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Chlamydia trachomatis pneumonia in the immune, athymic and normal BALB mouse

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Summary. This paper compares the histopathology of pneumonia due to murine *Chlamydia trachomatis* (MoPn, mouse pneumonitis agent) in susceptible athymic nude mice (nu/nu), resistant heterozygous littermates (nu/+) and very resistant immunized nu/+ mice. While all groups had an early heterophil response, successful host defence correlated with the presence of large numbers of plasma cells, lymphocytes, monocytes, and lipid laden macrophages. Reticulate bodies were seen in all groups, predominantly in type I alveolar epithelial cells. By 24 h in the immune nu/+ group, no intact organisms were visible. Optimal control of infection was thus rapid and not clearly related to heterophils. These studies show that the histopathology of chlamydial infection may be quite atypical in the immunocompromised host, mononuclear cells seem critical in host defence, and B cell activation with plasma cell infiltration is dependent on intact T cell function in this model.

Keywords: Chlamydial pneumonia, athymic mouse, T cell function, B cell response, histopathology, ultrastructure

In earlier reports, a mouse model of *Chlamydia trachomatis* pneumonia caused by the mouse pneumonitis biovar of *C. trachomatis* (MoPn) has been described (Williams *et al.* 1981; 1982; 1984 *a,b*). We have employed the athymic nude 'nu/nu' mouse and its furred heterozygous 'nu/nu' littermate. To date, we have shown that the nu/+ mouse with its relatively intact T cell function is more resistant to MoPn than the nu/nu mouse, but resistance to MoPn can be transferred to nu/nu recipients by immune T cells from nu/+ donors. In addition, nu/+ mice develop both delayed hypersensitivity to *C. trachomatis* antigen and antigen specific lymphocyte transformation, whereas, athymic nude mice do not. Finally, nu/+ mice can be immunized by sublethal infection to be resistant to subsequent lethal challenge with MoPn while nu/nu mice cannot, and stimulation of cell mediated immunity by prior infection with *Histoplasma capsulatum* increases the resistance of nu/+ mice to subsequent challenge with MoPn, but does not in nu/nu mice. These data suggest that immunity to pneumonia due to *C. tracho-*

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matis is T cell dependent and that cell mediated immunity is important in host defence. Since antibody to MoPn is also T cell dependent and antibody seems to provide some protection to nu/nu mice, T cell dependent host protection is probably polyfactorial (Williams *et al.* 1984*b*).

During our earlier studies in which the pathogenesis and immunological characterization of the mouse pneumonitis agent were investigated, a marked disparity in the histological responses of the lung was noted among immunologically distinct study groups, the results of which form the basis of this report (Williams *et al.* 1984*a*). Furthermore, since *Chlamydia trachomatis* pneumonia occurs, albeit infrequently, in the immunocompromised human as well (Tack *et al.* 1980; Ito *et al.* 1982) these studies may have some relevance to human immunopathology.

Materials and methods

Specific pathogen-free nu/nu and nu/+ mice with a BALB/c background were obtained and maintained as previously described (Williams *et al.* 1981), except that the mice used in these experiments were rendered germ free by giving them to germ free foster mothers, and were then introduced to a defined limited bacterial flora of organisms nonpathogenic to mice. This procedure eliminated any colonization with aerobic gram-negative organisms. These mice were shown to be free of prior chlamydial infection both by culture of homogenized lung tissue and by serological methods (Williams *et al.* 1981; 1982). Six-to-eight-week old mice of both sexes were used in experiments. A third study group was an immune nu/+ group; these animals had been infected by the intranasal route three months previously with a sublethal dose of MoPn. Challenge studies showed these mice to be solidly immune with no deaths in mice given MoPn at a dose which would otherwise have been lethal in 100% of nonimmunized recipients.

The mouse pneumonitis biovar of *C. trachomatis* (MoPn) was obtained as described previously (Williams *et al.* 1982) and maintained in the yolk sac of embryonated hens' eggs. Dilutions of the organism for intranasal inoculation were prepared in McCoy's modified 5A medium (Difco Laboratories, Detroit, MI) (Williams *et al.* 1981). Undiluted titre of the MoPn agent was 4×10^9 inclusion-forming units (ifu) per ml.

Groups of three to five mice under pentobarbitol anaesthesia were inoculated intranasally with 0.05 ml of MoPn agent (Williams *et al.* 1981). The infecting dose was 5×10^4 to 5×10^5 ifu of MoPn. A dose of 5×10^4 was lethal to 50% of nonimmunized recipients; 5×10^5 to 100%. For sham controls, groups of two to five mice were inoculated with 0.05 ml of yolk sac material without organisms.

For the pathological studies the experimental groups and study times are displayed in Table 1. Following killing with pentobarbitol anaesthesia, the lungs were removed and instilled intratracheally with phosphate buffered 4% formaldehyde—1% glutaraldehyde (4CF-1G) at 10–15 cm H_2O pressure (McDowell & Trump 1976). Following fixation the lungs were cut sagittally, embedded in paraffin, sectioned at 4μm, and stained with haematoxylin and eosin, trichrome elastica and Giemsa. Additional sections for immunocytochemical studies (Williams *et al.* 1984*a*) were stained with rabbit antibodies to mouse IgG, IgM and IgA (Cappel Laboratories, West Chester, PA), using direct and indirect immunoperoxidase methods (after Sternberger 1979). Ten blocks were randomly taken from the lungs of each study animal for transmission electron microscopy, and embedded in Polybed and Spurr. Thick specimens were obtained from all the blocks; three blocks per animal were chosen for thin sectioning. Thin sections were stained with lead citrate and uranyl acetate and studied with a Phillips 301 or Jeol 100-CX electron microscope. An average of 11 low power fields was taken from each grid plus additional fields at higher magnifications, yielding an average of 35

Table 1. Time after injection or challenge with yolk sac material

	0	2 h	10 h	24 h	48 h	5 days	14 days	21 days
Sham infected control groups								
immune/nu+	5*			2	2			2
nu/+	5			2	2			2
nu/nu	5			2	2			2
Infected groups								
immune/nu+			5	3	3	3	5	5
nu/+	3		5	3	3	5	5	5
nu/nu	3		5	3	3	3	5	5

* Number of mice in each group.

prints per study animal and no less than 90 prints per study group.

Pulmonary lavage material was obtained as previously described (Williams *et al.* 1984*a*). Direct fluorescent antibody staining for chlamydia was performed on these specimens with monoclonal genus specific anti-chlamydial antibody following the manufacturer's instruction (Ortho Diagnostics, Raritan, NJ).

Results

Light microscopy

Zero time and 2 h study specimens had normal histology apart for the immune/nu+ group in which peribronchiolar and perivascular collections of intermixed monocytes, lymphocytes and plasma cells were present. At 10 h, mouse heterophils (neutrophils) were evident in all three infected study groups but not in controls given yolk sac material without organisms. There were small numbers of heterophils in the lumina of small bronchioles and surrounding alveolar spaces, and scattered within the alveolar walls.

By 24 h, the immune/nu+ study group did not show the early heterophil accumulations at the respiratory bronchiolar/alveolar duct sites evident in the other two study groups. However, by 48 h heterophils were evident in the inflammatory exudates of all three study groups. The nu/nu study animals showed fewer mononuclear cells within the focal exudative lesions (Fig. 1). In both the nu/+ and nu/nu groups, basophilic stained cytoplasmic inclusions were easily identified in the alveolar epithelium (Fig. 2) at 48 h and only rarely in bronchiolar epithelium.

Five day study specimens showed a more confluent bronchopneumonia pattern in the nu/+ and nu/nu study animals, whereas in the immune/nu+ group, the perivascular-peribronchiolar pattern of inflammation persisted. A decrease in the number of heterophils was seen in the immune/nu+ lesions, whereas heterophils and alveolar macrophages predominated in the lesions of the other two study groups.

Abundant alveolar epithelial and rare bronchiolar cytoplasmic inclusions were observed in the nu/nu and nu/+ groups at this time period.

By 14–21 days, immune/nu+ animals showed nodules of abundant lymphocytes and fewer plasma cells and lymphoblasts with only rare sites of active alveolitis. nu/+ animals had developed persistent nodules of

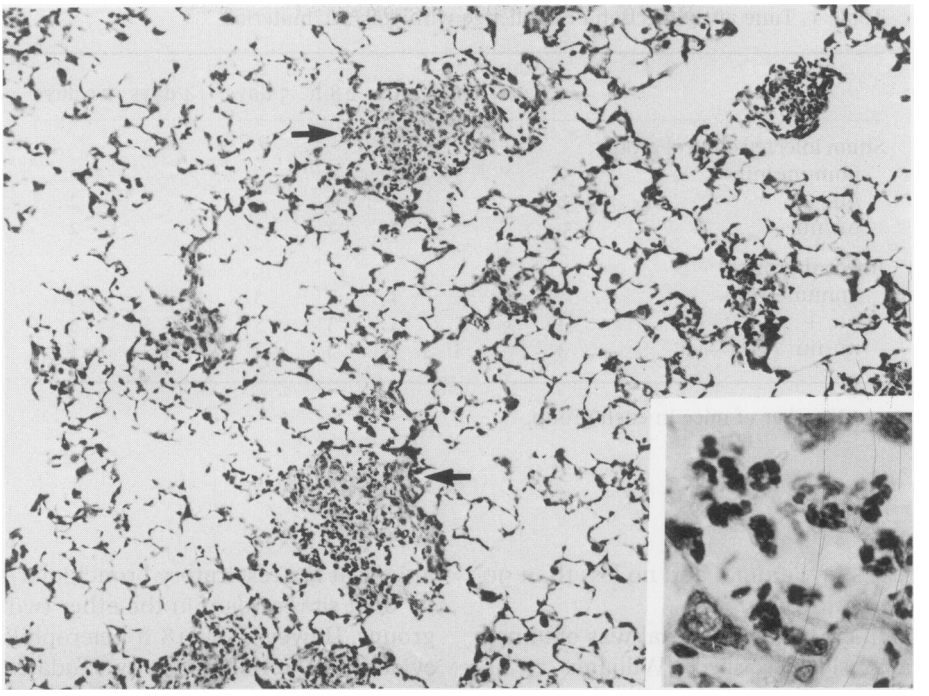

Fig. 1. nu/nu; 48 h specimen. Focal accumulations of abundant heterophils and lesser numbers of alveolar macrophages are evident at the alveolar duct and alveolar levels (arrow). Cellular details are depicted in the insert. Hematoxylin and eosin (H & E) × 160 and 140 respectively.

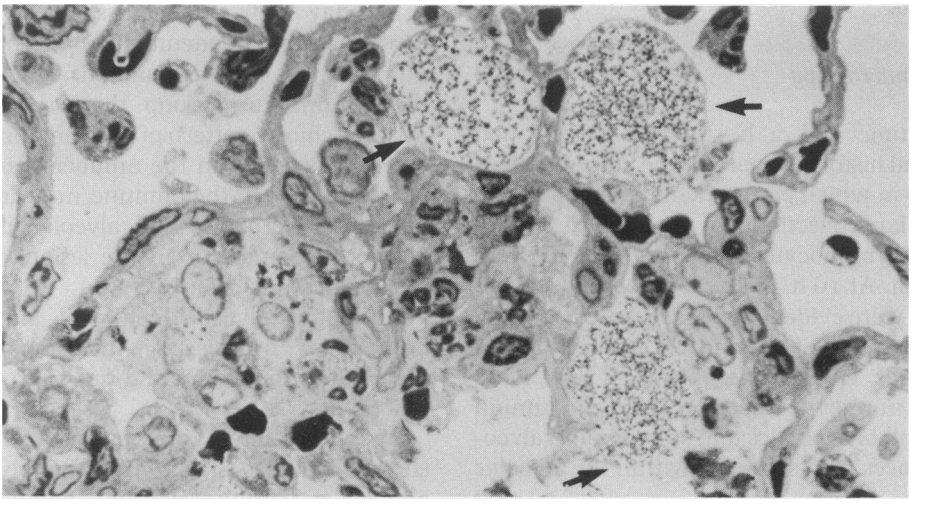

Fig. 2. nu/nu; 48 h specimen. The epithelial cytoplasmic inclusions (arrows) are well visualized in 2μm resin thick sections. A, Alveolus. Toluidine blue, × 750.

chronic inflammatory cells at airway and vascular sites which contained predominantly lymphoblasts and plasma cells with lesser numbers of small lymphocytes. In addition, this group had persistent focal alveolar abnormalities consisting of oedema, a few heterophils, plasma cells and alveolar macrophages (Fig. 3*a*). The nu/nu animals still showed large numbers of heterophils and macrophages (Fig. 3*b*). The cytoplasmic inclusions were seen often in the 14 day nu/nu study specimens, but were less frequent in the 21 day study animals. The trichrome elastica stained preparations showed only a slight increase in collagen deposition, with no differences evident among the three study groups.

Electron microscopy

Despite a large inoculum of organisms and an intensive ultrastructural search, entry of infective elementary bodies into the bronchiolar and/or alveolar epithelia was not seen at 2 h. The first evidence of infection was found in the 10 h specimens. A common finding in all three groups was reticulate bodies, usually numbering two to four in endosomes, within predominantly type I alveolar epithelial cells (Fig. 4*a*,*b*). The sparse type I cytoplasmic organelles were normal. Occasional heterophils and even fewer alveolar macrophages also contained some phagocytosed organisms. The surface epithelium was focally oedematous. Many heterophils and frequent platelet thrombi were in the capillaries. There was no endothelial disruption. Separate group findings included the finding of more interstitial monocytes and plasma cells in the immune/nu+ group. Organisms were easily found from field to field in the nu/nu and nu/+ groups, but were infrequently seen in the immune nu/+ groups.

Twenty-four hour nu/nu and nu/+ specimens showed large cytoplasmic inclusions, chiefly in Type I cells (Fig. 5). All stages of chlamydial development were evident: reticulate bodies, intermediate forms, and elementary bodies. Endocytotic membrane integrity varied. The sparse cytoplasmic organelles when seen were normal. In the immune/nu+ group, no organisms were seen, only abundant alveolar macrophages, most with secondary lysosomes or multiple lipid droplets.

By 48 h, there were more abundant numbers of organisms in the nu/nu and nu/+ groups. The cytoplasmic inclusions were very large, many were ruptured (Fig. 6) or sloughed 'free' in alveolar spaces. Abundant free elementary bodies and fewer reticulate bodies were in the alveoli, and were in phagosomes of heterophils (Fig. 7) and macrophages. Focal epithelial disruption and scattered fibrin strands were present. The immune/nu+ group did not contain any epithelial cytoplasmic inclusions with organisms at this time period; heterophils and alveolar macrophages contained many secondary lysosomes, but no recognizable organisms were identified.

The immune/nu+ animals at 5 days had multiple intra-alveolar lipid laden macrophages and infrequent heterophils. No organisms were found. In the nu/nu group, alveolar macrophages were seen encircling cytoplasmic inclusions that contained multiple organisms (Fig. 8). In the nu/+ and nu/nu groups, many epithelial endocytotic vesicles containing organisms were still present (Fig. 9). Heterophils and free organisms were in the alveolar spaces. Many of the organisms in the phagolysosomes of the macrophages were degenerated, only the organisms' outlines were evident. Rarely, mononuclear cells with intracytoplasmic organisms were in the interstitium.

By 14 days in the nu/+ and immune/nu+ groups large numbers of plasma cells, lymphocytes and monocytes were in the interstitium. The alveolar spaces contained numerous lipid laden macrophages, fewer lymphocytes and rare heterophils (Fig. 10). No intact organisms were evident at this study period. Direct fluorescent antibody staining with monoclonal antibody to chlamydia of alveolar lavage material from these

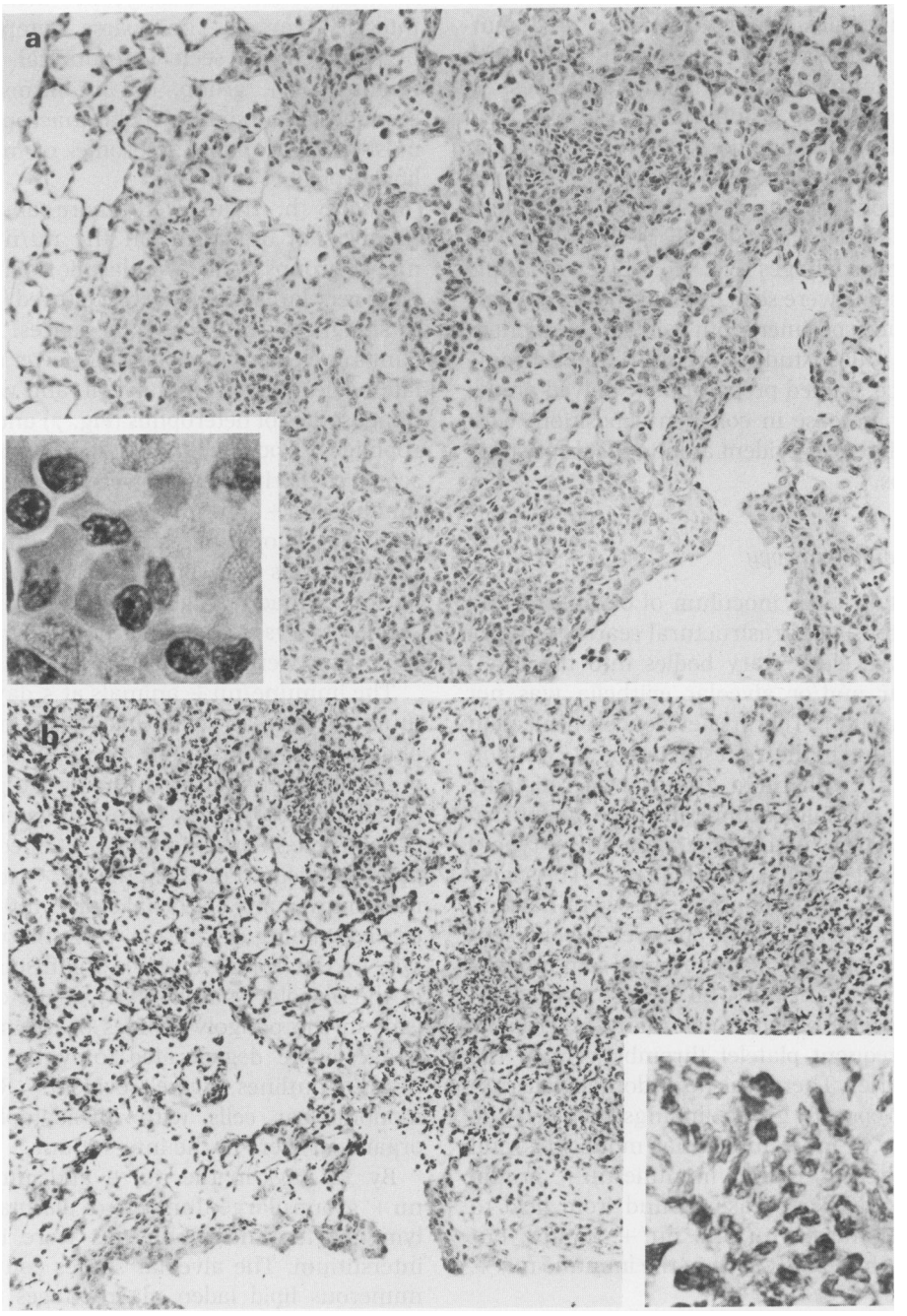

Fig. 3. *a*, A nu/+, 21 day post-infection specimen, peribronchiolar accumulations of plasma cells, lymphoblasts and a few lymphocytes (see insert) plus intraalveolar macrophages and occasional heterophils are present. *b*, a nu/nu, 21 day post-infection specimen, numerous heterophils and alveolar macrophages are evident (see insert) but lymphocytes are not present. H & E, × 160 and 640 respectively.

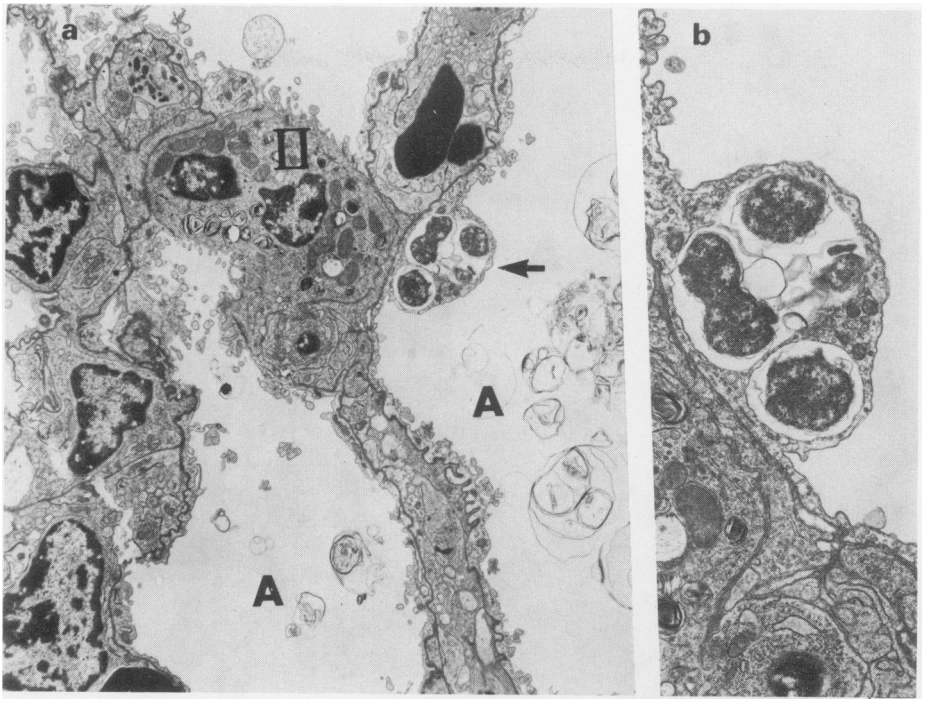

Fig. 4. nu/+, 10 h specimen. *a*, A type II cell (II) is seen on one side of the alveolar wall whereas on the other, a type I epithelial cytoplasmic extension contains several reticulate bodies in endosomes (arrow), details of which are seen in *b*. A, Alveolus. Uranyl acetate and lead citrate, × 3400 and 8500 respectively.

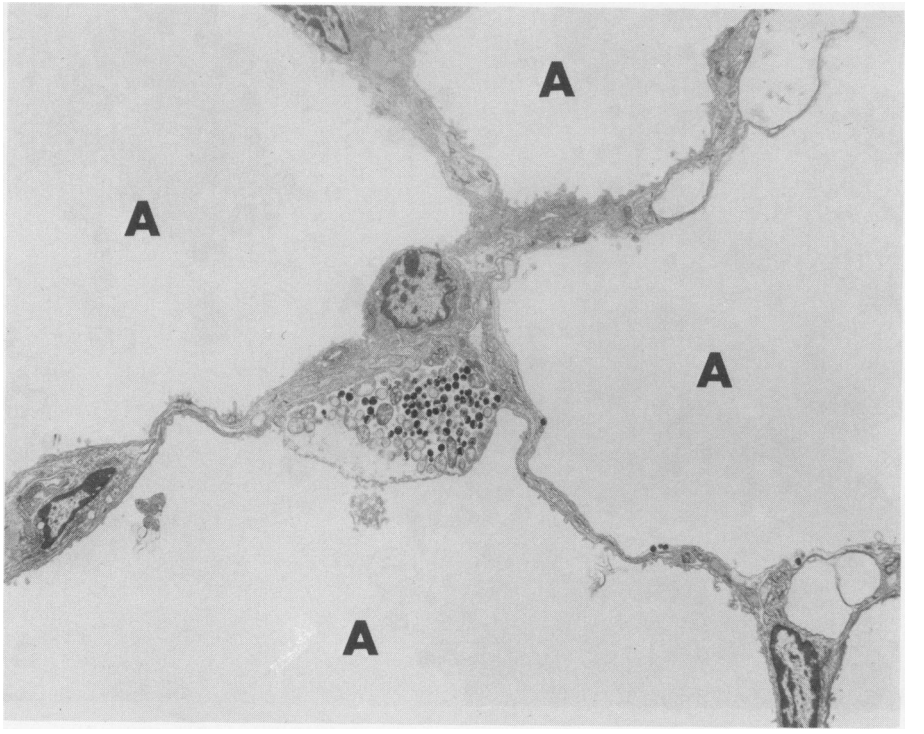

Fig. 5. nu/nu; 24 h specimen. The cytoplasmic inclusion contains reticulate bodies, intermediate forms and elementary bodies. The alveolar spaces (A) are devoid of cells. Uranyl acetate and lead citrate, × 2850.

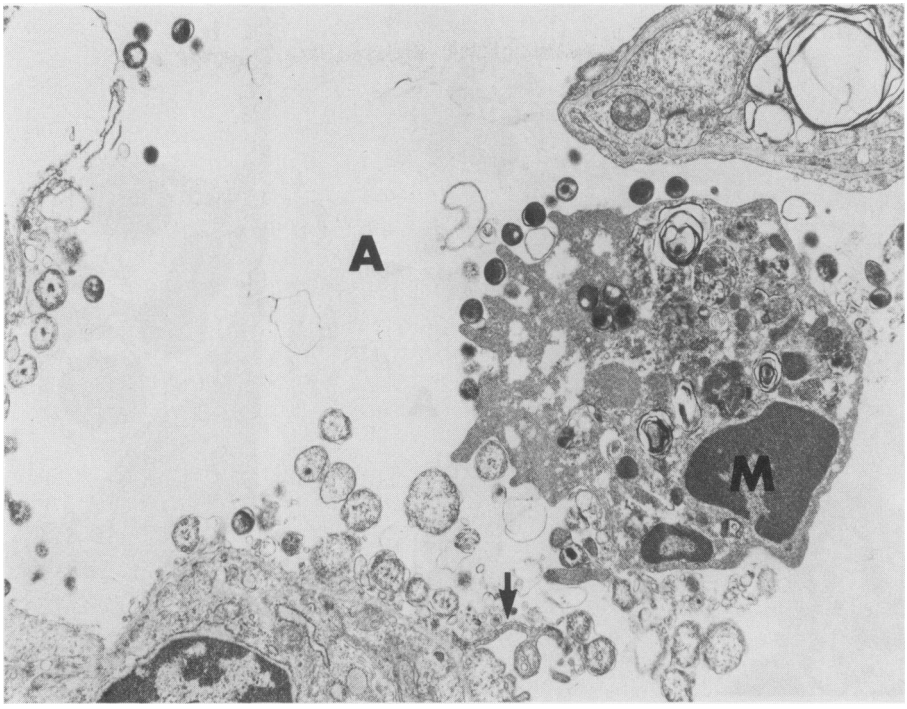

Fig. 6. Nu/+; 48 h specimen. The epithelial cytoplasmic membrane has ruptured (arrow) with release of chlamydial forms into the alveolar space (A). The monocyte (M) contains phagosomes with organisms and along its cytoplasmic surface many chlamydia are seen. Uranyl acetate and lead citrate, × 8500.

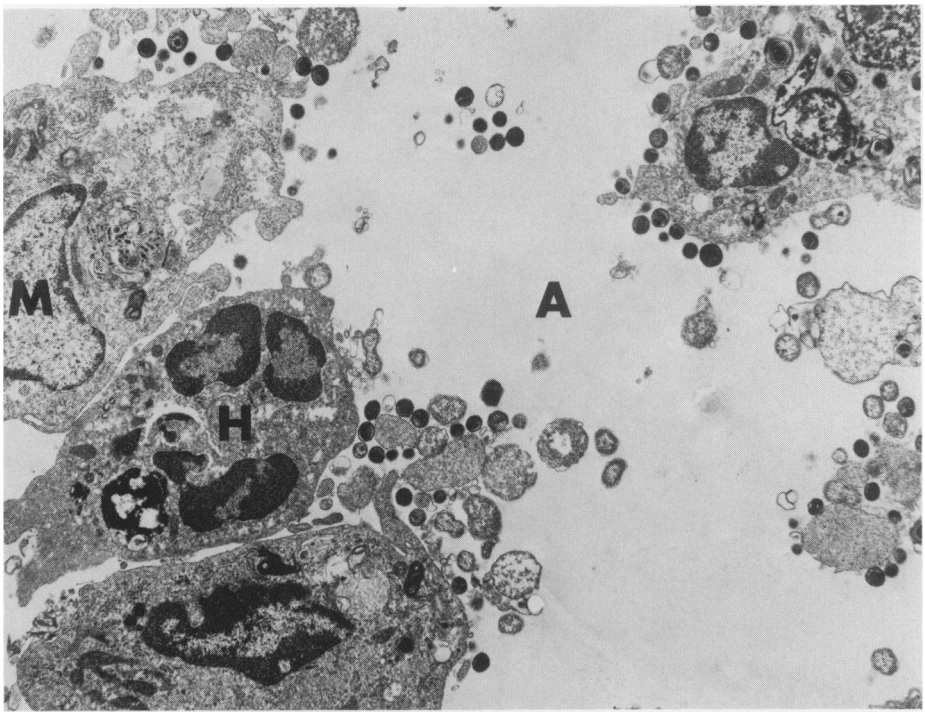

Fig. 7. nu/+; 48 h specimen. Within the alveolar space (A) multiple heterophils (H) and a monocyte (M) show multiple phagosomes. Many chlamydial forms are 'free' in the alveolar space. Uranyl acetate and lead citrate, × 5600.

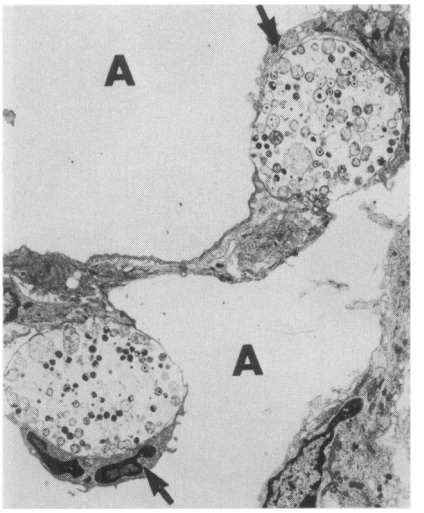

Fig. 8. Nu/nu; 5 day specimen. Two epithelial cytoplasmic inclusions are 'encircled' by alveolar macrophages (arrow). Fibrin strands are intermixed with an alveolar macrophage in the alveolar space (A). Uranyl acetate and lead citrate, × 3200.

mice showed markedly positive material within macrophages suggesting that some of the lipid material was of chlamydial origin. This material persisted in lavaged macrophages until at least day 41 post-infection. The nu/nu study group still showed numerous organisms within the interstitium, epithelium and free in the alveolar spaces. The most abundant inflammatory cell was the heterophil with fewer alveolar macrophages.

At 21 days the plasma cells in the nu/+ and immune/nu+ groups showed widely dilated endoplasmic reticulum. Lipid laden macrophages were in the alveolar spaces and interstitium. In the nu/nu group, residual organisms were in phagosomes of the persistent heterophil population. Free fibrin strands and alveolar macrophages were also in the exudate.

Sham inoculated animals of the study groups did not show any of the induced histopathological lesions described above.

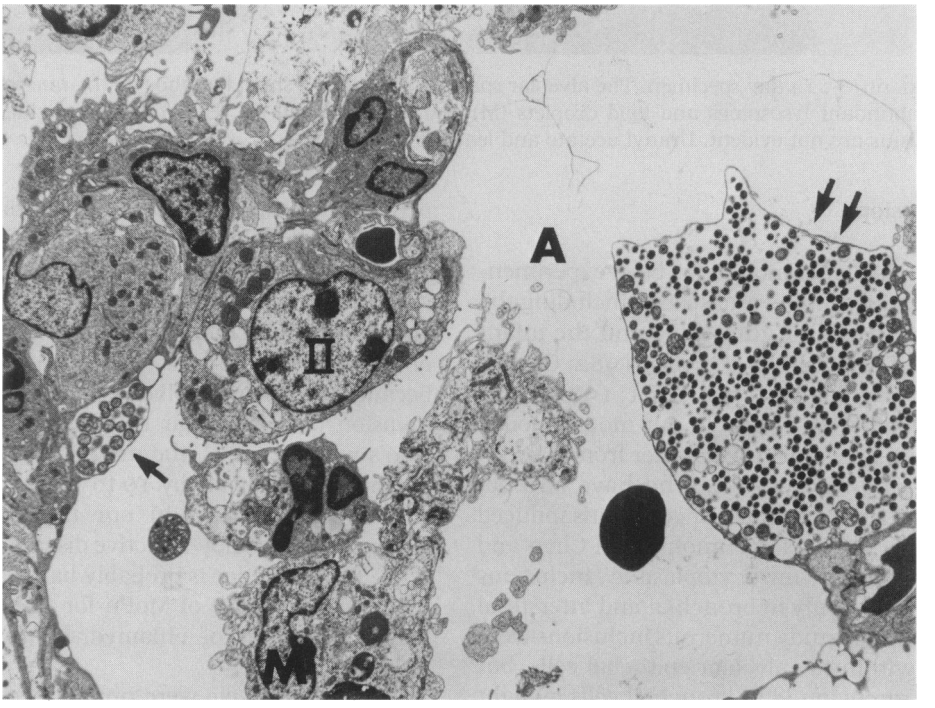

Fig. 9. Nu/+; 5 day specimen. Within the phagocytes (M) in the alveolar space (A), secondary lysosomes with probable residual chlamydial forms are evident. Two cytoplasmic inclusions, one with reticulate bodies (arrow), the other with multiple forms (double arrows) are seen. The lamellar bodies in the type II cell (II) are 'empty' or contain neutral lipid. Uranyl acetate and lead citrate, × 3200.

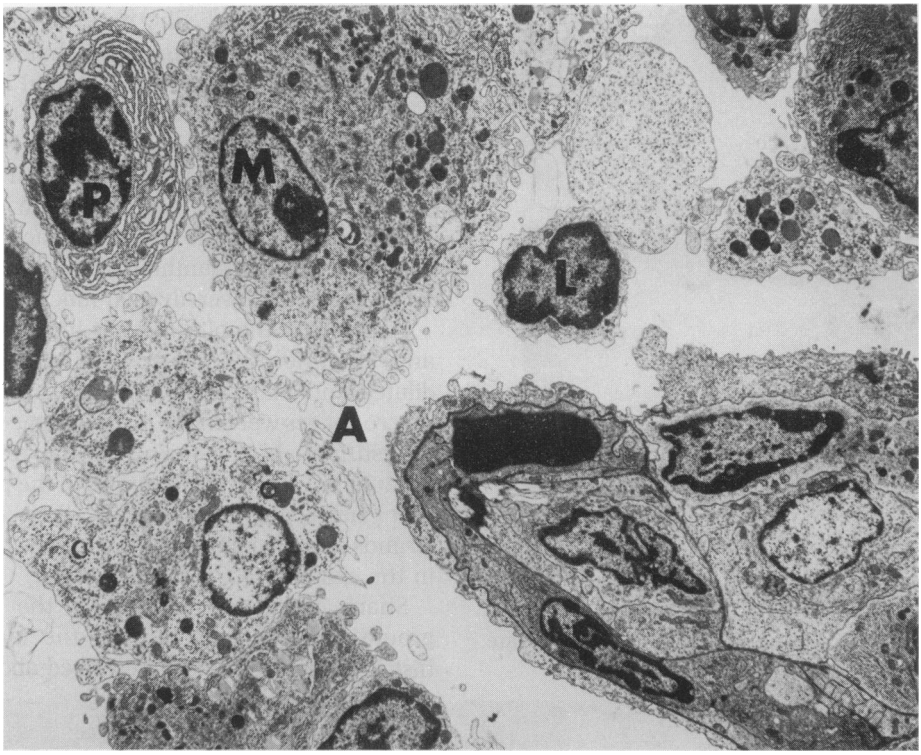

Fig. 10. nu/+; 14 day specimen. The alveolar space (A) contains a small lymphocyte (L), macrophages with abundant lysosomes and lipid droplets (M) and a plasma cell (P). Heterophils and chlamydial organisms are not evident. Uranyl acetate and lead citrate, × 3400.

Discussion

Chamydial pneumonia has been experimentally induced in several species including the mouse, newborn guinea pig and the infant baboon (Harrison *et al.* 1979; 1982; Chen & Kuo 1980; Williams *et al.* 1981; 1982; 1984 *a,b*; Rank *et al.* 1985). In our mouse model, the nu/+ group findings differ from those of Chen and Kuo (1980), who have also described a mouse model pneumonitis induced by *C. trachomatis* pneumonitis. In Chen and Kuo's model intracytoplasmic inclusions were found only in bronchial and interstitial cells. In this study numerous inclusions were seen within the alveolar epithelial cells, but were rarely found in bronchial cells by light microscopy (a few in 2 and 5 day study animals) and none were found at the ultrastructural level. Only in the late study periods was an occasional interstitial cell with inclusions found. Our results are more consistent with those described by Weiss (1949) in his classic study of psittacosis agents which included the MoPn agent. He observed that mouse pneumonitis first became established in alveoli, followed by the invasion of bronchiolar cells. In Chen and Kuo's model (1980), the mouse lungs were normal histologically by 10 to 14 days post-inoculation, whereas in our nu/+ study group, lung still showed active disease at this time. Ths difference is probably based on the increased virulence of MoPn for mice compared with that of chlamydial strains of human origin.

In our studies we were unable to demonstrate the uptake of chlamydia by the alveolar cells. In-vivo studies of chlamydial infections in the bovine intestine (Doughri *et al.*

1972; 1973; Todd *et al.* 1976) demonstrated that chlamydial elementary bodies were absorbed onto the microvilli which induced phagocytosis of the elementary bodies with subsequent formation of the endosome. Release of organisms occurred with degeneration of the cell or cytoplasmic fragments which contained the chlamydia organisms. Using in-vitro systems, Ward and Murray (1984) demonstrated that chlamydia bind to cell membranes by multiple weak and probably nonspecific interactions and enter the cell by a microfilament dependent zipper mechanism, not via the receptor mediated endocytosis of viruses.

Endosomes containing the chlamydia were seen at 10 h in predominantly type I epithelium and occasional type II cells. Type I epithelium cells are metabolically more quiescent than type II cells and contain only a few small mitochondria, a limited number of cisternae of endoplasmic reticulum and a small Golgi complex. No lysosomes are evident. The type I cell would only have limited protein synthetic capabilities as compared to the type II cell. The degeneration and/or necrosis of organelles surrounding the endosomes, described by other investigators, was not seen in type I epithelium. However, these organelles are so sparse that changes could have been missed.

An important finding of this study is that distinct histopathological changes were evident at different time periods among the three study groups. The presence of abundant heterophils in the 2 to 3 week specimens of the nu/nu group, clearly separated it from the nu/+ and immune/nu+ findings of chronic mononuclear infiltrates. In immunocompromised adults, pathological findings of neutrophilic infiltrates in one case report and interstitial pneumonitis in another have been reported but remain controversial and need confirmation in larger numbers of patients (Tack *et al.* 1980; Ito *et al.* 1982). The lesion in infants has been characterized by nodular aggregates of eosinophils, lymphoid and plasma cells with neutrophils present in alveolar and bronchial lumina (Arth *et al.* 1977; Beem & Saxon 1977; Frommel *et al.* 1977). Our results would indicate potential variability in histopathological responses to chlamydia based on the immunological status of the infected host.

The immune nu/+ study group did not have organisms within the type I epithelium at the later time periods. It appears that defence mechanisms are activated early, probably immediately following inoculation of the organisms which decrease the number of organisms available to invade the alveolar epithelium. It is interesting in this regard that the immune animals had peribronchiolar and perivascular collections of mononuclear cells at the time of infection. Heterophils were present in all three groups at 10 h within the alveolar space, but by 48 h the susceptible animals had fewer mononuclear cells than the other groups. In the immune animals the infection appears to be effectively controlled before the appearance of heterophils. At later time periods successful host defence correlated with a mononuclear cell response. Large numbers of replicating (apparently viable) organisms in the nu/nu mouse were associated with a continuing large number of heterophils. Since the nu/nu mouse is clearly more susceptible to MoPn than the nu/+ groups (Williams *et al.* 1981; 1982; 1984*a*,*b*), the morphological data suggests that the mouse heterophil is a relatively ineffective host defence mechanism at least in the absence of antibody and active cell-mediated immunity (CMI). Effective host defence was characterized by the presence of lymphocytes, monocytes, lipid laden macrophages and plasma cells.

In regard to the latter, *C. trachomatis* organisms have been shown *in vitro* to stimulate human peripheral blood B lymphocytes to proliferate and secrete polyclonal immunoglobulins (Bard & Levitt 1984). The effect was much more pronounced if autologous T cells were present suggesting a two-signal model of B cell differentiation. Our nu/nu data are consistent with this hypothesis since extensive plasma cell infiltration was

not seen in nu/nu mice which lack T cells and nu/nu mice did not show evidence of antibody production by immunoperoxidase staining (Williams *et al.* 1984*a*). The persistence of MoPn antigen in lipid laden macrophages in nu/+ mice may provide the stimulus for ongoing B cell stimulation in mice with intact T cell function.

Our findings, therefore, demonstrate at least three important features. First, in the immunocompromised host the histopathology of chlamydial infection may be quite atypical and the organisms should be sought despite lack of classic findings of a mononuclear and plasma cell response. The histopathology may also vary in the immune host. Secondly, from a morphological perspective, successful host defence is characterized by a response containing lymphocytes, monocytes, lipid laden macrophages and plasma cells, a defence which in the immune animal is able to control pulmonary infection at a very early stage, apparently before epithelial cytoplasmic inclusions become evident and before polymorphonuclear leucocytes appear able to play a major role. Thirdly, the prominent plasma cell response (B cell response) *in vivo* is dependent on intact T cell function in the host and not simply the orgnaism acting a a direct nonspecific B cell mitogen.

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References

ARTH, C., VON SCHMIDT B., GROSSMAN M. & SCHACHTER J. (1978) Chlamydial pneumonitis. *J. Pediatr.* **93**, 447–449.

BARD J. & LEVITT D. (1984) *Chlamydia trachomatis* stimulates human peripheral blood B lymphocytes to proliferate and secrete polyclonal immunoglobulins *in vitro*. *Infect. Immun.* **43**, 84–92.

BEEM M.O. & SAXON E.M. (1977) Respiratory-tract colonization and a distinctive pneumonia syndrome in infants infected with *Chlamydia trachomatis*. *N. Engl. J. Med.* **296**, 306–310.

CHEN W. & KUO C. (1980) A mouse model of pneumonitis induced by *Chlamydia trachomatis*. *Am. J. Pathol.* **100**, 365–382.

DOUGHRI A.M., ALTERA K.P. & STORZ J. (1973) Host cell range of Chlamydial infection in the neonatal bovine gut. *J. Comp. Pathol.* **83**, 107–114.

DOUGHRI A.M., STORZ J. & ALTERA K.P. (1972) Mode of entry and release of Chlamydiae in infections of intestinal epithelial cells. *J. Infect. Dis.* **126**, 652–657.

FROMMELL G.T., BRUHN F.W. & SCHWARTZMAN J.D. (1977) Isolation of *Chlamydia trachomatis* from infant lung tissue. *N. Engl. J. Med.* **2196**, 1150–1152.

HARRISON H.R., ALEXANDER E.R., CHIANG WEN-TSUO., GIDDENS W.E. JR., BOYCE J.T., BENJAMIN D. & GALE J.L. (1979) Experimental nasopharyngitis and pneumonia caused by *Chlamydia trachomatis* in infant baboons: histopathologic comparison with a case in a human infant. *J. Infect. Dis.* **139**, 141–146.

HARRISON H.R., LEE S.M. & LUCAS D.O. (1982) *Chlamydia trachomatis* pneumonitis in the C57BL/KsJ mouse: pathologic and immunologic features. *J. Lab. Clin. Med.* **100**, 953–964.

ITO J.I., J.R., COMESS K.A., ALEXANDER E.R., HARRISON H.R., RAY C.G., KIVIAT J. & SOBONYA R.E. (1982) Pneumonia due to *Chlamydia trachomatis* inan immunocompromised adult. *N. Engl. J. Med.* **307**, 95–98.

MCDOWELL E.M. & TRUMP B.F. (1976) Histologic fixatives suitable for diagnostic light and electron microscopy. *Arch. Pathol. Lab. Med.* **100**, 405–414.

RANK R.G., HOUGH A.J. JR., JACOBS R.F., COHEN C. & BARRON A.L. (1985) Chlamydial pneumonitis induced in newborn guinea pigs. *Infect. Immun.* **48**, 153–158.

STERNBERGER L.A. (1979) *Immunocytochemistry*. 2nd ed. New York: John Wiley. pp. 82–129.

TACK K.J., PETERSON P.K., RASP F.L., O'LEARY M., HANTO D., SIMMONS R.L. & SABATH L.D. (1980) Isolation of *Chlamydia trachomatis* from the lower respiratory tract of adults. *Lancet* **i**, 116–120.

TODD W.J., DOUGHRI A.M. & STORZ J. (1976) Ultrastructural changes in host cellular organ-

elles in the course of the Chlamydial developmental cycle. *Zentralbl. Bakteriol. Mikrobiol. Hyg. [A].* **236**, 359–373.

WARD M.E. & MURRAY A. (1984) Control mechanisms governing the infectivity of *Chlamydia trachomatis* for HeLa cells: mechanisms of endocytosis. *J. Gen. Microbiol.* **130**, 1765–1780.

WEISS E. (1949) The extracellular development of agents of the psittacosis-lymphogranuloma group (Chlamydozoaceae). *J. Infect. Dis.* **84**, 125–149.

WILLIAMS D.M., SCHACHTER J., COALSON J.J. & GRUBBS B. (1984*a*) Cellular immunity to the mouse pneumonitis agent. *J. Infect. Dis.* **149**, 630–639.

WILLIAMS D.M., SCHACHTER J., DRUTZ D.J. & SUMAYA C.V. (1981) Pneumonia due to *Chlamydia trachomatis* in the immunocompromised (nude) mouse. *J. Infect. Dis.* **143**, 238–241.

WILLIAMS D.M., SCHACHTER J., GRUBBS B. & SUMAYA C.V. (1982) The role of antibody in host defense against the agents of mouse pneumonitis. *J. Infect. Dis.* **145**, 200–205.

WILLIAMS D.M., SCHACHTER J., WEINER M.H. & GRUBBS B. (1984*b*) Antibody in host defense against mouse pneumonitis agent (murine *Chlamydia trachomatis*). *Infect. Immun.* **45**, 674–678.