A Mouse Model of Pneumonitis Induced by *Chlamydia trachomatis*

Morphologic, Microbiologic, and Immunologic Studies

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Swiss-Webster white mice were infected with *Chlamydia trachomatis* organisms through intranasal inoculation. It was found that a typical interstitial pneumonitis could be induced. Histopathologic findings showed that the lung infiltration was predominantly polymorphonuclear cells and was most prominent on Day 2. The cellular infiltrate gradually changed to mononuclear cells after Day 3. Intracytoplasmic inclusions were frequently found in the interstitial cells and occasionally in the bronchial epithelial cells. Typical chlamydial bodies (elementary, intermediate, and reticulate forms) were identified by electron microscopy. The organisms were recovered from mouse lungs on Days 1-7, with the highest yields on Day 2. This correlated with the peak of lung infiltration seen by histologic examination. Antibodies specific to the infecting immunotype began to appear between Day 7 and Day 10 after inoculation and lasted until Day 35 without a decline in titers. A delayed hypersensitivity reaction was observed by footpad test from Day 5 to Day 21, with the peak reaction at Day 7. This study showed that the mouse model could be used to study the immunopathogenesis of *C trachomatis* infection. (Am J Pathol 1980, 100:365-382)

INFECTION caused by *Chlamydia trachomatis* has become increasingly important in recent years.[1,2] Major human diseases are caused by the trachoma–inclusion–conjunctivitis (TRIC) group of organisms, including well-recognized infections of the eyes (trachoma and inclusion conjunctivitis) and the urogenital tract (urethritis, cervicitis, epididymitis, and salpingitis). Recently, Beem and Saxon [3] defined a distinctive infant pneumonitis syndrome caused by this group of organisms. The other group of *C trachomatis* organisms consists of the lymphogranuloma venereum (LGV) strains, which are biologically distinct from trachoma strains [1,2] and cause a distinctive sexually transmitted disease, LGV, which is relatively uncommon in the United States.

In light of the increasing clinical importance of *C trachomatis* as causative agents for human diseases, the need for an animal model for the study of the immunopathogenesis of *C trachomatis* infection has become apparent. The development of experimental models has been limited by the fact that man is the natural host for *C trachomatis*. Only nonhuman primates

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have been used successfully as experimental animals.[1] Monkeys have been used extensively for studies of ocular trachoma,[4,5] and recently grivet monkeys have been used for studies of salpingitis [6] and infant baboons for pneumonitis studies.[7] The monkey experiments have provided valuable information on the immunology and pathogenesis of *C trachomatis* infection, especially for ocular trachoma.[4] However, primate experiments are becoming increasingly expensive, because the availability of primates is decreasing. It would be of great help if an experimental model could be developed in a small laboratory animal.

Infection of small laboratory animals with *C trachomatis* of human origin in the past has been successful only with LGV organisms [1] and with "fast-killing" TRIC strains,[8,9] which are now regarded as LGV strains.[10] In 1965 Graham reported that both the fast-killing and regular TRIC strains were infective and lethal upon inoculation into the lungs of TO mice at high concentration.[11] With the availability of more advanced laboratory techniques for *C trachomatis*, we explored the usefulness of the mouse lung model. The preliminary results of these studies have been reported.[12] This communication presents the details of histopathologic, microbiologic, and immunologic findings of mouse pneumonia caused by non-LGV *C trachomatis* of human origin and correlation of these findings with the course of the disease.

Materials and Methods

Inoculation of Mice

Swiss-Webster white mice 4–5 weeks old (Simonsen Laboratories, Gilroy, Calif.; or Charles River Breeding Laboratories, Wilmington, Mass) were inoculated intranasally while under light ether anesthesia. A total of 4 drops of inoculum (0.4 ml) were delivered to the nostrils with a 23-gauge needle and syringe. Control mice were inoculated with HeLa cell material, which was prepared from uninfected cell cultures processed by the same procedure as that of the infected cell culture. Trachoma organisms inactivated by ultraviolet irradiation were also used in some experiments. Ultraviolet (UV) irradiation was done with a germicidal lamp (Westinghouse G30T8) at a distance of 24 cm for 30 minutes.

C trachomatis Strains

The *C trachomatis* strains used were two ocular and two genital TRIC strains, B/TW-5/OT, C/TW-3/OT, D/UW-3/Cx, and G/UW-57/Cx,[13,14] and one LGV strain, L_2/434/Bu.[12] The inocula for mouse infection were organisms grown in HeLa 229 cell culture.[16] The titers of these inocula usually contained 10^9 inclusion-forming units (IFU) of organisms per milliliter.

Light and Electron Microscopy

For light-microscopic study, the excised lungs were fixed immediately in 10% formalin and processed in the usual way. Standard specimens stained with hematoxylin and eosin

(H&E) were used for histologic studies, and the Giemsa stain was used to identify the inclusion bodies. For electron-microscopic (EM) study the animals were anesthetized with sodium pentobarbital (0.1 ml intraperitoneally), and 3% glutaraldehyde was infused through the trachea to inflate the lungs. After several hours, the lungs were sliced into sections, 1–2 mm thick. From consolidated areas multiple small sections were taken and later diced into pieces 1 cu mm thick. These tissues were then postfixed in 1% osmium tetroxide in phosphate buffer (pH 7.4). The tissues were dehydrated and embedded in Epon 812. Ultrathin sections were obtained with an ultramicrotome with a diamond knife and were examined by an AEI 801 electron microscope.

Quantification of Lung Infection

The mice were exsanguinated by axillary bleeding under ether anesthesia. The thoracic cage was opened after disinfection of the skin with tincture of iodine. The whole lungs were excised intact, together with the trachea and bronchus, weighed, minced by cutting with scissors, homogenized with mortar and pestle to make a 10% wt/vol suspension in sucrose–phosphate–glutamate (SPG) medium, centrifuged at 500g for 10 minutes to remove tissue debris, and stored at −65 C. Yields of infectious organisms from the lungs were assayed by titration in HeLa 229 cell culture [17] and the infectivity titers expressed as IFU per gram of lung. Usually 5 mice were inoculated per specimen, and the average of the yield from the lungs was taken. Lung reisolates were immunotyped by a previously described method.[18]

Serology and Delayed Hypersensitivity Test

Serum antibody to *C trachomatis* was measured by the micro-IF test as described by Wang.[19] Delayed hypersensitivity was measured by the footpad test,[20] which was done by injecting 0.02 ml of heat-inactivated (56 C/10 min) TW-5 organisms (1×10^9 IFU/ml) into the footpad of one hind leg and heat-inactivated HeLa cell material into the footpad of the other hind leg. Footpad thickness was measured by a sliding caliper at 24 and 48 hours. Increase of footpad thickness was obtained by subtracting the thickness of the control footpad from the test footpad, the results expressed in millimeters.

Results

Morphologic Study of Pneumonitis

On opening the thoracic cavity the control lungs (inoculated with HeLa cell material only) revealed no apparent gross changes. Microscopically, mild congestion was noted (Figure 1). The lung infection with TW-5 showed moderate congestion with patchy consolidation, which was most prominent on Day 2 and Day 3. Figure 2 represents the characteristic histologic changes of interstitial pneumonitis, showing the intensive infiltration of polymorphonuclear leukocytes. In many areas the alveolar spaces are obliterated by the exudates. In general, the bronchioles are spared, even though the bronchial lumen is filled with polymorphonuclear exudate (Figure 3). Intracytoplasmic inclusions are frequently seen in the interstitial cells on Day 2 and Day 3 (Figure 4). These inclusions were shown to contain chlamydiae in the various stages of developmental cycle by EM (Figures 5 and 6). Inclusions were occasionally seen within the

cytoplasma of the bronchial epithelial cells (Figure 7). After Day 3 the cellular infiltrates were gradually changed to mononuclear cells (Figure 8). No eosinophils were ever observed in any of the sections. Minimal infiltrate was still noted on Day 5. The histologic characteristics of the lung returned to normal by Days 10–14.

Microbiologic and Immunologic Studies

Course of Pneumonia

Experiments were conducted to study the course of pneumonia as shown by the symptoms, the persistence of organisms in the lung, the appearance of antibody, and the pathologic features of the lung. Groups of mice were inoculated with TW-5 organisms at 3×10^9 or 3×10^8 IFU/ml concentration and the controls with HeLa cell material. Two mice from each group were killed every day from Day 1 to Day 5 and on Day 7, Day 10, and Day 14. Blood was saved for antibody assay. One lung was fixed in 10% formalin for pathologic study, and the other lung was used for infectivity assay. Only a few mice showed weakness on the first day but had recovered by the following day. One mouse died on the third day. The organisms could be recovered from the lungs from Day 1 through Day 7, including the 1 dead mouse (Table 1). The infection had cleared by Day 14. The highest yield of infectious organisms from the lung were obtained on Day 2. It was decided to assay lung infection 2 days after intranasal inoculation in the subsequent experiments. Micro-IF antibodies, which were not detected from Day 1 to Day 5, started to appear between Day 7 and Day 10.

Table 1—Recovery of *C trachomatis* B/TW-5/OT From Infected Mouse Lungs on Various Days After Inoculation

Experiment	Inoculum concentration* (IFU/ml)	Yield of organisms (log_{10} IFU/g lung)† days after inoculation						
		1	2	3	4	5	7	14
1	3×10^9	5.15	5.68	5.69	4.30	2.00		
2	3×10^9	4.41	7.20	6.52	3.81	2.78		
3	3×10^9		6.70				(+)‡	0
4	3×10^8		6.84				0.93	0

* Inoculation by delivery of 4 drops to the nostrils.

† Results of pools of lungs from 2 mice assayed by titration in HeLa 229 cell culture. Blanks mean "not tested."

‡ Inclusions detected only in the second passage of cell culture.

Table 2—Variation in Susceptibility of Individual Mice to Lung Infection With Different Inoculum Concentrations of *C trachomatis* B/TW-5/OT

Inoculum concentration (IFU/ml)	Yield of organisms (log_{10} IFU/g lung)*					
	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Average ± SD
3×10^9	7.08	7.08	7.04	6.40	C†	6.90 ± 0.33
3×10^8	6.36	6.20	6.15	5.79	4.59	5.82 ± 0.81
3×10^7	5.48	5.34	5.08	4.89	4.41	5.04 ± 0.60
Control	0	0	0	0	0	0

* Culture 2 days after inoculation.

† C = yield not assessable due to culture contamination.

Virulence

Mortality following intranasal inoculation of TRIC organisms was low. It was 3% (3/90) for 10^8 IFU/ml inoculum and 4% (3/76) for 10^9 IFU/ml inoculum with the mice from one commercial breeder, and 6% (7/118) for 10^8 IFU/ml inoculum and 0% (0/12) for 10^7 IFU/ml inoculum with the mice from the other commercial breeder. The denominators in these figures include only the mice kept for more than 7 days of observation. TRIC organisms were reisolated from the lungs of all of 5 dead mice in which isolation was attempted. Most of the mice died on the second day and none after the seventh day. Mice that died had severe dyspnea, ruffled fur, and emaciation. The surviving mice appeared normal, but their weight gain was slower than that of the control mice. None of over 200 control mice inoculated with HeLa cell material died.

Inoculum Concentrations and Individual Variations

An experiment was designed to determine the concentrations of inoculum that can infect mice homogeneously and to observe individual variations in susceptibility and infectivity titer. Groups of 5 mice each were infected with inoculum concentrations of 3×10^9, 3×10^8, and 3×10^7 IFU of TW-5 organisms per milliliter and 5 control mice with HeLa cell material. The mice were killed two days after inoculation and the lungs titrated separately. The results showed that three inoculum dilutions caused infection in all the mice (Table 2). The yields of infectious organisms from the lungs were in proportion to the inoculum concentrations. Individual variations were 0.33, 0.81, and 0.60 log of the standard deviation (SD) of IFU/g lung for the three inoculum concentrations.

Table 3—Effect of Mouse Age From 4 to 9 Weeks on Susceptibility to Lung Infection by *C trachomatis* B/TW-5/OT

Age of Mice (wks)	Yield of organisms ($\log_{10}$ IFU/g lung)* inoculum dose	
	1×10^9	3×10^8
4	6.32 ± 0.84†	6.45 ± 0.45
5	4.14‡	7.17 ± 0.22
6	6.86 ± 0.81	6.22 ± 0.94
7	7.35 ± 0.32	6.72 ± 0.48
8	6.95 ± 0.55	6.63 ± 0.22
9	5.79 ± 3.15§	5.34 ± 2.20

* Culture 2 days after inoculation.
† Average ± SD; 5 mice in each age group.
‡ One mouse not infected; 2 mice excluded from average due to culture contamination.
§ One mouse not infected.

Age Susceptibility

Susceptibility of mice to lung infection at various ages was studied by inoculating mice from the same original group weekly from 4 to 9 weeks of age. Each week 5 mice were challenged with TW-5 organisms and 2 mice with HeLa cell material. The mice were killed 2 days after inoculation. The lungs were titrated separately. Table 3 shows the results of two experiments using different inoculum concentrations of 1×10^9 and 3×10^8 IFU/ml. Mice were equally susceptible from 4 to 8 weeks of age, yielding 6 to 7 log of IFU/g lung. Somewhat lower titers, 5 log of IFU/g lung, were obtained at 9 weeks of age. All except 2 of the 60 inoculated mice were infected. Individual variations were small in 4–8-week-old mice, with an SD of 0.22 to 0.94 (average 0.54) log and a standard error of mean (SE) of 0.10 to 0.42 (average 0.22) log of IFU/g lung. The variation was large in 9-week-olds, with an SD of 3.15 and 2.20 and an SE of 0.98 and 1.41 log, respectively, in two experiments.

Growth of Organisms in the Lung

For an estimate of the growth of trachoma organisms in the mouse lung, 5 mice were killed immediately (within 30 minutes) after inoculation and another 5 mice on Day 2. Each lung was weighed and titrated separately. Table 4 shows the results of two experiments. Only 0.25 to 1.9% of inoculated organisms were recovered as viable organisms from the lungs 30 minutes after intranasal inoculation. Six to 53 times more infectious organisms were recovered from the lungs on Day 2.

Serum Antibody Response

Groups of 7 mice each were inoculated either with TW-5 (3×10^9 IFU/ml) or HeLa cell material. Two mice from each group were killed on Day 2. Blood was saved for an antibody test and lungs for an infectivity assay. Serial blood samples were obtained from the remaining 5 mice on Day 7 or Day 10 and every 7 days thereafter for up to 35 days. Blood from 5 mice was pooled and tested against immunotypes B and C by micro-immunofluorescence. Three separate experiments were done (Table 5). Antibody specific to the infecting immunotype B started to appear on Day 7 or Day 10 and was consistently present after Day 14 until the longest follow-up on Day 35 without decline in titer.

Delayed Hypersensitivity

Mice inoculated intranasally with either TW-5 organisms or HeLa cell material were tested with heat-inactivated TW-5 organisms in the footpad at various intervals of 3, 5, 7, 14, and 21 days after inoculation. Weak

Table 4—Recovery of Infectious Organisms From the Lungs of Mice Immediately and 2 Days After Inoculation With *C trachomatis* B/TW-5/OT

Experiment	Time after inoculation	No. of ogranisms inoculated (log_{10} IFU)	Yield of organisms (log_{10} IFU/g lung)	Weight of lungs (g)	No. of organisms in lung (log_{10} IFU)	% of organisms inhaled	Increase of infectious organisms (fold)
1	Time 0	7.08	5.0	0.30	4.48	0.25	
	Day 2		6.67	0.34	6.20		53
2	Time 0	7.08	6.0	0.23	5.36	1.9	
	Day 2		6.67	0.28	6.11		6

NOTE: Four drops (0.04 ml) of inoculum (3×10^8 IFU/ml) were delivered to the nostrils. Mice were killed at Time 0 (within 30 minutes) and Day 2. We titrated the lung homogenates in HeLa 229 cell culture to determine the number of infectious organisms. The figures were derived from the average of 5 mice.

Table 5—Microimmunofluorescence (Micro-IF) Antibody Response of Mice to Lung Infection by *C trachomatis* B/TW-5/OT

Experiment*	Intensity of lung infection at Day 2 (log_{10} IFU/g lung)	Micro-IF antibody†						
			Titer‡ on days after inoculation					
		Immunotype	2	7	10	14	21	28	35
1	4.77	B	0	0	32	64			
		C	0	0	0	0			
2	6.22	B	0	64		64	64	128	
		C	0	0		0	0	0	
3	4.66	B	0	0		128	128	128	128
		C	0	0		0	0	0	0

* Inoculum concentration = 3×10^9 IFU/ml.
† By tail bleeding; pool of 5 mice. Control mice inoculated with HeLa cells were negative.
‡ Reciprocal of twofold serum dilution; $0 \leqq 1:16$.

delayed footpad reaction was observed in the mice from 5 to 21 days after inoculation, the peak reaction at 7 days. The average increases of the footpad thickness at 24 hours after the test were 0.14 mm (5 mice) on Day 3, 0.27 mm (9 mice) on Day 5, 0.30 mm (13 mice) on Day 7, 0.22 (9 mice) on Day 14, and 0.21 mm (9 mice) on Day 21. The swelling subsided at 48 hours after the test. The control mice had negative results for the footpad test. Of 44 control mice tested, only 4 had an increase of 0.1 mm; the remaining 40 showed no increases.

Inoculation with UV-Inactivated Trachoma Organisms

Mice were inoculated with UV-inactivated TW-5 organisms (3×10^8 and 2×10^9 IFU/ml, before UV irradiation). Inoculated mice looked normal, and no deaths were observed. The micro-IF serum antibody was not demonstrated on Days 2–5 or on Day 7, Day 14, or Day 21 after inoculation. TRIC organisms were not isolated from the lung, and the lung was histologically normal on Day 2.

Lung Infection With Various TRIC Immunotype Strains

The pathogenesis of four non-LGV *C trachomatis* strains (B/TW-5/OT, C/TW-3/OT, D/UW-3/Cx, and G/UW-5/Cx) for the mouse lung were compared. Four-week-old mice were inoculated with 3×10^8 IFU/ml of the test strains. An infectivity assay and a pathologic study of lungs were done on Day 2. Lung reisolates were immunotyped. The four strains were equally pathogenic to the mouse lung, yielding similar titers of infectious organisms (Table 6). The reisolates were confirmed as the original inoculating strains by immunotyping. The histopathologic findings of the lungs were essentially the same as described above for the TW-5 strain. Typical inclusions were also detected in the lung with each strain.

Lung Infection With the LGV Strain

The pathogenesis of LGV for mouse lung was studied with the L_2/434/Bu strain. LGV was found more virulent than TRIC strains. When 3×10^8 IFU/ml inoculum concentration was used, all 13 inoculated mice were very sick on Days 1 and 2. Two died on Day 1 and 7 on Day 2. The remaining mice were killed on Day 2 for pathology and reisolation studies. When an inoculum of 3×10^7 IFU/ml was used mice looked normal on Day 1. However, 8 of 18 inoculated mice died on Day 2. The average yield of LGV organisms from the lungs of 5 surviving mice on Day 2 was 7.44 IFU/g lung (Table 4), which was 100-fold greater than that of TRIC strain TW-5 at the same inoculum concentration (Table 2). Lung infiltration was more extensive than that of TRIC pneumonitis induced with

Table 6—Susceptibility of Mice to Lung Infection by Various *C trachomatis* Strains

Infecting strain	Inoculum concentration (IFU/ml)	Yield of organisms from lungs* (log_{10} IFU/g lung)†	Immunotype of reisolate
Trachoma	3×10^8		
B/TW-5/OT		6.45 ± 0.45	B
C/TW-3/OT		6.56 ± 0.55‡	C
D/UW-3/Cx		6.96 ± 0.22	D
G/UW-57/Cx		7.36 ± 0.51	G
LGV	3×10^7		
L_2/434/Bu		7.44 ± 0.18	L_2

* Two days after inoculation as assayed by titration in HeLa 229 cell culture.
† Average of 5 mice ± SD.
‡ Not including 2 dead mice that had titers of 7.18 and 2.00.

the same inoculum concentration. Otherwise the histologic picture was the same.

Discussion

This study provided the pathologic, microbiologic, and immunologic evidence together to demonstrate that non-LGV *C trachomatis* strains can cause infection in mice by intranasal inoculation. Pneumonitis was specific to TRIC infection as shown by the development of serum antibody to the inoculating strain, the recovery of the same TRIC organisms from the lung, and the demonstration of TRIC inclusions in the interstitial cells and occasionally bronchial epithelial cells of the lung. These findings were negative in the control mice inoculated with either HeLa cell material or UV-inactivated TRIC organisms.

The use of the cell culture method has simplified the infectivity assay. It is a better quantitative method than the mean day of death and the mean score of lung consolidation used in the earlier reports with fast-killing strains.[11,21] The assay of infected lungs showed that the variation between individual mice was small with the same inoculum concentration either within the same experiment or between the different experiments. This indicates that the methods of inoculation and assaying were reasonably reproducible and the susceptibility of individual mice was quite predictable, regardless of the breeder.

Although it is possible to infect mouse lung with TRIC organisms, multiplication of TRIC organisms in the mouse lung seems to be inefficient. The yield of the organisms in the lung reached its peak on the second and third day after inoculation and declined drastically over the next few days, indicating that TRIC organisms undergo only limited cycles of repli-

cation in the mouse lung. Comparison of TRIC and LGV strains in the mouse lung infection showed that the LGV strain tested was more virulent than TRIC strains with regard to lethality and growth in the lung. This is in agreement with observation in human disease and other reported biologic characteristics of the organisms.[1] Graham's report [21] that the virulence in mice after intranasal inoculation was the same for fast-killing and regular TRIC strains as well as the JH strain of LGV was certainly not substantiated by our study.

Light-microscopic histologic findings in the lung are similar to those with fast-killing and regular TRIC strains described by Graham [21] and with *C trachomatis* mouse pneumonitis described by Gogolak.[22] Somewhat different from their findings is the intactness of the bronchial trees seen in our studies. Even in the area of heavy infiltrate or where the inclusions were seen in the bronchial epithelial cells, the bronchial epithelium appeared normal. Histologic studies showed that *C trachomatis* organisms were able to colonize the bronchial epithelial cells and the interstitial cells of lung parenchyma. EM histologic findings in lung have never before been described with *C trachomatis*. One striking difference between the inclusions seen in the lung infiltrate and those seen in the epithelial cells of the conjunctivum [23] and the uterine cervix [24] in man and in the cells of chick embryo yolk sac [23] was that inclusions in the interstitial cells of the lung infiltrate contained only a small number of mature elementary bodies even at the stage when the lung yielded the highest titers of organnisms. This finding partially explains the microbiologic results of low yields of organisms from the lung and the short duration in which the organisms can be isolated from the lung, and the self-limiting course of pneumonitis. Our earlier studies [25] also showed that mouse peritoneal macrophages were insensitive to the growth of TRIC organisms in the *in vitro* culture. The EM studies failed to identify the nature of the interstitial cells bearing the inclusions because the entire cytoplasmic space was occupied by the inclusion vesicle, preventing the markers that might have been of help in identifying cell types from being seen. However, the histologic features suggest that the inclusion-bearing cells in the lung infiltrate were mononuclear phagocytic cells.

The relationship of the *C trachomatis* mouse pneumonitis model to the human disease, the infant pneumonitis syndrome, is not clear. The histologic features of the lung in mice were characterized by patchy interstitial pneumonitis with polymorphonuclear cell infiltration in the acute stage and mononuclear cell infiltration in the recovery stage. In infant pneumonitis caused by *C trachomatis* the characteristic histologic picture at biopsy is interstitial pneumonitis with mixed infiltration of polymorpho-

nuclear and mononuclear to predominantly mononuclear cells and occasional eosinophils, depending on the stage of the disease when the biopsy was performed.[7,26] Clinically, mouse pneumonitis is mild and of short duration, and the agent is cleared from the mouse lung in a relatively short period, while infant pneumonitis usually has a prolonged course.[3] Observations in infant pneumonitis of an increased absolute eosinophil count in the peripheral blood [3,27] and occasional eosinophil infiltration in the lung infiltrate [7,26] and the difficulty of isolating the agent from the lung biopsy tissue [3] have led Beem and Saxon [3] to postulate that hypersensitivity may play a major role in infant pneumonitis syndrome caused by *C trachomatis*.

Good correlation between the histopathologic features of the lung and the recovery of organisms from the lung were seen. Both were maximum on Day 2 to Day 3 and gradually decreased over the next 4 days. The decline of infection seemed to coincide with the appearance of delayed hypersensitivity, which was first detected on Day 5. Serum antibody was not detected until after the decline of infection Day 7 to Day 10. The model system presented here may provide a useful alternative to the nonhuman primate model for studies of the immunopathogenesis of *C trachomatis* infection. The model system will allow the determination of *in vivo* antibiotic susceptibility testing and studies of the effect of immunosuppression on the infection.

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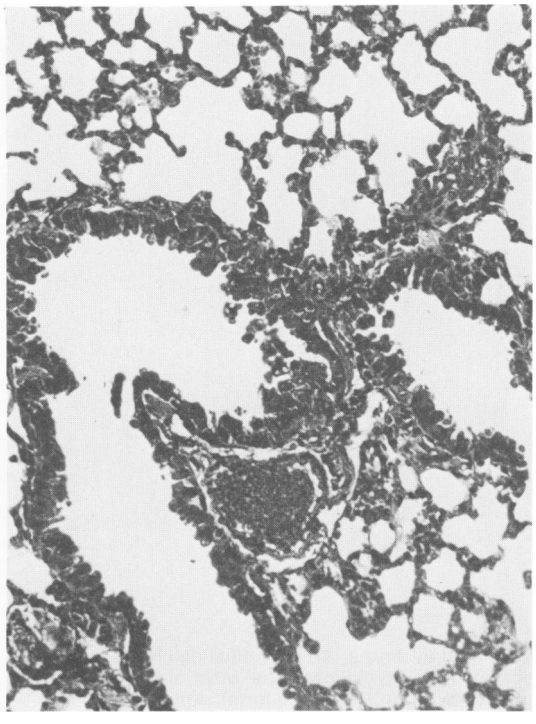
Figure 1—A lung inoculated with HeLa cell suspension shows no evidence of inflammatory reaction. However, mild congestion is noted. (×125) (With a photographic reduction of 3%)

Figure 2—A lung section 2 days after inoculation with the TW-5 strain of *Chlamydia trachomatis* grown in HeLa cells. Note the massive polymorphonuclear cell infiltrate in the interstitium and the terminal bronchiole. (×125) (With a photographic reduction of 3%)

Figure 3—A section of lung through an infiltrated area showing polymorphonuclear exudate and some mononuclear cells in the bronchial tree. Note the intactness of the bronchial epithelial cells. (×300) (With a photographic reduction of 3%)

Figure 4—Higher magnification of two intracytoplasmic chlamydial inclusions (vesicles). In one cell the nucleus is pushed to one side by the ballooning vesicle (*arrow*). Some chlamydial bodies are discernible within the vesicle. (×400) (With a photographic reduction of 3%)



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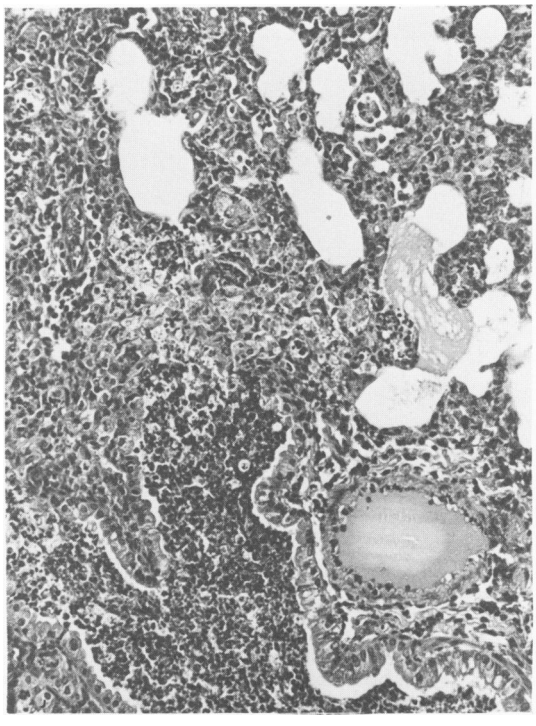

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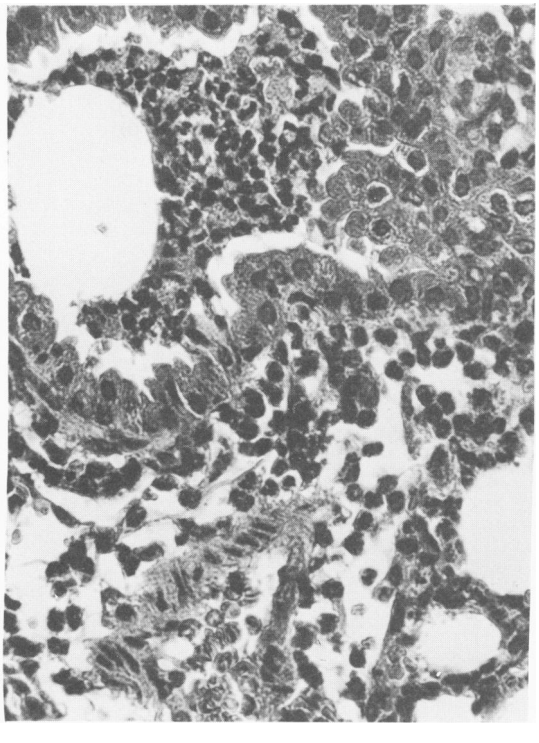

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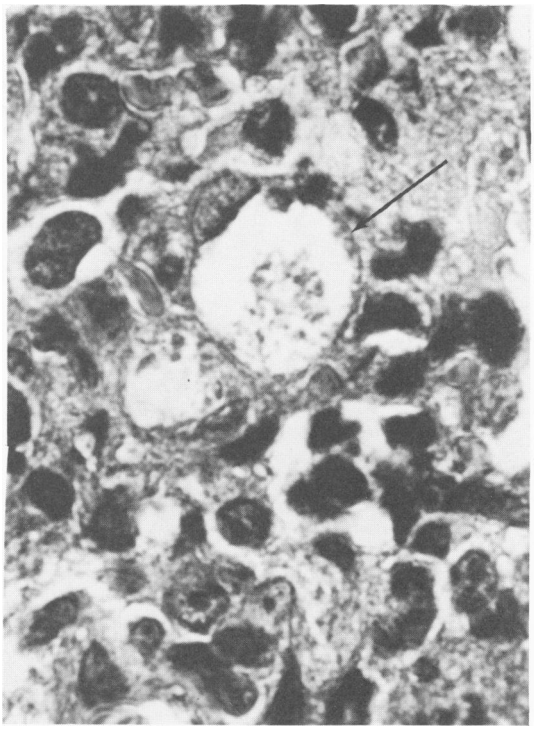
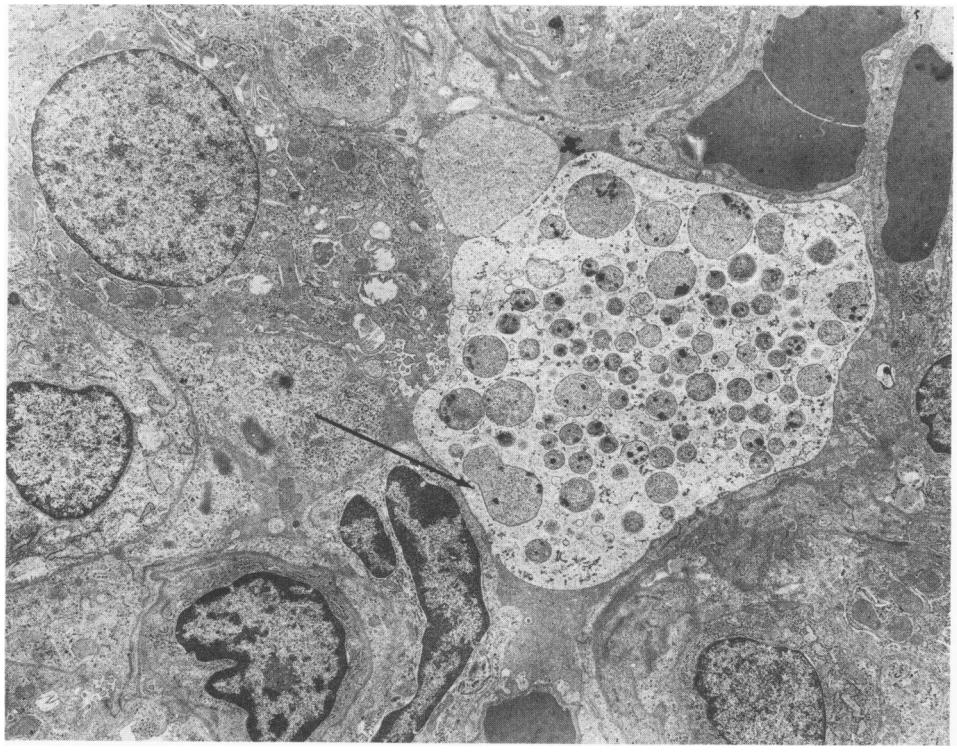


4

Figure 5—An electron-microscopic section through an infiltrated area. An interstitial cell is occupied by an intracytoplasmic vesicle containing numerous chlamydial particles. Most of these particles are reticulate bodies. A few have a dark nucleoid body (intermediate form); some contain more than one nucleoid. Divisions of reticulate bodies are also noted (*arrow*). A cell to the left of this inclusion can be identified as a Type II pneumocyte with markers of microvilli and lamellar bodies. (×2500)

Figure 6—Higher magnification of one of the intracytoplasmic vesicles, showing various developmental forms: reticulate bodies (*R*), intermediate bodies (*I*), and elementary bodies (*E*). Distinct cell wall and cytoplasmic membrane are discernible in these chlamydiae. (×10,000)



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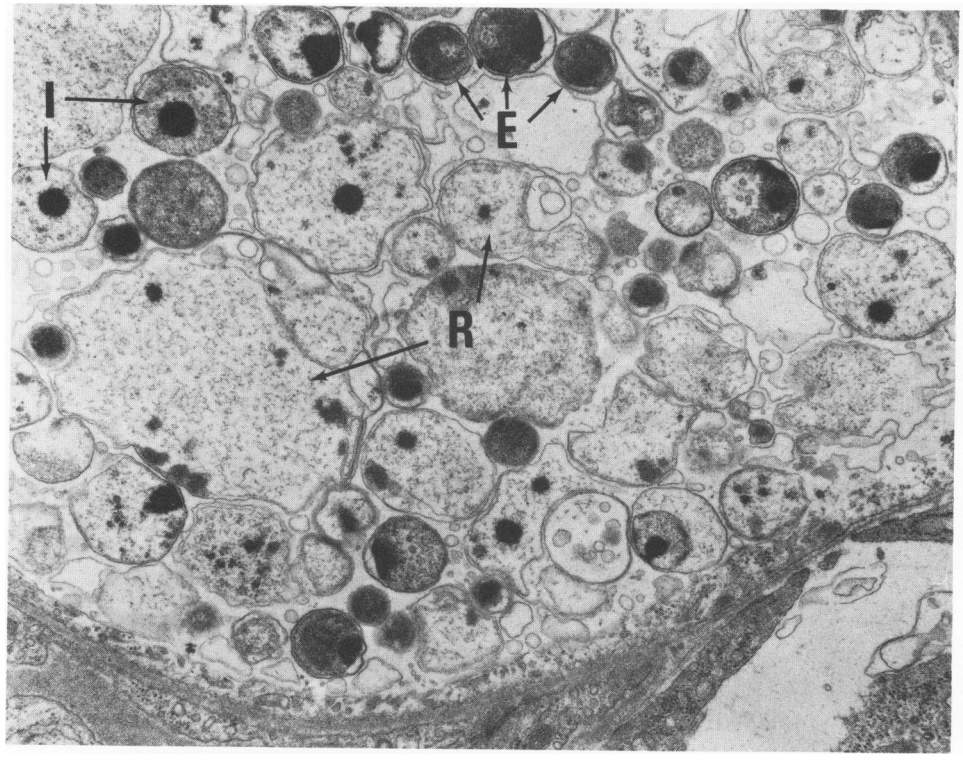

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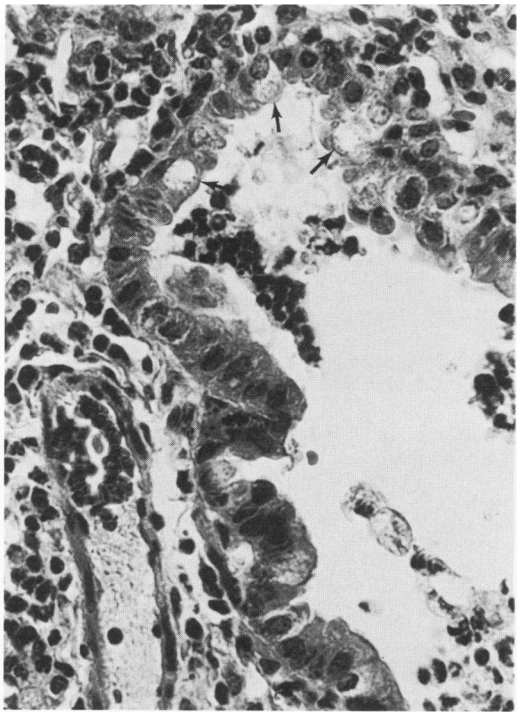

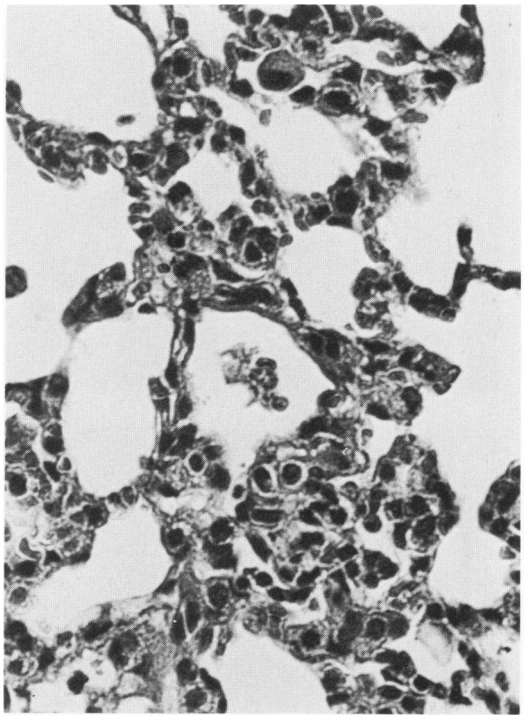

Figure 7—Occasionally the intracytoplasmic inclusions are seen in the bronchial epithelial cells (*arrow*). Dark chlamydial particles are present within these vesicles. (×300) **Figure 8**—A lung section 5 days after chlamydial infection. The acute polymorphonuclear infiltrate has disappeared. The lung has returned to normal histologically except in a few areas where mild mononuclear cell infiltrate is still present. No eosinophils are noted. (×300) (Both with a photographic reduction of 3%)