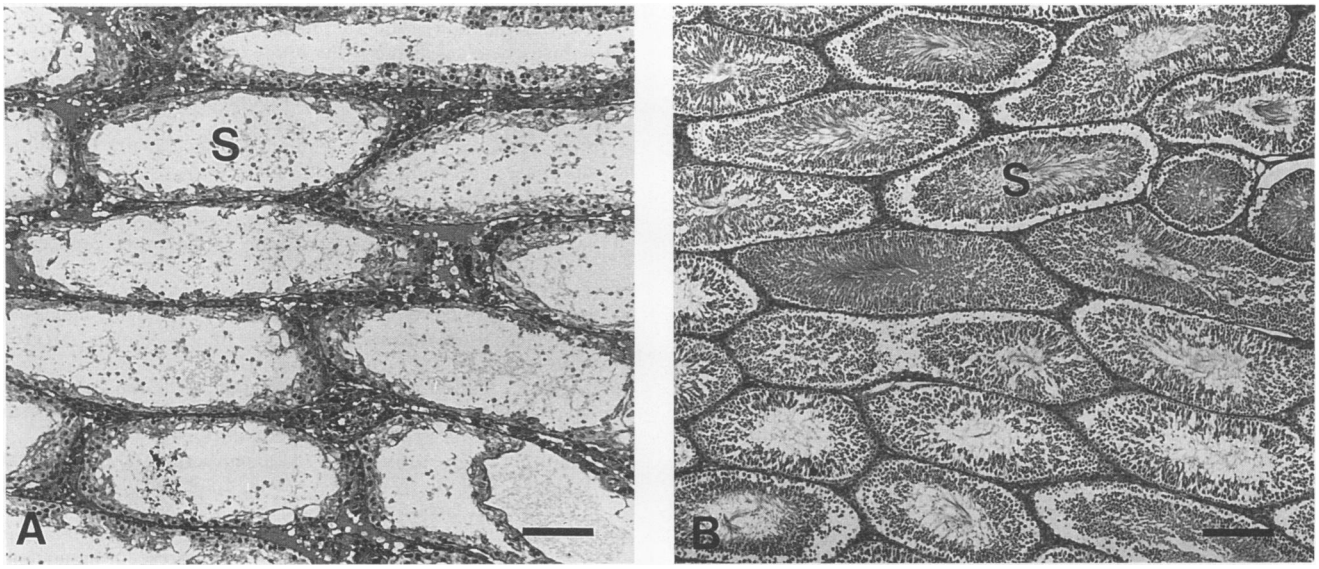

FIG. 3. (A) Photomicrograph of the testis from a rat with ipsilateral *C. trachomatis* epididymitis at 30 days p.i. Note the severe loss of the spermatogenic epithelium. (B) Photomicrograph of the testis from a control animal. No reduction in spermatogenesis is evident. S, seminiferous tubule. Hematoxylin and eosin stain. Bars, 300 μm.

spermatogenesis, were frequently observed. These changes were first noted at 7 days p.i., when epididymides were significantly enlarged. It is likely that the decreased spermatogenesis results from testicular hypoperfusion. Enlargement of the inflamed epididymis may cause either arterial occlusion or venous thrombosis of the testicular vessels (16). Whether unilateral occlusion of the epididymal duct and decreased spermatogenesis lead to an impairment of fertility remains to be determined.

An unexpected finding in this study was the persistence of chlamydiae, demonstrated both by culture and immunohistochemistry until the end of the observation period at 90 days p.i. Chlamydial antigen was detected in the epididymal epithelium, intratubular macrophages, and macrophages in the stroma. The duration of infection is in disagreement with results obtained from experimental female genital tract infections in mice (1, 15). In these studies, clearance of the MoPn agent was complete by days 7 and 14. These discrepancies may be attributable to differences in the spread and site of replication of the organism in the female and male genital tracts. The immunological status and immunological reactions in the epididymis are not well documented. To date, it is unknown whether the epididymis is an immunologically privileged site. However, persistent infection of rat epididymides with the MoPn agent represents a new finding that requires further study.

It is generally assumed that chlamydiae spread canalicularly through the vas deferens from the urethra to the epididymis (3). Further intralumenal spread to the testis may be restricted by the length of the epididymal duct. However, organisms may reach the testis through the lymphatic vessels. Infection of the testis was observed in two animals at 30

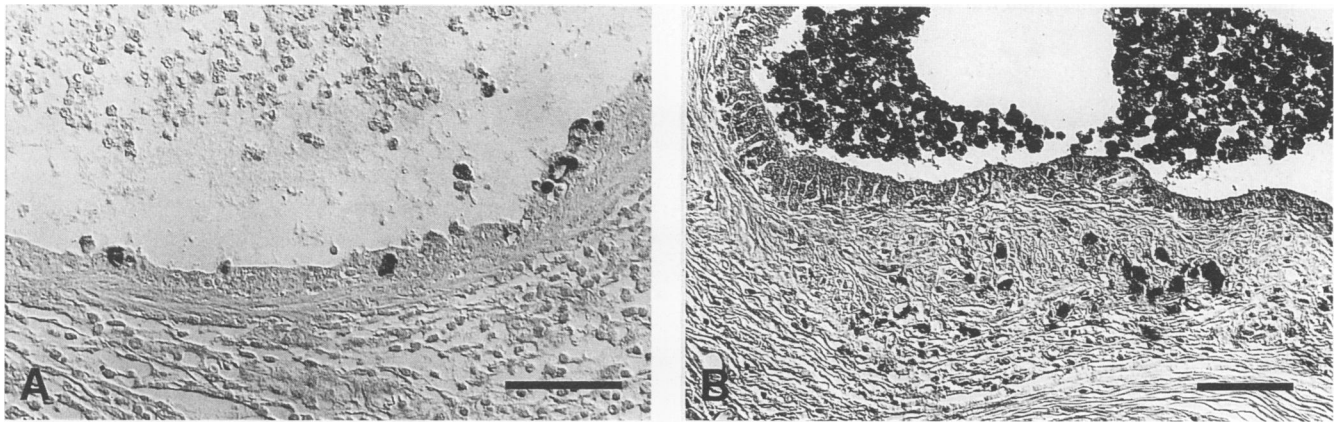

FIG. 4. (A) Immunoperoxidase photomicrograph of rat epididymis at 7 days after infection with *C. trachomatis*. Darkly staining chlamydial antigen is seen in inclusion bodies within the epididymal epithelium and within single intratubular macrophages. (B) Immunoperoxidase photomicrograph of rat epididymis at 30 days p.i. Demonstration of darkly staining chlamydial antigen in numerous intratubular macrophages and in macrophages within the surrounding interstitium. Nomarski optics; avidin-biotin-peroxidase complex. Bars, 50 μm.

days p.i. Testicular invasion by the organisms was associated with pyogranulomatous changes. In humans, involvement of the testis by infection with *C. trachomatis* is not documented (4, 13). Though the present observation has been made in an animal model, future studies of chlamydial epididymitis in men should address the possible infection of the testis.

Previous studies of experimental chlamydial epididymitis have used human strains of *C. trachomatis* in nonhuman primates and mice. Inoculation of *C. trachomatis* serovar K into the vasa deferentia of monkeys induced an inflammatory response in all layers of the vas deferens and in the lumen of the epididymal duct. Inflammatory infiltrations and lesions were not observed in the epididymis. Chlamydiae were recovered from the vas deferens but not from the epididymis (11). After direct inoculation of *C. trachomatis* serovar E into the epididymides of mice, organisms were reisolated for up to 21 days p.i. Histopathologically, an inflammatory cellular infiltrate in the stroma and a flattening of the epididymal epithelium were noted (9). Results of these two studies differ from those of the present study with respect to the microbiological and histopathological findings. The reason for these discrepancies may be differences in animal species, size of the inoculum, and pathogenicity of the different *C. trachomatis* strains.

The murine biovar of *C. trachomatis* which was used in this study is a natural parasite of mice and not known to infect humans. Lack of human pathogenicity of this strain may be regarded as a potential limitation of this model. However, the usefulness of any model depends on its relevance to the human disease. Clinical and histopathological findings in infected rats are similar to those seen in the human situation. We believe that this model offers the opportunity to examine the mechanisms of pathogenesis, the immune response, and the sequelae of epididymitis caused by *C. trachomatis*.

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