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Mouse Strain-Dependent Variation in the Course and Outcome of Chlamydial Genital Tract Infection Is Associated with Differences in Host Response

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Whether there is a pathogenic or protective outcome to chlamydial infection may be defined by the host response. We infected C57BL/6 (C57) and C3H/HeN (C3H) mice with the human biovar of *Chlamydia trachomatis*, serovar E, and, in select experiments, with the mouse pneumonitis agent of *C. trachomatis* (MoPn). We compared the courses of infection, histopathology, and host responses that resulted from these infections. The duration of infection with either chlamydial biovar was significantly increased in the C3H strain of mice. The intensity of infection was examined in mice infected with serovar E, and it was significantly increased in the C3H strain. Histopathology revealed the incidence of severe hydrosalpinx to be significantly greater in C3H mice than in C57 mice. In contrast, severe distention of the uterine horns was observed in all infected C57 mice compared to none of the C3H mice infected with serovar E and only 25% of those infected with MoPn. Acute inflammation was significantly increased in the uterine horns of C57 mice compared to that of C3H mice. Examination of antigen-specific responses revealed qualitatively similar responses in the two strains. Determination of gamma interferon- versus interleukin 4- producing cells revealed the predominance of a Th1 response in both strains. Serum enzyme-linked immunosorbent assays for immunoglobulin G1 (IgG1) and IgG2a revealed a predominance of IgG2a antibody in both strains, although the levels of antibody were significantly greater in C3H mice. Lymphocyte proliferation studies revealed increased proliferation in the iliac nodes of both strains at 1 to 3 weeks after infection. Because of the early eradication of infection observed in the C57 strain, we explored the relative production of tumor necrosis factor alpha (TNF-α) in the two strains. TNF-α levels were significantly increased in the genital tract secretions of C57 mice compared to that of C3H mice during the first week of infection. Increased TNF-α may be beneficial to the host by leading to earlier eradication of infection, thereby preventing infection of the oviduct and thus the major disease sequelae associated with chlamydial infection of the genital tract.

In women, *Chlamydia trachomatis* is a major cause of salpingitis, which may lead to infertility or ectopic pregnancy (14). It is estimated that 200,000 women per year in the United States become involuntarily infertile as a result of chlamydial salpingitis (20). Studies have demonstrated a higher rate of isolation of chlamydiae from the endometrium in humans than previously anticipated (10), indicating that upper tract infection may be a relatively common event subsequent to lower tract infection. Since only a small percentage of individuals develop upper tract pathology upon infection (3), a genetic predisposition to the development of pathology may exist.

In animal models of experimental infection, genetically controlled differences in susceptibility to chlamydial infection have been observed among inbred mouse strains (5–7, 23). Data from de la Maza et al. (5) revealed the development of different degrees of infertility in different inbred mouse strains infected with the mouse pneumonitis agent (MoPn) by intravaginal inoculation. In addition, Tuffrey et al. (19) found genital tract changes in C3H mice infected with human serovars of *C. trachomatis* via intrauterine inoculation to be more severe than those in other inbred strains. The mechanisms underlying these genetically determined differences have not been defined.

Using a murine pneumonia model induced by intranasal inoculation of MoPn, Yang et al. (23) compared cytokine patterns and immune responses elicited in C57BL/6 and BALB/c mice. BALB/c mice had a significantly higher mortality rate and slower clearance of the MoPn agent from the lungs than did C57BL/6 mice. Their study demonstrated excessive interleukin 10 (IL-10) production in BALB/c mice in association with decreased Th1-like responses, including gamma interferon (IFN-γ) expression and the delayed-type hypersensitivity response to chlamydial infection, with a consequent delay in the resolution of infection. Thus, this study suggested a protective role for Th1-like responses and the delayed-type hypersensitivity response as regards chlamydial pneumonia.

The immunopathogenesis of trachoma and of genital tract disease due to *C. trachomatis* may be more complicated than the disease process in the lung. The immune response itself may be a double-edged sword, leading to eradication of infection on the one hand but promoting the development of pathological tissue damage on the other. The component(s) of the response to chlamydial infection that causes tissue damage and whether it can be separated from those that allow destruction of the pathogen itself remains to be determined. We have observed prompt eradication of chlamydial genital tract infection in C57BL/6 mice, compared to a relatively prolonged

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infection of greater intensity in C3H mice. The enhanced level of infection seen in C3H mice is associated with increased pathology in the oviducts of this strain. In this study, we sought to determine whether mouse strain-dependent variation in the course and outcome of chlamydial genital tract infection is associated with differences in nonspecific and/or specific host responses.

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MATERIALS AND METHODS

Animals. Female C3H/HeN (C3H; *H-2*^{k}) and C57BL/6 (C57; *H-2*^{b}) mice (6 weeks old) were purchased from Harlan Sprague-Dawley, Indianapolis, Ind. The mice were given food and water ad libitum in an environmentally controlled room with a cycle of 12 h of light and 12 h of darkness. Female mice 7 to 10 weeks of age were used throughout the study.

Infection. Mice received 2.5 mg of medroxyprogesterone acetate (Depo-Provera; Upjohn, Kalamazoo, Mich.) subcutaneously at 10 and 3 days before vaginal infection and again at 30 days of infection. The mice were infected by placing 30 μl of 250 mM sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (pH 7.2; SPG) containing 10^7 inclusion-forming units (IFU; 2,000 50% infective doses) of McCoy cell-grown *C. trachomatis* human serovar E into the vaginal vault. In select experiments, McCoy cell-grown MoPn (10^7 IFU) was used for infection. Infection was administered with the mice under sodium pentobarbital anesthesia. Control mice of each strain were inoculated with SPG. In certain experiments, mice who had recovered from a primary infection were rechallenged intravaginally following one injection of medroxyprogesterone acetate given 7 days prior to inoculation of chlamydiae. The course of infection was monitored by swabbing the vaginal vault and ectocervix with a Calgiswab (Spectrum Medical Industries, Los Angeles, Calif.) at various times following infection and by enumerating IFUs by isolation on McCoy cell monolayers (11). Isolations from the upper genital tract were determined by removing the upper one-third of the uterine horn, the oviduct, and the ovary from the rest of the genital tract and then performing isolations solely on tissue homogenates of the upper tract. The right and left uterine horns were processed independently. The tissues were removed and frozen at −70°C in 1.0 ml of sucrose-phosphate buffer (17) until processed. Tissues were homogenized with an automated tissue homogenizer at low speed for 10 s, and then 0.5 ml of homogenized specimen was transferred to a 15-ml centrifuge tube. This was water sonicated for 30 s and centrifuged at 300 × *g* for 10 min at 4°C. The supernatant was diluted in Eagle's minimal essential medium containing 10% fetal bovine serum, 5% glucose, 100 μg of vancomycin per ml, 50 μg of gentamicin per ml, 2.5 μg of amphotericin B per ml, and 0.5 μg of cycloheximide per ml. Individual wells were inoculated in duplicate with 200 μl of the solution described above, and isolations were performed by a standard method (11). Inclusions were visualized by indirect immunofluorescence (11). Immune serum from serovar E-infected or MoPn-infected mice was used as the primary antibody, followed by fluorescein-tagged goat anti-mouse immunoglobulin G (IgG; ICN Immunobiologicals, Irvine, Calif.). The number of inclusion bodies within 20 fields (40×) was counted under a fluorescent microscope, and the numbers of IFU per milliliter were calculated.

Histopathology. Mice were sacrificed at various times following infection or inoculation of buffer; the entire genital tract was removed en bloc, fixed in 10% buffered formalin, and embedded in paraffin. Longitudinal 4-μm sections were cut, stained with hematoxylin and eosin, and evaluated by a pathologist blinded to the experimental design. Each anatomic site (ectocervix, endocervix, uterine horn, oviduct, and mesosalpinx) was assessed independently for the presence of acute inflammation (neutrophils), chronic inflammation (lymphocytes), plasma cells, and fibrosis. In addition, luminal distention of the uterine horns and dilatation of the oviducts were graded from 1 to 4. Right and left uterine horns and right and left oviducts were evaluated individually. A four-tiered semiquantitative scoring system was used to quantitate the inflammation and fibrosis, as follows: 0, normal; 1+, rare foci (minimal presence) of parameter; 2+, scattered (1 to 4) aggregates or mild diffuse increase in parameter; 3+, numerous aggregates (>4) or moderate diffuse or confluent areas of parameter; 4+, severe diffuse infiltration or confluence of parameter.

Evaluation of serum antibody responses. Sera from mice were collected at various times by retroorbital bleeds and stored at −20°C until analyzed. Antibody responses were assayed by enzyme-linked immunosorbent assays (ELISAs). Microtiter plates were coated with 5 μg of UV-inactivated serovar E antigen per ml grown in HeLa cells and purified on a Renografin gradient in 0.5 M $NaHCO_3$ overnight at room temperature and then blocked with a 5% solution of horse serum in phosphate-buffered saline for 30 min. Serum samples were applied to the plate in serial dilutions, incubated at 37°C for 1 h, and washed with phosphate-buffered saline–Tween 20. Preimmune sera were used as negative controls. Either goat anti-mouse IgG (1:10,000 dilution), IgG1 (1:5,000 dilution), or IgG2a (1:10,000 dilution) conjugated to horseradish peroxidase (class and subclass specific; Southern Biotechnology Associates, Birmingham, Ala.) was added to the plate, incubated at 37°C for 1 h, and then washed. The bound antibody was then visualized by adding the substrate 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid (Sigma Chemical Co., St. Louis, Mo.) in 0.1 M citrate buffer (pH 4.35) at a concentration of 0.3 mg/ml. Optical densities were read after 1 h at 405 nm. The titer for individual mice was determined as the highest serum dilution with an optical density value greater than that of the control wells.

Preparation of genital tract MNCs. Mice were sacrificed by cervical dislocation, and their genital tracts were removed, weighed, and placed in RPMI 1640 containing 0.6 mg of collagenase type I (Sigma) per ml. The genital tracts were minced extensively with a scalpel, and the suspension was incubated for 1 h at 37°C in 5% CO_2. The cell suspensions were filtered through a 70-μm-pore-size nylon cell strainer (Becton Dickinson, Franklin Lakes, N.J.), and mononuclear cells (MNCs) were obtained by centrifugation of the cell suspension over a layer of Lympholyte M (Cedarlane Laboratories, Hornby, Ontario, Canada). These cells were washed twice and resuspended in RPMI 1640 containing 10% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2 mM L-glutamine, and 50 mM 2-mercaptoethanol (complete medium). The yield of MNCs from murine genital tracts varied depending on the time after genital infection when killed. To compensate for the potential variation due to these parameters and to obtain a satisfactory number of cells for the assays, tissues from at least 10 mice were pooled at each time point.

Preparation of MNCs from lymphoid tissue. Iliac nodes which were pooled from 10 mice were placed separately in RPMI 1640, and single-cell suspensions were prepared by filtering them through a sterile cell strainer. These suspensions were treated with 0.17 M Tris-buffered NH_4Cl (pH 7.3) to lyse erythrocytes, washed twice, and then resuspended in complete medium. MNCs were then used in in vitro proliferation assays and enzyme-linked immunospot (ELISPOT) assays.

Proliferation assays. Cells were seeded into 96-well tissue culture plates (Falcon, Becton-Dickinson, Lincoln Park, N.J.) at 2×10^5 cells per well. UV-inactivated serovar E antigen, purified on a Renografin gradient (2), was added to the wells at 5 μg/ml. In some cases, as a positive control, concanavalin A (Sigma) was added to cultures at 2.5 μg/ml. Control wells received medium alone for the determination of background response in the absence of antigen or mitogen. The proliferative response was measured by the incorporation of 1 μCi of [*methyl*-^{3}H]thymidine per well over the last 18 to 24 h of a 5-day culture period. Cells were harvested onto Packard (Meriden, Conn.) Self-Aligning RG glass fiber filters with a Packard FilterMate cell harvester. Uptake of [*methyl*-^{3}H]thymidine was measured by a determination of total counts over a 6-min time period with a Packard Matrix 96 Direct Beta Counter. The mean response was obtained from an average of triplicate cultures. Data were expressed as mean antigen-specific counts after subtraction of background counts.

ELISPOT assay. The ELISPOT assay for detection of IFN-γ- and IL-4-secreting cells was that described by Cain and Rank (1). Briefly, nitrocellulose-based 96-well plates (Millititer HA; Millipore Corp., Marlborough, Mass.) were coated overnight with a primary antibody (2 μg/ml) directed against either murine IFN-γ (R46A2) or murine IL-4 (1D11). Coating and detecting pairs of antibodies were purchased from PharMingen (San Diego, Calif.). MNCs (10^7/ml) were stimulated with 5 μg of E antigen per ml or 2.5 μg of concanavalin A per ml overnight before being added to the plates. Cells were added at various dilutions ranging from 2×10^5 to 5×10^3 per well to the plate in triplicate. After 20 h of incubation, the plates were washed and then incubated overnight with a secondary biotinylated antibody (4 μg/ml) directed against either IFN-γ (XMG1.2) or IL-4 (24G2). The plates were then washed and incubated with 2.5 μg of avidin-peroxidase (Vector, Burlingame, Calif.) per ml for 1 h; color development with 3-amino-9-ethylcarbazole (Vector) followed. Spots indicative of locations of cells secreting cytokines were enumerated with the aid of a dissecting microscope. The mean number of cytokine-secreting cells counted in triplicate samples at a single dilution was used to calculate the spot-forming-cells per million.

Measurement of TNF-α in genital tract secretions. To determine the kinetics of tumor necrosis factor (TNF) secretion, genital tract secretions were collected from mice on multiple days throughout the course of serovar E infection and analyzed for TNF by ELISA or bioassay. At intervals before and after infection or inoculation of buffer, an aseptic surgical sponge (2 by 5 mm; Weck Ophthalmologicals, Atlanta, Ga.) was inserted into the vagina of an anesthetized animal and retrieved 30 min later. The sponges were held at −70°C until they were eluted in either 80 μl of PBS containing 0.05% Tween and 0.05% Na azide for use in a murine TNF alpha (TNF-α) ELISA (Factor-Test-X; Genzyme Immunobiologicals, Cambridge, Mass.) or in 100 μl of Eagle's minimal essential medium for use in a bioassay. Eluates of sponges from the same strain and time point were pooled for assays.

The ELISA for TNF-α was performed as described in the manufacturer's instructions. Precision was >95% between duplicate samples. The L929 cytotoxicity assay was used to determine levels of biologically active TNF present in genital tract secretions. As described previously (4), mouse L929 fibroblasts were plated in 96-well plates at a concentration of 4×10^4 cells per well. After an overnight incubation, samples were added to the plates and serially diluted. To determine the specificity of the assay, appropriate dilutions of selected samples or standard recombinant murine TNF-α (Genentech, San Francisco, Calif.) were preincubated with 5 neutralizing units of polyclonal rabbit anti-mouse TNF-α

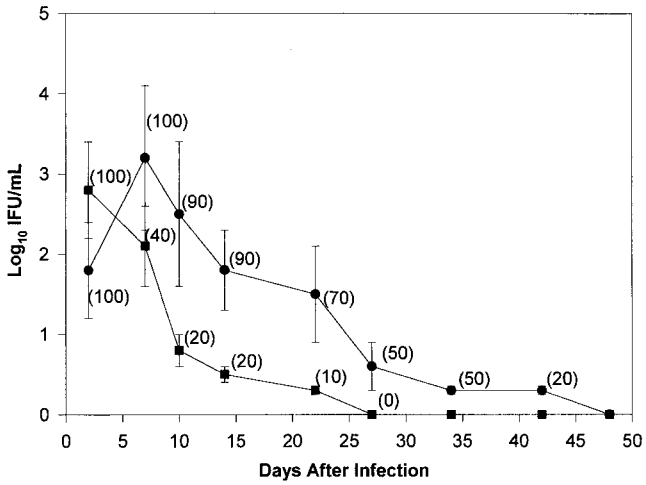

FIG. 1. Intensity and duration of primary lower genital tract infection with human serovar E of *C. trachomatis* in C3H (●) and C57 (■) mice. Quantitative isolations were performed on vaginal swabs collected at various times after infection. Numbers of inclusions were visualized and enumerated in McCoy cell monolayers by indirect immunofluorescence. Data points represent means ± standard errors of the means of duplicate determinations with 10 animals examined on each day. Numbers in parentheses are percentages of animals positive for infection on that day.

(Genentech) or nonspecific rabbit IgG for 4 h at 4°C before addition to the plates. Actinomycin D (Sigma) was added to each well, and the plates were incubated overnight. Samples were decanted from the adherent L929 cells, and monolayers were washed twice with PBS. Residual cells were stained with 0.05% crystal violet in 20% ethanol for 10 min, washed, dried, and eluted with 100 μl of methanol per well. The optical density at 570 nm was determined in a microplate reader. The optical density of L929 cells incubated with medium alone represents 0% lysis, and that of cells treated with 3 M guanidine hydrochloride represents 100% lysis. One unit of TNF is defined as the amount of TNF required to produce 50% lysis.

Statistics. Statistical comparisons between the two murine strains for level of infection and TNF-α production were made by a two-factor (days and murine strain) analysis of variance with repeated measures (RM ANOVA) on one factor (days). Differences between the two murine strains in lymphocyte proliferation and ELISPOT cytokine data were analyzed by the Student *t* test. The Wilcoxon rank sum test was used to compare the duration of infection in the respective strains over time. The Kruskal-Wallis one-way ANOVA on ranks was used to determine significant differences in the pathological data between groups. Comparisons of pathological data within groups were made by use of the Student-Newman-Keuls method of all-pairwise multiple comparisons. The *z* test for determination of significant differences in sample proportions was used to compare frequencies of pathological findings between specific groups.

RESULTS

Course of lower genital tract infection with serovar E in C57 versus C3H mice. The durations and levels of lower genital tract infection with human serovar E of *C. trachomatis* were compared for C57 and C3H mice. Two groups of 10 mice of each strain were vaginally infected, and quantitative isolations were performed on vaginal swabs taken at intervals throughout infection (Fig. 1). Vaginal isolations from C57 mice were all negative for infection by day 27, whereas 2 of 10 C3H mice remained positive on day 40. Thus, the duration of infection was significantly prolonged in the C3H strain ($P = 0.004$ for C57 versus C3H mice; Wilcoxon rank sum). The intensities of infection over time were compared for the two groups by two-way RM ANOVA, and the intensity in the C3H strain was found to be significantly increased when compared to that of the C57 strain ($P < 0.0005$). In C57 mice, the numbers of organisms detected in the lower genital tracts declined rapidly. Earlier studies in our lab examining the duration of MoPn chlamydial infection in these two strains of mice also found it to be significantly prolonged in C3H mice compared with that in C57 mice ($P = 0.003$) (data not shown).

Course of upper genital tract infection with serovar E in C57 and C3H mice. We performed an experiment to determine the relative invasiveness of serovar E in the two strains. Twenty-five mice of each strain were infected intravaginally with serovar E, and groups of five mice of each strain were sacrificed at intervals (days) after infection. Upper genital tract tissue homogenates were cultured for chlamydiae, and quantitative isolations were compared for the two strains (Fig. 2). Only two of five C3H mice were positive for infection in the upper genital tract 7 days postinoculation, compared to all five of the C57 mice sacrificed on that day. Thus, it took longer for the chlamydiae to ascend to the upper tracts of the C3H mice, but once established in the upper uterine horns, the infection was significantly prolonged in this strain ($P = 0.03$ for C3H versus C57 mice). Organisms continued to be isolated from the upper tracts of C3H mice through day 35. The intensity of upper tract infection over time was significantly increased in the C3H strain when compared to that of C57 ($P = 0.01$ by two-way RM ANOVA) (Fig. 2). These data indicate that although serovar E readily establishes infection in the upper tracts of both strains, the duration and level of infection are significantly increased in the C3H strain when compared to that in the C57 strain.

Chronic histopathology in uterine horns of C57 versus C3H mice. We sought to determine whether the different courses of infection found for these two strains of mice were reflected in differences in long-term pathology. Therefore, eight mice of each strain that had been inoculated with human serovar E or with MoPn were sacrificed after 42 days and their genital tract tissues were examined for histopathology. Figure 3E to H show representative examples of the uterine horns from both strains of mice sacrificed 42 days after intravaginal inoculation with human serovar E. Figure 3A to D show uterine horns of control mice for comparison. Infected animals of both strains had a persistence of acute and chronic inflammatory cells in the submucosal epithelium, with chronic inflammatory cells being the predominant infiltrate. There was greater destruction of

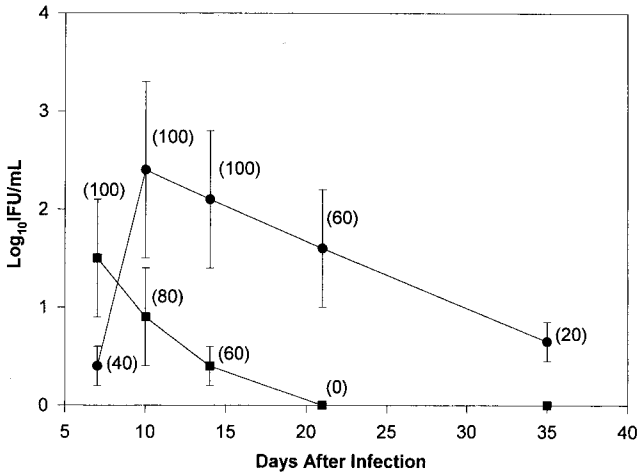

FIG. 2. Intensity and duration of primary upper genital tract infection with human serovar E of *C. trachomatis* in C3H (●) and C57 (■) mice. Quantitative isolations were performed on homogenized tissues from the upper uterine horns of the respective strains collected at various times after infection. Numbers of inclusions were visualized by indirect immunofluorescence. Data points represent means ± standard errors of the means for duplicate determinations, with five animals examined on each day. Numbers in parentheses are percentages of animals positive for upper tract infection on that day.

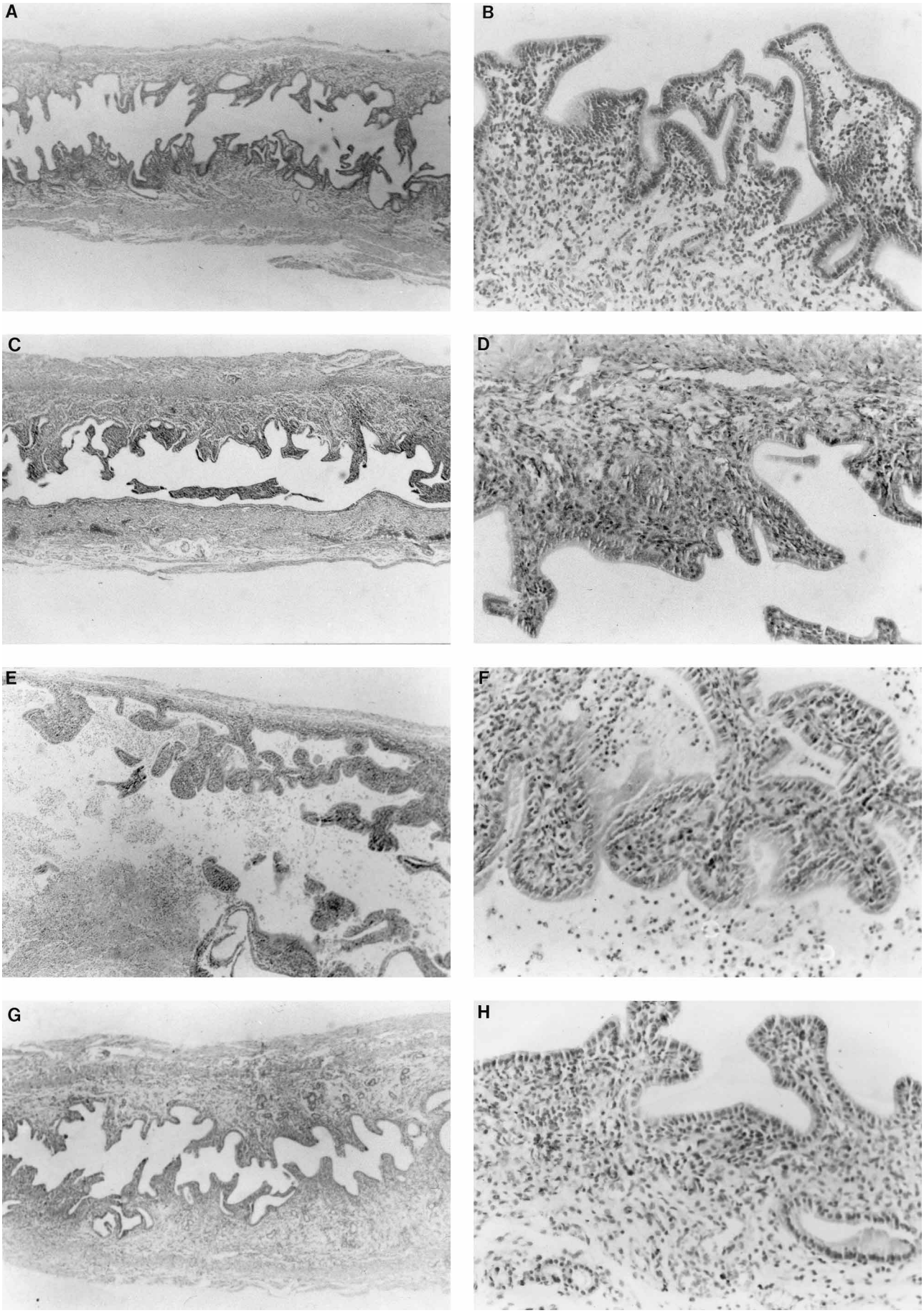
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FIG. 3. Histopathological evaluation of uterine horn tissue from C3H and C57 mice. Micrographs are representative examples from noninfected C57 mice (A and B), noninfected C3H mice (C and D), C57 mice 42 days after infection with serovar E (E and F), and C3H mice 42 days after infection with serovar E (G and H). Magnification, ×50 (A, C, E, and G) and ×100 (B, D, F, and H).

the uterine horn epithelium in C57 mice (Fig. 3E and F) than in C3H mice (Fig. 3G and H). Marked mucosal edema in the horns of C57 mice caused marked distention of the horns themselves. Control groups included (i) animals receiving medroxyprogesterone acetate 10 and 3 days before and 30 days after vaginal inoculation of buffer (Fig. 3A to D), (ii) animals not receiving medroxyprogesterone acetate but inoculated intravaginally with buffer, and (iii) untreated, uninoculated animals. There were no pathologic changes in any of the control animals.

When the respective mouse strains were infected with MoPn, the same pattern occurred (data not shown). There was greater destruction of the uterine horn epithelium in the C57 strain than in the C3H strain, and the uterine horns of the C57 mice were more edematous and distended.

The frequencies of severe uterine horn distention were compared for the two mouse strains. After serovar E infection, 16 of 16 uterine horns (100%) from C57 mice were severely distended, compared to 0 of 16 uterine horns (0%) from C3H mice. Similarly, in mice infected with MoPn, 16 of 16 (100%) of the C57 uterine horns were severely distended compared to only 3 of 16 (19%) of the uterine horns from C3H mice. Thus, surprisingly, the highest degree and frequency of uterine horn pathology occurred in C57 mice, the strain with the shortest course of infection.

Chronic histopathology in oviducts of C57 versus C3H mice. No inflammatory infiltrates were found in the oviducts of either murine strain 42 days after infection with serovar E (data not shown). In both C57 and C3H mice, the structure and integrity of the oviduct epithelium were intact and there were only mild degrees of dilatation. Mild oviduct dilatation occurred in 12 of 16 (75%) oviducts from C3H mice and 8 of 16 (50%) oviducts from C57 mice. Thus, chronic pathology was minimal in the oviducts of mice infected with serovar E, and there was no difference between the two strains.

In mice infected with MoPn and sacrificed on day 42, there was a continued presence of acute and chronic inflammatory cells in the oviducts of C3H mice, whereas inflammatory cells were notably absent from the oviducts of C57 mice (Fig. 4C and D). In addition, although there was some degree of oviductal dilatation in all of the mice of both strains, 16 of 16 (100%) oviducts from C3H mice had severe dilatation or hy-

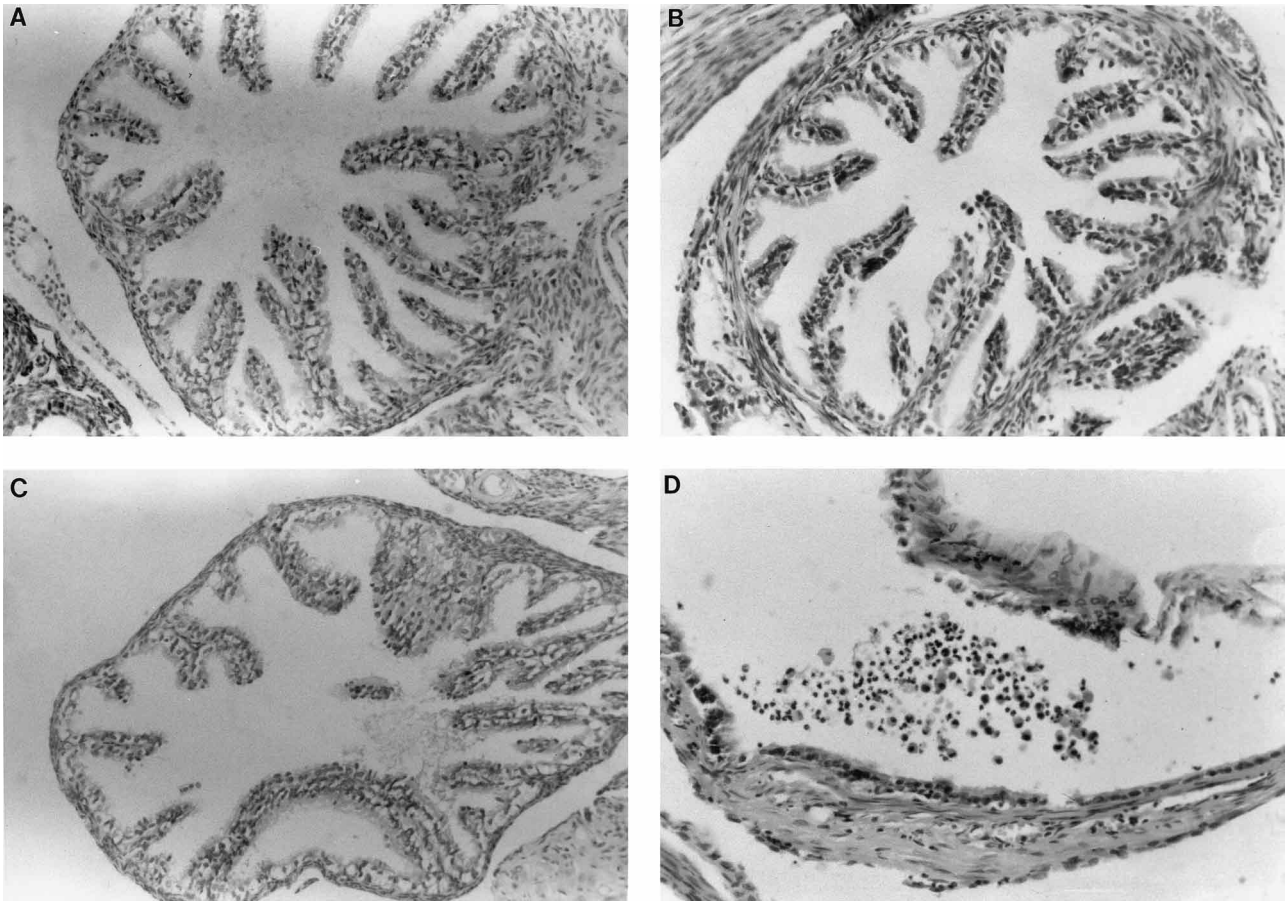

FIG. 4. Histopathological evaluation of oviducts from C3H and C57 mice. Micrographs are representative examples from noninfected C57 mice (A), noninfected C3H mice (B), infected C57 mice (C), and infected C3H mice (D) sacrificed 42 days after vaginal inoculation with the MoPn agent of *C. trachomatis*. Magnification, ×100.

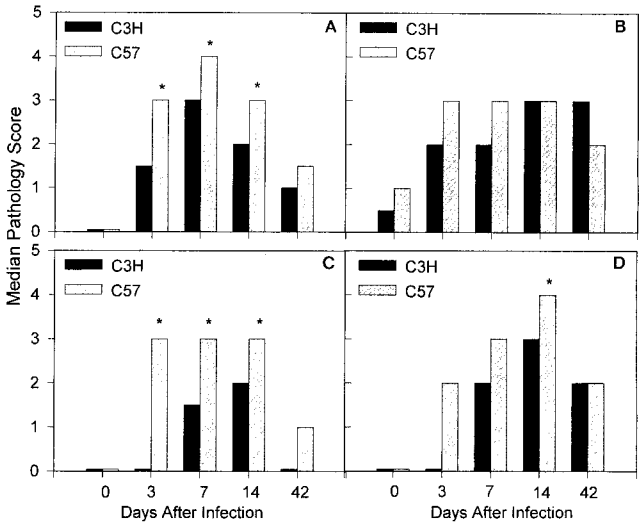

FIG. 5. Comparison of the relative degrees of acute (neutrophilic infiltrates) (A and C) and chronic (lymphocytic infiltrates) (B and D) inflammation in the uterine horn tissues of C3H and C57 mice over the course of infection with human serovar E of *C. trachomatis* (A and B) and MoPn of *C. trachomatis* (C and D). Bars represent median pathology scores calculated from eight mice of each strain sacrificed at each time point. *, $P < 0.05$ for C3H versus C57.

drosalpnix (Fig. 4D) compared with 6 of 16 (38%) oviducts from C57 mice ($P = 0.005$ for C3H versus C57). The majority of C57 mice had only mild (6 of 16) or moderate (4 of 16) degrees of oviductal dilatation (Fig. 4C). Thus, there was a higher degree and a greater frequency of pathology in the oviducts of C3H mice, the strain with the longest course of infection. There was no pathology in medroxyprogesterone acetate-treated animals inoculated with buffer and then sacrificed at 42 days (Fig. 4A and B).

Cellular infiltrates in uterine horns of C57 versus C3H mice. We examined the kinetics and the relative degrees of acute inflammation (neutrophils) and chronic inflammation (lymphocytes) in the uterine horns and oviducts of the respective strains over the course of infection. Regardless of the biovar used for infection, moderate levels of acute inflammation were observed in the uterine horns of C57 mice as early as day 3 of infection. In contrast, in the C3H strain, the influx of acute inflammatory cells was delayed until day 7 (Fig. 5). Significantly higher degrees of acute inflammation occurred on days 3, 7, and 14 in the uterine horns of C57 mice infected with serovar E or with MoPn (Fig. 5A and C). Thus, regardless of the biovar used for infection, acute inflammatory infiltrates were increased in C57 mice in the uterine horns. Inflammation declined in both strains by day 42. A comparison of the median levels of chronic inflammatory cells in the uterine horns of the respective strains revealed similar degrees of chronic inflammation in both groups (Fig. 5B and D). Plasma cell infiltrates paralleled those of chronic inflammatory cells, and interestingly, fibrosis was not observed in any animal (data not shown).

Cellular infiltrates in oviducts of C57 versus C3H mice. No acute inflammation was observed in the oviducts of C57 mice infected with serovar E, and although present, acute inflammation was very mild in C3H mice (Fig. 6A). Chronic inflammatory cells were also few in number (Fig. 6B). When MoPn was used for infection, an influx of acute inflammatory cells was observed in the oviducts of both strains by day 7. Higher numbers of acute inflammatory cells occurred in the C3H strain on days 7, 14, and 42 than in the C57 strain, although the differences between the strains were not significant (Fig. 6C). The chronic inflammatory responses observed after MoPn infection were similar for both strains (Fig. 6D). Moderate degrees of chronic inflammation were present on day 14, with levels approaching baseline by day 42. Plasma cell infiltrates again paralleled those of chronic inflammatory cells, and fibrosis was not observed in any animal (data not shown).

Antigen-specific responses in C57 versus C3H mice. We next sought to determine whether the variation seen in the course of infection and pathological outcome was associated with differences in antigen-specific host responses. Lymphocyte proliferative responses of iliac node lymphocytes to UV-inactivated elementary bodies were examined over the course of infection with serovar E. The ELISPOT assay for detection of cytokine-secreting cells was also used to determine frequencies of antigen-specific IFN-γ- and IL-4-secreting cells in the genital tract tissues and iliac nodes, thus determining whether a predominant Th1 or Th2 response developed in the respective strains. Serum IgG1 and IgG2a antibody levels to E antigen were analyzed by ELISA. Table 1 summarizes the results from these experiments. Iliac node cells from both strains exhibited high proliferative responses to serovar E antigen on day 7, with peak levels being observed on day 14 for both strains. On day 18, the proliferative responses had declined in both strains. The predominance of a Th1 cytokine response was suggested in both strains by the detection of high numbers of IFN-γ-producing cells and very few IL-4-producing cells. The numbers of IFN-γ-producing cells were similar in both strains (data not shown). Total serum IgG was higher and more prolonged in the C3H strain than in the C57 strain. Titers of IgG2a were significantly higher than IgG1 titers in C3H mice during primary infection (data not shown). In the C57 strain, IgG levels were too low during primary infection to determine a predominant subtype. A challenge infection revealed a predominance of IgG2a in both strains (Table 1). Thus, no difference existed in the quality of the T-cell response nor in the production of antibody that could explain the variation in the course and outcome of infection.

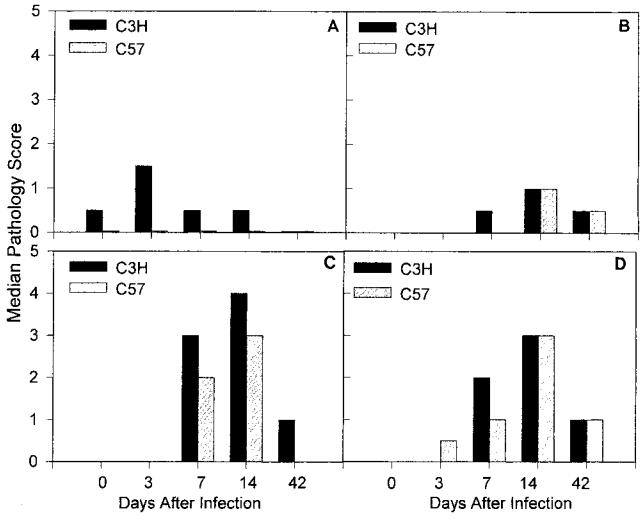

FIG. 6. Comparison of the relative degrees of acute (neutrophilic infiltrates) (A and C) and chronic (lymphocytic infiltrates) (B and D) inflammation in the oviducts of C3H and C57 mice over the course of infection with human serovar E of *C. trachomatis* (A and B) and MoPn of *C. trachomatis* (C and D). Bars represent the median pathology scores calculated from eight mice of each strain sacrificed at each time point. *, $P < 0.05$ for C3H versus C57.

TABLE 1. A comparison of antigen-specific responses in C3H and C57 mice[a]

Type of antigen-specific response	Strain comparison	Response in:	
		C3H mice	C57 mice
Iliac node proliferation response	Similar responses in both strains	Increased on days 7, 14, and 18; peak on day 14 (mean responses of 42,000 [day 7], 74,000 [day 14], and 27,000 [day 18] counts)	Increased on days 7, 14, and 18; peak on day 14 (mean responses of 31,000 [day 7], 70,000 [day 14], and 23,000 [day 18] counts)
T-helper phenotype (Th1 vs. Th2)	No difference	Th1 predominant (IFN-γ>>IL-4)	Th1 predominant (IFN-γ>>IL-4)
Antibody response[b]	C3H>>C57	IgG2a>>IgG1	IgG2a>>IgG1

[a] The following antigen-specific responses were compared in C57 and C3H mice after infection with human serovar E of *C. trachomatis*: iliac node lymphocyte proliferative response, Th phenotype of iliac nodes and genital tract tissues (numbers of IFN-γ- and IL-4-producing cells), and serum antibody response (levels of total IgG, IgG1, and IgG2a specific for serovar E antigen).
[b] Antibody response detected after challenge infection.

TNF-α response in C57 versus C3H mice. The earlier resolution of chlamydial infection by the C57 strain combined with the increased degree of acute inflammation observed in the uterine horns of the C57 mice suggested the possibility of enhanced mechanisms of innate immunity in this strain compared to that of C3H. Thus, we next sought to determine the relative levels of an important proinflammatory cytokine, TNF-α, in the respective strains. Levels of TNF-α in endocervical secretions were determined by ELISA and by bioassay with murine L929 fibroblast cells. When TNF-α levels detected over the course of infection were compared between the two strains by a two-factor (days and strain) RM ANOVA on one factor (days), C57 mice had significantly higher levels of TNF-α ($P = 0.04$). Figure 7 shows the combined TNF-α results from two separate experiments analyzed by ELISA. As early as 2 days after infection, high levels of TNF-α were present in the C57 strain, and levels remained elevated through day 6, when the peak TNF-α response was detected (4,250 ± 430 pg/ml). In contrast, in the C3H strain, TNF-α levels gradually increased through day 5, where a peak level was determined to be only 1,650 ± 140 pg/ml. In both strains, a decline in TNF-α was observed by day 7 of infection. Bioassay for TNF-α revealed similar kinetics of the responses in both strains, and TNF-α activity was significantly increased in the C57 strain when compared to that of C3H (data not shown).

DISCUSSION

In this study, we determined that the duration of serovar E genital tract infection in C3H/HeN ($H\text{-}2^k$) mice is significantly prolonged when compared to that of C57BL/6N ($H\text{-}2^b$) mice. Also, greater numbers of organisms were isolated from the genital tracts of C3H mice than from those of C57 mice. Moreover, histopathological studies revealed marked differences in the outcome of chlamydial infection in these two murine strains. Chronic tissue changes detected at 42 days revealed oviduct pathology to be most severe in the C3H strain, whereas uterine horn pathology was most severe in the C57 strain.

de la Maza et al. (5) observed an increased rate of infertility in C3H mice infected intravaginally with MoPn when compared to C57 mice (30% pregnancy rate in C3H compared to 75% pregnancy rate in C57). They also observed more pathology in the oviducts of C3H mice than in those of C57 mice (three of four C3H mice had unilateral or bilateral hydrosalpinx compared to one of four C57 mice). This study together with ours suggests that the C3H strain of mice may be prone to increased tissue damage in the oviduct when compared to the C57 mouse strain.

Progesterone was used in these studies to enhance the initial infection rate. Progesterone treatment is not essential but enhances genital tract infection with chlamydiae by altering the estrous cycle to maintain the target epithelium. In addition, the numbers of inflammatory cells present in the genital tract, particularly polymorphonuclear leukocytes (PMNs), fluctuate with the estrus cycle. Thus, a valid comparison between numbers of inflammatory cells among individual mice could not be made without control of the estrus cycle. Mice of both strains injected with progesterone and vaginally inoculated with buffer exhibited no pathology greater than that of naive animals. Thus, although we cannot rule out an effect of progesterone treatment on the pathology that occurred in the presence of infection, progesterone treatment in and of itself did not result in any pathological change in the genital tract tissues.

The low degree of pathology observed in the oviducts of mice infected with serovar E was not surprising. Ito et al. (8) inoculated progesterone-treated CF-1 mice intravaginally with seven different human serovars of *C. trachomatis*, and although serovars D and E were found to ascend to the upper uterine horns, ovarian and oviduct infections were not documented. Tuffrey et al. (18, 19) documented the absence of oviduct

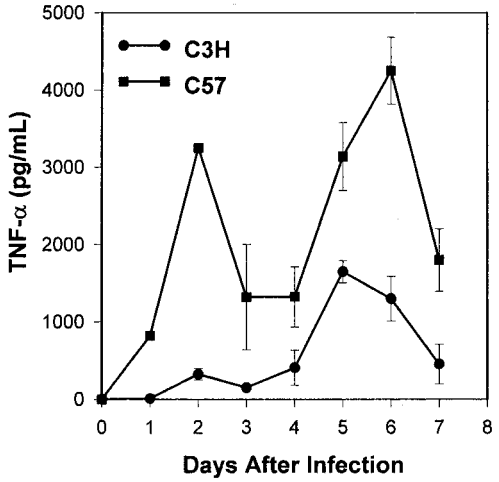

FIG. 7. TNF-α levels (means ± standard errors of the mean) in genital tract secretions of C3H and C57 mice over the course of primary infection with human serovar E of *C. trachomatis*. Levels of TNF-α were determined with an ELISA kit specific for murine TNF-α. Data represent the combined results of two separate experiments ($N = 5$ per strain per experiment). Each sample was run in duplicate.

infection in mice inoculated via the intravaginal route with human serovars of *C. trachomatis* and so resorted to injection of the ovarian bursa or of the upper uterine horn to produce salpingitis. Our use of MoPn for select experiments allowed us to examine the effect of genetically defined host response differences on oviduct pathology. The reason(s) for the decreased level of infection attained by the human serovars when used in the mouse has not been determined.

The significance of these studies lies in the potential determinations of critical points in the complex process of host-chlamydia interaction where the paths towards healing and tissue destruction depart. Less oviduct pathology occurred in the C57 strain, where the duration and intensity of infection were significantly less. Thus, in this model, host responses that promote early eradication of infection are associated with a decreased risk of chronic pathology in the oviduct. Of course, one must be cautious in extrapolating these data to chlamydia-induced fallopian tube pathology in the human. The lack of fibrosis in this murine model is evidence for dissimilarities in pathogenesis in the mouse as compared to the human, where scarring and fibrosis occur as a result of tissue damage (15).

An influx of acute inflammatory cells occurred earlier and to a significantly higher degree in the uterine horns of infected C57 mice than in infected C3H mice. In contrast, the chronic inflammatory responses were very similar in the two strains. The enhanced acute inflammatory response in the uterine horns of C57 mice may be beneficial by leading to earlier eradication of infection, preventing infection of the oviducts and thus the occurrence of hydrosalpinx. On the other hand, severe distention of the uterine horns occurred in all of the infected C57 mice, compared to none of the C3H mice infected with E and only 25% of the C3H mice infected with MoPn. There was greater destruction of the uterine horn epithelium in the C57 strain, and mucosal edema was frequent. Although not statistically significant, there was a suggestion of higher numbers of acute inflammatory cells in the oviducts of the C3H strain, the strain with the highest degree and frequency of hydrosalpinx. These data suggest an important role for the acute inflammatory response and its products in the development of tissue damage associated with a primary chlamydial infection in the murine model. Of course, our study was conducted after a single infectious episode; thus, we cannot comment on the relative importance of acute and chronic inflammatory responses with repeated infections.

When antigen-specific responses were explored for differences that might explain the marked variation seen in the duration and outcome of infection between these two strains, no significant differences were found. Antigen-specific T-cell proliferative responses in the iliac nodes were similar in degree and kinetics. Both strains exhibited a predominance of a Th1 response, as evidenced by high numbers of IFN-γ-producing cells in the presence of very few IL-4-producing cells in the iliac nodes as well as in the genital tract tissues. Further evidence for a Th1 response being dominant in both strains is the relative preponderance of IgG2a compared to IgG1. The higher levels of total IgG and of IgG2a during primary infection in the C3H strain compared to those in the C57 strain likely reflect the prolonged presence of organisms in this strain. Earlier studies by Cain and Rank (1) demonstrated the induction of Th1-like responses in the genital tract and draining iliac nodes of BALB/c mice infected intravaginally with MoPn.

The decreased duration and level of infection observed in the C57 strain and the relative lack of oviduct pathology in this strain imply that the C57 strain has some advantage over the C3H strain with regard to the protective host response to chlamydial infection. Although no significant differences were found in antigen-specific responses between the two strains, the innate response of TNF-α production was definitely increased in the genital tracts of C57 mice. Of course, TNF-α levels were determined from genital tract secretions collected from the lower genital tract, and we cannot be certain that these levels reflect those of the upper uterine horns and oviducts.

Williams et al. (21) demonstrated with a murine model of chlamydial pneumonia that exogenous administration of anti-TNF-α antibody significantly accelerated mortality and caused a borderline increase in MoPn counts in the lung. TNF-α is secreted primarily by macrophages, under the influence of lipopolysaccharide, and it has frequently been documented as an initiatory cytokine released upon bacterial invasion of a host. It can induce a significant influx of neutrophils and has a strong activating effect on these cells, in addition to other leukocytes and endothelial cells. We have documented high levels of TNF-α in the genital tract secretions of guinea pigs during primary chlamydial infection at a time of marked neutrophil influx (4).

The increased levels of TNF-α observed in the C57 strain may promote the accelerated acute inflammatory response seen early on in the uterine horns of C57 mice, effecting earlier eradication of infection and thereby protecting the oviduct from chlamydial invasion. In other animal models of disease, monocytes and macrophages from C57 mice have been reported to have enhanced bactericidal activity in comparison to other inbred murine strains (22). In both the mouse and guinea pig models (4), the peak TNF-α activity in vaginal secretions occurs just prior to the time of marked neutrophil influx associated with primary chlamydial infection. Resident macrophages and resident neutrophils may be among the first to release TNF-α. Such TNF-α release could then stimulate PMN influx, adherence, and further production of TNF-α. Of course, with respect to C57 mice, such increased TNF-α activity in the face of an accelerated acute inflammatory response may be a double-edged sword, causing increased pathology in the uterine horn tissue but protecting the oviduct in the process.

The reason for enhanced TNF-α production in C57 mice is not clear. In a study by Jacob et al. (9), a genetic basis for differential production of TNF-α was explored. When, under conditions that induce optimal levels of TNF-α, identical numbers of macrophages from various mouse strains were activated with IFN-γ and lipopolysaccharide, the different mouse strains produced either low, intermediate, or high levels of TNF-α. The variability in TNF-α production appeared to be major histocompatibility complex associated, and studies with *H-2* recombinant mouse strains suggested a linkage between TNF-α production and *H-2*. Interestingly, the C3H strain was found to produce levels of TNF-α twice those of the C57 strain. We have determined similar results with peritoneal macrophages from uninfected C3H and C57 mice. Yet, when we obtained peritoneal macrophages from animals after intraperitoneal injection of serovar E elementary bodies, the macrophages from C57 mice produced higher levels of TNF-α when stimulated in vitro with IFN-γ and lipopolysaccharide than did macrophages from C3H mice (unpublished data). Thus, it seems that it is the chlamydial infection itself that activates macrophages in the C57 murine strain to produce higher levels of TNF-α. Although macrophage production is a likely candidate, it is possible that the higher levels of TNF-α observed in vivo in the C57 mice are due to increased PMN numbers and activity in this strain.

In addition to TNF-α, it is possible that differential production of other proinflammatory cytokines may be involved. Data from a murine model of chlamydial pneumonia have demon-

strated the induction of IL-1 and IL-6 (12) and of colony-stimulating factors (13) early on during the course of chlamydial infection. Recent data from Rasmussen et al. (16) demonstrated in vitro production of IL-1 and IL-8 by human epithelial cells infected with *C. trachomatis* or *Chlamydia psittaci*. These and other mediators of the innate host response need to be examined and compared in these two strains of mice as well as in other congenic murine strains.

A delineation of responses that promote immune clearance from those that are pathogenic is critical to the rational development of effective methods of chlamydial disease prevention. The data we have gathered to date suggest that nonspecific host responses determine the differential course and outcome of chlamydial infection observed in these two strains of mice. A role for TNF-α as a cytokine important in host defense against primary chlamydial infection is suggested in this murine model. But, as in other disease states, although TNF-α and other products of the acute inflammatory response may help to eradicate infection, they may also promote lasting tissue damage.

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