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Chlamydicidal Activity of Human Alveolar Macrophages

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Pneumonia due to *Chlamydia trachomatis* is a disease limited mainly to infants under 6 months of age. Rare cases have been reported in immunocompromised adults. One possible reason for the propensity of the pneumonia to occur in the very young may be related to differences in the phagocytic and bactericidal capacity of alveolar macrophages (AMs) in young infants and adults. At birth a function of AMs is clearance of surfactant-related material from the alveolar surface. Studies in animals have suggested that engorgement of AMs with surfactant-related lipids may reduce the microbicidal capacity of these cells. In the present study we determined that AMs obtained from healthy, nonsmoking adults were capable of killing both human biovars of *C. trachomatis*, with complete killing observed by 48 h after inoculation. Preincubation of AMs from adults with surfactant did not reduce the capacity of the cells to kill *C. trachomatis*.

Pneumonia due to *Chlamydia trachomatis* is a disease limited for the most part to infants under 6 months of age. Available epidemiological data suggest that the respiratory tract of older children and adults is not a hospitable environment for *C. trachomatis*. Rare cases of pneumonia due to *C. trachomatis* have been reported in immunocompromised adults (persons with leukemia, bone marrow transplant recipients) (4, 6). The symptomatology and pathologic findings in this disease appear to be more the result of a host immune response to the organism rather than to a direct effect of *C. trachomatis*. Cultures of lung biopsies from infants with chlamydial pneumonia have frequently failed to yield the organism (2).

The diameter of the chlamydial elementary body is small enough to allow it to be inhaled into the alveolar sac, where it would encounter the alveolar macrophage (AM), which is thought to the major immune response-regulating cell of the lung, directing the inflammatory response to these same antigens or particles through its ability to synthesize and secrete various chemical mediators. The limitation of the occurrence of pneumonia due to *C. trachomatis* to early infancy could be in part due to possible maturational or functional differences between alveolar macrophages of infants and adults. At birth a function of the AM is to clear the alveolar surface of fetal lung fluid, which contains large amounts of surfactant-related lipids. Studies have suggested that the phagocytosis of this surfactant-related material may reduce the microbicidal activity of AMs in newborn animals (11).

The purpose of this study was to investigate the interaction of *C. trachomatis* with human AMs and to observe the effect of the surfactant-related lipid engorgement on the interaction.

MATERIALS AND METHODS

Growth and purification of *C. trachomatis*. Two strains of *C. trachomatis* were used for our study: L_2/434/Bu, a lymphogranuloma venerum biovar, and N_{18}, a clinical cervical isolate that had the growth characteristics of a trachoma biovar strain. Organism were cultivated in cycloheximide-treated HeLa 229 cell monolayers and purified by differential centrifugation followed by one-step centrifugation over a self-generating Percoll gradient (Sigma Chemical Co., St. Louis, Mo.) (3).

AMs. AMs were collected from normal, nonsmoking adult volunteers by standard fiberoptic bronchoscopy and bronchoalveolar lavage procedures. Lidocaine (2%, without preservative) was used for topical anesthesia. Immediately after the lavage the specimen was kept at 4°C. The volume was measured, and the fluid was filtered through two layers of sterilized surgical gauze. The total cell number was determined by using a hemacytometer, viability, was determined by trypan blue exclusion, and differential count was determined by using a hematoxylin-eosin (Diff-Quik)-stained cytocentrifuged preparation. The cells were separated from the bronchoalveolar lavage fluid by centrifugation (400 × *g*, 10 min) and washed twice in phosphate-buffered saline without ions (pH 7.2). The cells were suspended in Dulbecco modified Eagle essential medium with 20% human serum (chlamydia antibody negative), glutamine (2 mM), vancomycin (10 μg/ml), amphotericin B (2 μg/ml), and gentamicin (10 μg/ml).

The AMs were cultured in flat-bottom 96-well tissue culture plates with low evaporation lids (Falcon) at a density of 10^5 cells per well in room air with 5% CO_2 and 100% humidity at 37°C. The AMs were examined for adherence at 2 h, and the cell culture medium was changed. Thereafter, the medium was changed every 72 h until the AMs were used. The AMs were used within 48 to 72 h of collection.

Preparation of surfactant. Surfactant was prepared from the bronchoalveolar lavage supernatants by the method of Shelley et al. (8). The supernatant was added to NaBr to a final concentration of 16%. Ten milliliters of lung washing containing 16% NaBr was placed at the bottom of a 30-ml polyallomer centrifuge tube (Beckman Instruments Spinco Division, Palo Alto, Calif.) and then overlayered with 16 ml of 15% NaBr in 0.9% NaCl and 4 ml of 0.9% NaCl. The gradient was then centrifuged at 114,000 × *g* for 1 h at 4°C in an ultracentrifuge (Beckman L7). The surfactant formed an oily-appearing band near the top of the gradient. The surfactant band was then removed, aliquoted, and frozen at −80°C until used (8).

Infection of AMs with *C. trachomatis*. The AM monolayer

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TABLE 1. Activity of AMs against the human biovars of *C. trachomatis*

Biovar and expt	Titer (IFU/ml [10^5])		% Reduction
	0 h	48 h	
Trachoma (N_{18})			
1	0.7	0	100
2	4.0	0	100
3	5.0	0	100
4	0.2	0.01	95
LGV (L_2)			
1	0.7	0.05	99.9
2	0.03	0	100

was inoculated with a known strain of *C. trachomatis* at a multiplicity of infection of 1:1 or 10:1. The inoculated AM monolayers were then centrifuged (400 × *g*) for 10 min and incubated in room air with 5% CO_2 and 100% humidity at 37°C for up to 48 h. At each time point, the viability of the cells was assessed by trypan blue exclusion.

Killing assay. Killing of *C. trachomatis* by the AMs was determined by viability of the organism in the supernatant and cell lysate in McCoy cell monolayers. At 0, 1, 2, 4, 8, 16, 24, and 48 h after inoculation, the supernatant was removed; the AMs were collected by scraping and then disrupted by sonication for 30 s with a needle probe tip Branson sonifier (model 185; Branson Sonic Power, Danbury, Conn.) to release the chlamydia. Titers of viable organisms from the supernatant and the cell lysate were determined by serial dilutions in McCoy cell monolayers. The monolayers were then stained after 48 to 72 h of incubation with a fluorescein-conjugated monoclonal antibody (Kallestad Diagnostics, Chaska, Minn.). To determine the effect of phagocytosed surfactant on killing, AMs were also preincubated with surfactant 1 h before infection at a concentration of 0.2 ml of the surfactant band per 10^5 AMs. A control of infected McCoy cells was also run for each experiment and processed in the same manner as the AMs.

Preparation of cells for transmission electron microscopy. Samples for transmission electron microscopy were taken by removing the AMs from the walls of the 96-well plates by gentle scraping with a rubber spatula. The cells were then centrifuged at 500 × *g* for 10 min at 4°C and washed with phosphate-buffered saline (pH 7.2). The cells pellets were then placed in 2% Formalin–glutaraldehyde fixative and centrifuged at 500 × *g* for 10 min. The pellets were postfixed with 1% osmic acid for 1 h. The fixed pellets were then embedded in Epon, sectioned, and examined in a JEOL 100C transmission electron microscope.

RESULTS

AMs were collected from eight healthy adult volunteers (mean concentration, 1.84×10^7 AMs/ml). The results of six experiments are summarized in Table 1. Both biovars of *C. trachomatis* appeared to be readily ingested and killed by the AMs. By 48 h there was a 95 to 100% reduction in inclusion-forming units (IFU) per milliliter. A detailed killing curve was prepared with cells from one subject (Fig. 1). There was an approximate 1-log increase in chlamydia in the McCoy cell control. The chlamydia (strain N_{18}) appeared to be ingested over a 4-h period; after 4 h we could no longer detect viable chlamydia in the supernatant. By 8 h there was >90% reduction in viable chlamydia, with 100% killing at 48 h.

The chlamydia did not appear to have a toxic effect on the AMs; >90% of the cells were viable as determined by trypan blue exclusion. Figure 2a shows an electron micrograph of an uninfected AM. An electron micrograph (Fig. 2b) of a human AM at 1 h after inoculation with strain N_{18} shows many vacuoles containing chlamydial elementary bodies. At 48 h postinoculation (Fig. 2c), large vacuoles containing amorphous debris, signifying killing, were seen.

Two experiments comparing AMs incubated with and without surfactant-related material also showed 100% killing (4 to 5 log reduction in IFU per milliliter) by 48 h after inoculation. Ultrastructural studies were conducted to determine whether the surfactant vesicles were phagocytized by

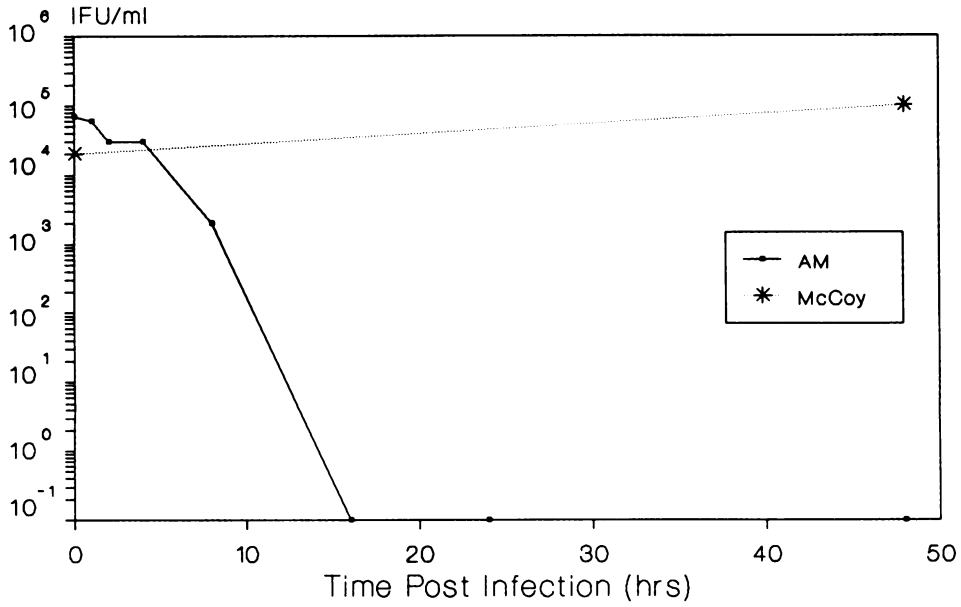

FIG. 1. Killing of *C. trachomatis* strain N_{18} by AMs from a healthy adult volunteer (■) compared to McCoy cells (*) at a multiplicity of infection of 1:1. Times of sampling were 1, 2, 4, 8, 16, 24, and 48 h after inoculation.

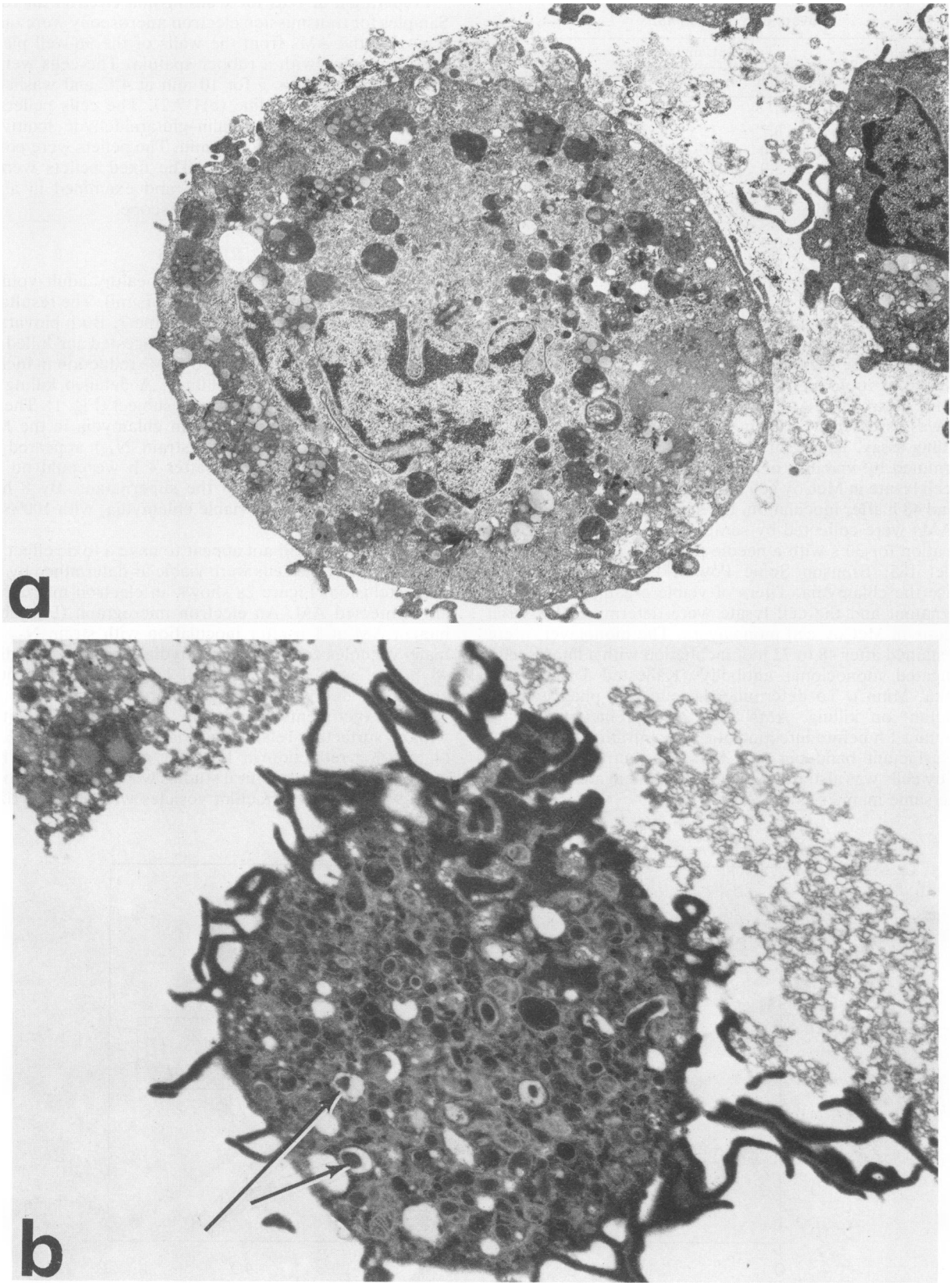

FIG. 2. Electron micrograph of uninfected AM. (b) Electron micrograph of human AM, 1 h after infection with *C. trachomatis* N_{18}, showing vacuoles containing elementary bodies (arrows). (c) Electron micrograph of AM, 48 h postinfection, demonstrating large vacuoles containing amorphous debris (arrows). Magnification, ×13,000.

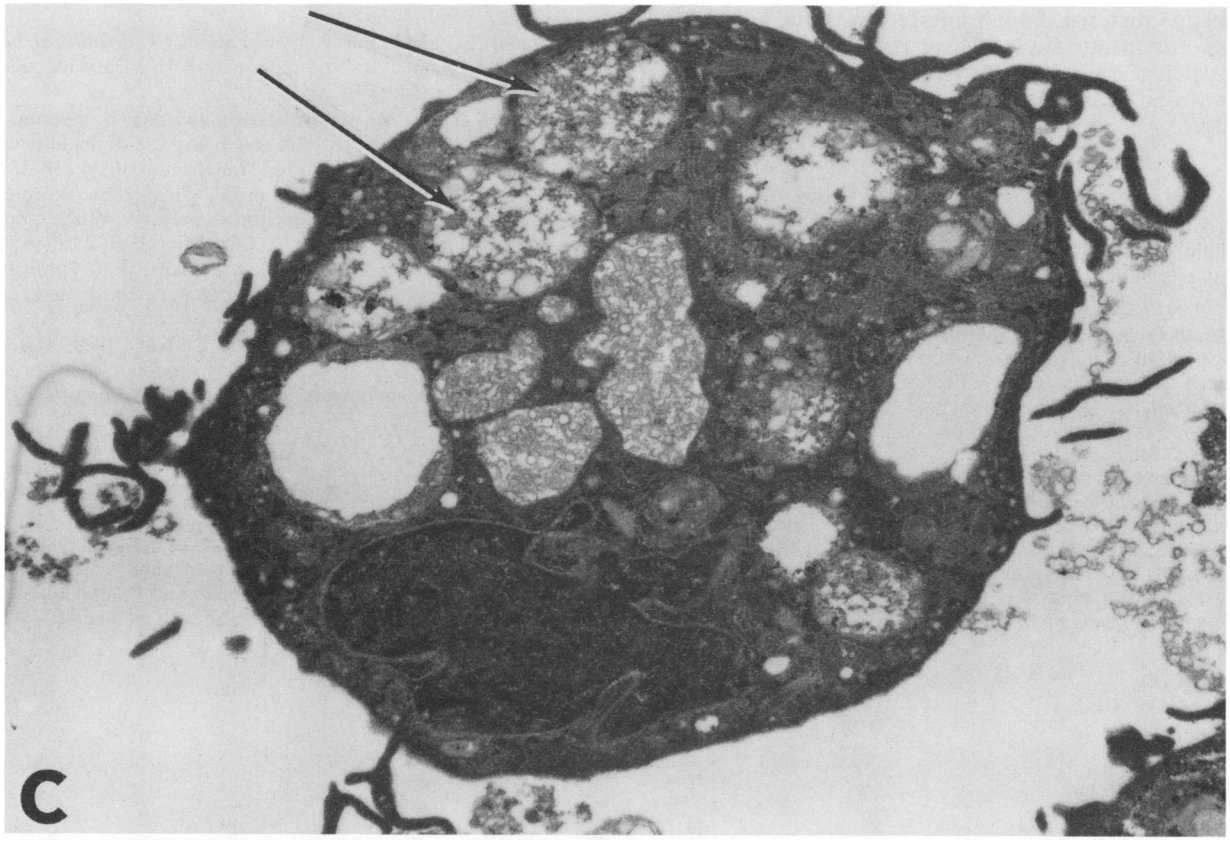

FIG. 2—*Continued*

the AMs and how long they persisted. After 1 h of incubation with surfactant, large membrane-bordered lipid vesicles were visible within the cytoplasm of the AMs. Light microscopic studies of thick sections stained with toluidine blue revealed that the lipid-laden vesicles were still present in the majority of AMs 48 h after incubation with the surfactant. At 1 and 48 h after inoculation, respectively, 62% ± 4.2% and 66.4% ± 7.1% of AMs per high-power field (mean of five fields) were engorged; the mean numbers of vesicles per AM were 2.9 ± 2 and 3.5 ± 3, respectively. AMs in culture that were not incubated with surfactant did not contain these vesicles. The surfactant was not toxic to the AMs; over 90% of the cells were viable as determined by trypan blue exclusion.

DISCUSSION

Our preliminary results suggest that AMs from normal adults readily ingest and kill both biovars of *C. trachomatis*. Manor and Sarov (5) reported that human peripheral blood monocyte-derived macrophages (cultured for >7 days) were not able to kill a lymphogranuloma venerum biovar strain of *C. trachomatis*. Yong et al. (9) found that human peripheral blood monocyte-derived macrophages cultured for 8 to 21 days were capable of killing a trachoma biovar strain but not the lymphogranuloma venerum biovar. Their ultrastructural studies of the macrophage-chlamydia interaction suggested that phagolysosomal fusion occurs when the chlamydiae are killed but that there is failure of fusion when they survive. Our finding of killing of both the trachoma and the lymphogranuloma venerum biovars by AMs from normal adults differs from these studies with monocyte-derived macrophages and once again points to differences between blood monocyte-derived macrophages and alveolar macrophages. Our results also may suggest why healthy adults do not develop pneumonia due to *C. trachomatis*. In a preliminary study we demonstrated that *C. trachomatis* was capable of causing productive infection in alveolar macrophages obtained from individuals with acquired immune deficiency syndrome (M. Nakajo, P. Roblin, M. Hammerschlag, P. Steiner, and M. Nowakowski, Pediatr. Res. **23**:337A, 1988). The best-documented cases of *C. trachomatis* pneumonia in adults have all been in immunosuppressed individuals. Although the organism was isolated from brush biopsies and bronchoalveolar lavage fluids of patients with acquired immune deficiency syndrome, it may have not had an etiologic association with diseases because the patients all also had infection with *Pnemocystis carinii* (7).

Data on the antimicrobial activity of alveolar macrophages from newborn infants are limited. Studies have demonstrated reduced candidacidal activity of human neonatal AMs compared with that of cells from adults (1). Studies with alveolar macrophages from newborn rabbits have also shown reduced microbicidal activity against *Staphylococcus aureus*, *Escherichia coli* (10), and *Candida albicans* (11). The bactericidal activity of the AMs increased with increasing age. It was hypothesized that the large quantities of phagocytized surfactantlike material present in AMs during the early postnatal period might be inhibiting their bactericidal activity. Pretreatment of AMs from 7- and 28-day-old animals with vesicles of surfactant-related material resulted in decreases in both chemotactic and candidacidal activity (11). Pretreatment of AMs from healthy adults with surfac-

tant-related material did not interfere with the killing of *C. trachomatis* in our study. There may be other defects in chlamydicidal capacity of AMs from human newborns that are yet to be defined and would require testing these cells directly.

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