Effects of Corticosteroids and Cyclophosphamide on a Mouse Model of *Chlamydia trachomatis* Pneumonitis

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Suppression of the inflammatory reaction with daily doses of cortisone acetate or cyclophosphamide substantially prolonged the pulmonary infection in mice which had been intranasally inoculated with a trachoma biotype of *Chlamydia trachomatis*. Titration of organisms recovered from the lungs of treated mice revealed a drop in titer after day 2 (postinfection), followed by a prominent increase on day 6. In cyclophosphamide-treated mice the infection was resolved after day 12, whereas in cortisone acetate-treated mice a significant titer remained after day 17. In contrast, no organisms were recoverable after day 6 in control mice treated with saline or in mice treated with hydrocortisone succinate. Histologically, the ability of cortisone acetate and cyclophosphamide to inhibit the inflammatory reaction correlated with the respective course of chlamydial pneumonitis. This study demonstrated that mice were intrinsically capable of sustaining a lung infection induced by a human strain of *C. trachomatis*.

*Chlamydia trachomatis* is recognized as a common cause of human eye and urogenital tract infection (7). In 1975, Schachter et al. first reported a case of *C. trachomatis* pneumonia in an infant (16). Since that report, *C. trachomatis* pneumonia in infants has been well defined and repeatedly described (2). It is now recognized as a major cause of pneumonia in infants (15). Recently, *C. trachomatis* pneumonitis has been reported in immunocompromised adults (17). To facilitate studies of the immunopathogenesis of these organisms in the lung, Kuo and Chen developed and refined a mouse model of *C. trachomatis* pneumonitis (3, 11). Although these studies unequivocally demonstrated chlamydial infection of the mouse lung, the duration of the pneumonia in the mouse was relatively short. The peak of the infection was observed by the 2nd day postinfection, followed by rapid elimination of the organisms. Chlamydiae are obligate intracellular bacteria whose biphasic growth cycle, in cell culture, takes 2 to 3 days to complete. Thus, in the mouse lung, only limited cycles of infection had taken place. Similarly, chlamydial inclusions observed histologically in the mouse lung seemed to contain only small numbers of mature elementary bodies. The mouse is not the natural host for trachoma biotypes of *C. trachomatis*, and these observations suggest that the cells in the mouse lung may be nonpermissive for growth of these organisms, thereby resulting in an apparent self-limiting infection.

To determine if the apparent self-limiting infection in the mouse model is caused by an intrinsically incompatible host-parasite relationship, or whether it is controlled by immunological effecter functions of the host, we studied the microbiological and histological course of *C. trachomatis* pneumonitis in immunosuppressed mice. The results of this study demonstrated that the mouse lung was permissive for repeated cycles of chlamydial replication, and immunosuppressive therapy with cortisone acetate (CA) or cyclophosphamide (Cy) resulted in a prolonged course of infection.

**MATERIALS AND METHODS**

**Organism.** Immunoype B, ocular *C. trachomatis* strain B/TF-5/OT grown in HeLa 229 cells was used for mouse inoculations (13). The inocula contained 5 × 10⁴ inclusion-forming units (IFU) of infectious organisms per ml.

**Inoculation of mice.** Six-week-old male Swiss-Webster white mice (Washington State University, Pullman) weighing 27 to 31 g were inoculated intranasally with 0.04 ml of *C. trachomatis* suspension or with a control suspension of HeLa 229 cell material prepared from uninfected cell cultures as previously described (11).

**Treatments of mice.** Mice were weighed and given doses of drug based on the mean weight. Mice whose weight deviated more than 4 g from the mean were not used in these studies. Mice were given daily 0.2-ml injections of one of the following: pyrogen-free saline, intraperitoneally or subcutaneously; hydrocortisone succinate (HC) (Solu-Cortef, The Upjohn Co., Kalamazoo, Mich.), 125 mg/kg subcutaneously; CA (Cortone; Merck Sharp & Dohme, West Point, Pa.), 125 mg/kg intraperitoneally; or Cy (Cytoxan; Mead Johnson Laboratories, Evansville, Ind.), 40 mg/kg intraperitoneally.
Treatments were begun 2 days (day -2) before infection with *C. trachomatis* and continued for 9 consecutive days (day 6 postinfection) with HC and Cy. Experiments using CA were administered similarly, except the treatment was terminated after 7 consecutive days (day 4) because the mice in this group became ill and further treatment was contraindicated.

**Quantification of lung infection.** Lungs from mice were removed, and a 10% (wt/vol) suspension of lung homogenate was prepared as previously described (11). These suspensions were assayed for infectious organisms by titration on monolayers of HeLa 229 cells (12), and the titers were expressed as log₁₀ IFU per gram of lung. Cages containing three to five mice per experimental group were randomly selected on the indicated day postinfection, and the mean titer of infectious organisms obtained from the lungs was determined. The initial experiments in this study were performed after HC administration, wherein five HC-treated and five saline-treated mice were assayed on days 2, 3, 4, 5, and 7. After the HC experiments, two additional sets of experiments were performed after administration of CA or Cy. In the first set, five mice from each experimental group (CA, Cy, or saline) were assayed on days 2, 4, 5, 6, and 7. In the second set, three mice were assayed from each group on days 2, 4, 6, 8, 10, 12, and 17. Additionally, the second set of experiments included HeLa cell-inoculated control mice treated with either CA or Cy. Two HeLa cell-inoculated control mice from each of these treatment groups were assayed on days 2, 6, 10, and 17.

Organisms that were reisolated on day 17 for the CA experiment, or on day 10 for the Cy experiment, were immunotyped by a previously described method (13).

**Histological studies.** Excised lungs were fixed immediately in 10% Formalin and processed by standard procedures. Specimens were stained with hematoxylin and eosin.

**Peripheral leukocyte counts.** Five mice from each experimental group (saline, CA, or Cy) were assayed on day 4 for total and differential leukocytes by standard hematological methods.

**Statistics.** Student's *t* test was used for comparison of mean lung titers.

**RESULTS**

**Effects of drug administration on peripheral leukocyte counts.** Figure 1 shows the absolute peripheral leukocyte counts of the granulocytic, lymphocytic, and monocytic series on day 4. There was a prominent leukocytosis in mice treated with CA which was entirely displayed in the granulocytic series. Furthermore, a reduction in the number of lymphocytes was noted which probably reflects the loss of a subpopulation of mouse lymphocytes sensitive to CA (4). In contrast, Cy caused a marked leukopenia which was particularly pronounced in the granulocytic series. There was also a decrease in the numbers of lymphocytes and monocytes. Cy is an alkylating agent which preferentially affects the more rapidly dividing cell populations. This coincided with the respective leukopenia observed and was in agreement with previous reports (19).

**Microbiological studies.** Previous studies (3, 11) showed that the highest yields of infectious organisms were obtained from the lung on day 2 postinfection; consequently, assays were begun on day 2. No statistically significant differences were observed between titers determined on day 2, regardless of experiment or treatment.

**Saline treatment.** In each saline experiment, the clinical course of pneumonia was virtually identical, and the same as previously described (3, 11). After the high titer of organisms recovered from the lung on day 2, the titer declined through days 3 and 4. After day 4, the titer dropped precipitously. After day 7, infectious organisms were no longer recoverable from the lungs (Fig. 2).

**HC treatment.** After 9 consecutive days of 125-mg/kg HC, no significant difference in titers of infectious organisms from the lungs was observed between saline- and HC-treated groups.

**CA treatment.** Although originally scheduled for 9 days of CA treatment, by the 7th day treated mice were very ill. Mice appeared lethargic, with ruffled fur and labored breathing, and therefore the treatment regimen was stopped at this point. After a modest decline in the titer, the highest yield of organisms was obtained on day 6, which was significantly greater than the titer on day 2 (*P < 0.01*, first experiment; *P < 0.05*, second experiment). The peak on day 6 was followed by a gradual decline in titer over the next 11 days; however, even by day 17 there remained a significant chlamydial burden in the lungs.
mouse lung (Fig. 2). Three mice died during the course of this treatment. One died on day 4 and demonstrated a chlamydial titer of 5.23 log₁₀ IFU/g of lung. The second died on day 5 with a titer of 5.84 log₁₀ IFU/g of lung. The third mouse died on day 8, but the lung was not recoverable for titration.

**Cy treatment.** Titer obtained from the lungs of mice treated with Cy demonstrated the typical peak on day 2, a slight decline through day 5, and a 10-fold increase by day 6. After day 6 the organisms were rapidly eliminated, and infectious organisms could not be recovered after day 12 (Fig. 2).

**Lung reisolates.** Infectious chlamydial organisms reisolated on day 17 (CA) or on day 10 (Cy) were immunotyped and found to be type B, the same immunotype as was used for initial inoculations.

*C. trachomatis* was not isolated from the lungs of control mice inoculated with HeLa cell material and treated with CA or Cy when assayed on days 2, 6, 10, and 17.

**Histological studies.** Lungs inoculated with HeLa cell material from mice treated with saline revealed no gross pathological changes. Microscopically, mild congestion was observed (Fig. 3A). Gross examination of lungs of saline-treated mice infected with chlamydial organisms displayed moderate congestion, with patchy consolidation most evident on day 2. Microscopic examination of these lungs revealed characteristic interstitial pneumonitis associated with intense infiltration of polymorphonuclear leukocytes which filled alveolar spaces and some bronchial lumen (Fig. 3B). Polymorphonuclear leukocyte infiltration was gradually replaced by mononuclear cells, and infiltrated foci became smaller through days 4 to 6. By day 7 only mild congestion was noted.

Gross examination of lungs from infected mice treated with Cy revealed only slight consolidation on day 2, appearing normal thereafter. Microscopically, moderate infiltration of mixed polymorphonuclear and mononuclear cells was observed, but sparing the bronchial lumen and many of the alveolar spaces (Fig. 3C). Subsequent specimens showed only rare foci of mononuclear cell infiltration (day 4 and 5) followed by mild congestion (days 6 and 7).

Lungs from infected mice treated with CA revealed no gross pathology. After day 6 (2 days after the last dose of CA), copious quantities of pleural fluid containing approximately 10⁵ mononuclear cells/ml were seen. Pleural fluid was not seen on any other occasion. Cultures of pleural fluids for *C. trachomatis* were negative. Microscopic examination of lung specimens from days 2 (Fig. 3D) through 5 displayed no infiltration and appeared normal. Samples from days 6 to 10 demonstrated a similar paucity of cellular reaction, except that rare interstitial pockets of mild polymorphonuclear cellularity were noted.

**DISCUSSION**

Immunosuppressive therapy with CA or Cy substantially prolonged the interstitial pneumonitis in mice infected with a trachoma biotype of *C. trachomatis*. These results demonstrated that mice were indeed capable of sustaining chlamydial lung infection in the absence or impairment of host effector cell systems. In contrast to the profound effect of CA on the course of chlamydial pneumonitis, the same concentration of HC had no effect. Similar differential effects between CA and HC have been previously observed. In a review by Bach (1), discordant results of the effects of corticosteroids on cell-mediated immunity were attributed to the relative ineffectiveness of HC. Similarly, studies by Hunninghake and Fauci on the effects of corticosteroids on alveolar macrophage function demonstrated no effect after administration of HC, yet profound effects with CA (10). CA is a depo-preparation which maintains high plasma cortisol levels for approximately 48 h, whereas HC increases plasma cortisol levels for only several hours (5), which may account for the differential effects observed.

The fact that immunosuppression prolonged chlamydial pneumonitis does not suggest, a priori, that repeated cycles of chlamydial infection had taken place. However, the characteristic drop in titer after day 2, with the subsequent peak repeatedly observed on day 6, is consistent...
FIG. 3. Hematoxylin-and-eosin-stained mouse lung sections. (A) Lung section from a mouse inoculated with HeLa cell material and treated with saline shows no evidence of inflammatory reaction. However, mild congestion is noted. (x150). (B) Lung section 2 days after inoculation with the TW-5 strain of *C. trachomatis* grown in HeLa cells from a mouse treated with saline. Note the extensive polymorphonuclear cell infiltrate in the interstitium and the terminal bronchiole. (x150.) (C) Section of lung 2 days after chlamydial infection from a mouse treated with Cy. Note the moderate mixed cellular infiltrate in the lung parenchyma. Bronchial lumen is spared. (x150.) (D) Section of lung 2 days after chlamydial infection from a mouse treated with CA. Note the paucity of cellular infiltrate. (x150.)
with the infectious cycle observed in cell culture.

The lung is immunologically unique from all other organs (14). Immunological responses in the lung are primarily lymphokine mediated, wherein circulating macrophages are recruited and activated, yet resident alveolar macrophages are not activated (18). CA efficiently eliminates the induction of cell-mediated immunity and precludes the accumulation of polymorphonuclear cells at infectious sites (20), even though it mobilizes polymorphs from the marrow (6). Cy has been shown to have moderate effects on the efferent limb in nonimmune animals by reducing cell numbers (19), but is most effective in limiting the response of the central limb through preferential elimination of rapidly dividing immune cells (19). The ability of these drugs to inhibit the inflammatory reaction correlated with the respective course of chlamydial pneumonitis observed in treated mice. Compared with the profuse cellular response and short duration of infection in the lungs of saline-treated mice, CA treatment prevented cellular infiltration and revealed the highest and most enduring chlamydial titers. Cy treatment displayed mild cellular infiltration with intermediate titers and duration of infection.

Our studies on the mouse model of chlamydial pneumonitis demonstrate that it is a useful model for investigating the immunopathogenesis of this pulmonary infection. The recent report by Williams et al. (21), using the mouse pneumonitis strain in nude mice, and preliminary results in our laboratory after treating mice with monoclonal antibody specific for Thy 1.1-bearing lymphocytes suggest that a T-cell-dependent response is necessary to control chlamydial lung infection. The sequence of events and the presumably important role of macrophages, polymorphs, and interferon (8) remain to be elucidated. Furthermore, this model could provide information on the effectiveness of various antibiotics, in situ, uncomplicated by host immune responses which appropriately reflects the state of individuals at risk.

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LITERATURE CITED