Fate of *Chlamydia trachomatis* in Human Monocytes and Monocyte-Derived Macrophages

ESTHER MANOR AND ISRAEL SAROV*

Virology Unit, Faculty of Health Sciences and Soroka Medical Center, Ben Gurion University of the Negev, Beer Sheva 84105, Israel

The fate of *Chlamydia trachomatis* ($L_2/434/Bu$) in human peripheral blood monocytes and human monocyte-derived macrophages was studied by transmission electron microscopy (TEM) and by measuring the yield of infectious *C. trachomatis* in one-step growth experiments. Two main types of phagosome were seen by TEM in the cytoplasm of *C. trachomatis*-infected human monocytes (1 h postinfection [p.i.]): one in which the elementary body (EB) was tightly surrounded by the membrane of the phagosome and another in which the EB appeared in an enlarged phagosome. Later, 24 to 48 h p.i., each phagosome contained a single EB-like particle, an atypical reticulate body, or a damaged particle. One-step growth experiments showed that infection of human monocytes with *C. trachomatis* results in a decrease of infectious particles between 24 and 96 h p.i., whereas infection of the monocytes by *C. psittaci* (6BC strain) results in productive infection with, however, a 3.5-log lower yield than in control MA-104 cells. In contrast to the abortive replication of *C. trachomatis* in monocytes, monocyte-derived macrophages permitted replication as indicated by one-step growth experiments and TEM. in *C. trachomatis*-infected, monocyte-derived macrophages 72 h p.i., inclusions of two kinds were observed by TEM. One was very similar to the typical inclusions appearing in infected MA-104 (control) cells; the other was atypical, pleomorphic, often contained "channels," and held relatively few EB and reticulate bodies, some of which appeared damaged or abnormal. The significance of the responses to infection with *C. trachomatis* in monocytes compared with monocyte-derived macrophages and the role of these cells in sustaining chronic or latent infection and in dissemination of the infection to various parts of the body is discussed.

Chlamydiae are obligate, intracellular, gram-negative bacteria with a genome of 660×10^6 daltons (25). The multiplication cycle of chlamydiae involves two distinct forms of microorganism: the elementary body (EB), which is the infectious extracellular form, and the reticulate body, which is the metabolically active, highly permeable, intracellular form (20). There are two known species of chlamydiae, *Chlamydia trachomatis* and *C. psittaci*. Different serovars of *C. trachomatis* have been associated with clinically distinct infections ranging from hyperendemic trachoma to sexually transmitted infections and pneumonia. *C. psittaci*, the etiologic agent of psittacosis and ornithosis, has a broad host range which includes avian and mammalian species as well as humans, in whom it causes pneumonia with systemic involvement (16a). The lymphogranuloma venereum serovar of *C. trachomatis* infects both epithelial and lymphoid tissue, whereas non-lymphogranuloma venereum serovars are thought to grow only in epithelial cells (11).

Cell-mediated and humoral immune responses to the chlamydiae have been recorded after infection in humans and in a variety of animal models (17), but the role of these responses in the control of acute, chronic, or asymptomatic *Chlamydia* infections is poorly understood.

Macrophages play an essential role in eliminating a wide variety of pathogenic microorganisms from the host and are essential for the maintenance of resistance to infection (18). However, there are very few studies in which the in vitro interaction between macrophages and *C. trachomatis* has been investigated. Kuo (16) showed that *C. trachomatis* B/TW-5/OT and $L_2/434/Bu$ (two different serovars of *C.*

trachomatis) grow to a limited extent in mouse peritoneal macrophages. Most research on this subject has been carried out with *C. psittaci* and mouse peritoneal macrophages (5, 6, 22, 28). Murray et al. (21) and Rothermel et al. (24) showed that *C. psittaci* readily replicates in human monocyte-derived macrophages by examining the percentage of *C. psittaci*-infected cells after Giemsa staining for the presence of chlamydial inclusions. In the present study we examined the interactions between human peripheral blood monocytes or monocyte-derived macrophages and the lymphogranuloma venereum $L_2/434/Bu$ serovar of *C. trachomatis*. The one-step growth curve technique was used to measure the yield of infective EB particles from infected monocytes or macrophages at specific time intervals. The fate of *C. trachomatis* in infected monocytes and macrophages was examined by transmission electron microscopy (TEM).

MATERIALS AND METHODS

Cells. MA-104 cells, an embryonic rhesus monkey kidney cell line, were grown in RPMI 1640 (Biological Industries, Kibbutz Beth Haemek, Israel) supplemented with 10% fetal calf serum (GIBCO Laboratories, Grand Island, N.Y.), 2 mM L-glutamine, 100 μg of streptomycin ml^{-1}, 10 μg of gentamicin ml^{-1}, and 10 μg of Fungizone ml^{-1} (all from Bio-Lab Ltd. Laboratories, Jerusalem, Israel). Human peripheral blood monocytes were prepared from heparinized blood of normal donors by gradient centrifugation on Ficoll-Hypaque (1.077 μg cm^{-2} at 25°C) as described by Böyum (4). Cells from the interface were washed three times, suspended in RPMI 1640 containing 10 mm HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer, and incubated in plastic culture dishes (Nunc, Roskilde, Denmark) for 2 to 3 h at 37°C in an atmosphere of 5% CO_2. Nonadher-

ent lymphocytes were decanted, and adherent cell layers were washed five times with RPMI 1640. Adherent monocytes were removed from the plate by gentle scraping with a rubber policeman. Freshly isolated adherent cells were greater than 95% monocytes as determined by the nonspecific esterase technique (29).

For preparation of monocyte-derived macrophages, monocytes were obtained as described above; the monocytes were removed from their 75-cm^2 flasks (Nunc) by vigorous agitation and seeded in 96-well plates (Nunc; 2×10^5 to 4×10^5 cells per well), 24-well plates (Nunc; 7×10^5 to 10×10^5 cells per well), or both in the same medium. The cells were incubated at 37°C in an atmosphere of 5% CO_2 for 7 to 14 days. Ninety-five percent were macrophages as determined by using Mac-1 monoclonal antibodies obtained from J. Gopas (Faculty of Health Sciences, Ben Gurion University, Beer Sheva, Israel) in the fluorescence technique described by Gathing et al. (13). Sera of all monocyte donors were examined for the presence of *C. trachomatis*-specific immunoglobulin G antibodies by the immunoperoxidase assay described by Cevenini et al. (9).

Preparation of infectious EB particles. *C. trachomatis* biovar lymphogranuloma venereum (L$_2$/434/Bu) and *C. psittaci* 6BC (obtained from J. Orfila, Laboratoire de Bacteriologie-Immunologie Generale, Amiens, France) were grown in MA-104 cells in RPMI 1640 supplemented with 5% fetal calf serum, 1% glucose, 0.15% bicarbonate, 2 mM L-glutamine, 100 μg of streptomycin ml^{-1}, 10 μg of Fungizone ml^{-1}, and 1 μg cycloheximide ml^{-1} as the growth medium. Chlamydiae were harvested from MA-104 monolayers grown in 175-cm^2 polystyrene flasks (Nunc) as described by Caldwell et al. (8). Purified EBs were suspended in SPG buffer (0.01 M sodium phosphate, pH 7.2, containing 0.25 M sucrose and 5 mM L-glutamic acid) and stored at −70°C until use.

Immunoperoxidase assay for titration of *C. trachomatis* and *C. psittaci*. *C. trachomatis* and *C. psittaci* were titrated on MA-104 cells as described by Shemer and Sarov (26). The cells were seeded at 2×10^4 to 3×10^4 cells per well in 96-microwell plates (Nunc). After 48 h, 10-fold dilutions of chlamydial inoculum were prepared in growth medium. Triplicate 50-μl samples of serial 10-fold dilutions of chlamydial inoculum were added. The plates were fixed 2 days later with 100% ethanol, and an immunoperoxidase assay modified for *Chlamydia* spp. (14, 26) was performed. The final results of titration were expressed as inclusion-forming units (IFU) per milliliter.

Chlamydial infection of monocytes, monocyte-derived macrophages, and control cells. (i) Monocytes and control MA-104 cells were infected in suspension with either *C. trachomatis* or *C. psittaci* at an input multiplicity of infection (MOI) of 10 to 10^{-1} infectious particles per cell in siliconized test tubes at a cell concentration of approximately 5×10^6/ml. After 1.5 h at 37°C with gentle agitation at 10-min intervals, cell suspensions were washed with RPMI 1640 and cultured in 96- or 24-well plates in RPMI 1640 containing 5% fetal calf serum. Original MA-104 suspensions were prepared by dispersion of monolayers with trypsin versene (0.25% trypsin, 0.05% EDTA; Biological Industries). For titration at the times indicated, triplicate samples were removed from individual wells in 96-well plates by scraping and frozen at −70°C.

(ii) Infection of monocyte-derived macrophages was done in the original wells. Infectious EB particles were added to the cells at an MOI of 1 in 50-μl volumes per well for 96-well plates and 200 μl per well for 24-well plates. After 1.5 h with
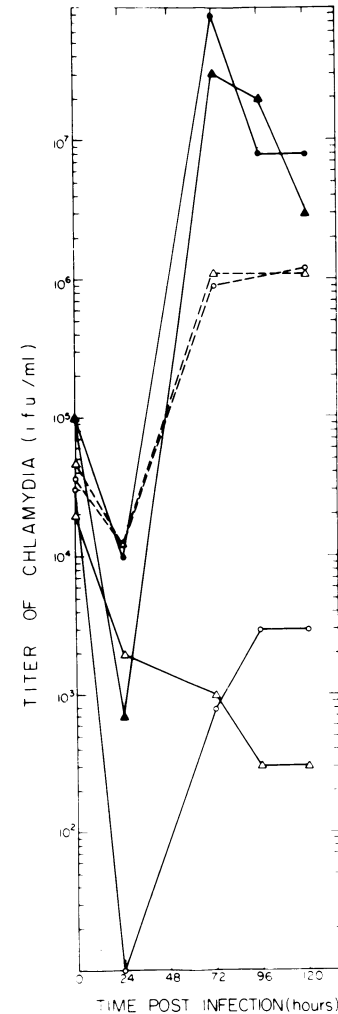

FIG. 1. One-step growth curves: infection of monocytes (solid lines, open symbols), monocyte-derived macrophages (dashed lines, open symbols), or control (MA-104) cells (solid lines, solid symbols) infected with either *C. trachomatis* (triangles) or *C. psittaci* (circles) at an MOI of 1 IFU per cell. Four repetitions of this experiment yielded similar results.

gentle agitation every 10 min, the inoculum was washed off and fresh medium was added to the wells.

Preparation of cells for TEM. Samples for TEM were taken by removing the cells from wells of 24-well plates with trypsin versene (Bio-Lab) and washing them in phosphate-buffered saline (pH 7.4). The samples were taken at 1, 5, 10, 24, and 48 h after infection. Monocyte-derived macrophages were fixed for TEM in the original culture wells. Infected and uninfected cells were fixed in 0.1 M sodium cacodylate buffer (pH 7.2) containing 2% (wt/vol) glutaraldehyde for 1 h at room temperature and post fixed for 1 h with 0.1 M (wt/vol) osmium tetroxide in cacodylate buffer. Fixed-cell pellets were embedded in Araldite, sectioned, and examined with a Philips 201C TEM after staining with uranyl acetate and lead citrate as described by Biberfeld (3).

RESULTS

A one-step growth curve of *C. trachomatis* and *C. psittaci* in peripheral blood monocytes and in MA-104 cells (control)

at an input MOI of 1 IFU per cell is illustrated in Fig. 1. In monocytes inoculated with *C. trachomatis*, the number of infectious chlamydiae detected decreased considerably from 24 h post infection (p.i.) until 96 h p.i., with no further change in the number of infectious chlamydiae at 120 h p.i. No difference in the fate of *C. trachomatis* in monocytes was observed whether the infection was carried out at an MOI of 0.1 or 10 (data not shown). In contrast, monocytes inoculated with *C. psittaci* supported replication. At 24 h p.i. no infectious chlamydiae were detected, but after 72 h p.i. a rise in the number of infectious chlamydiae was found. Their numbers continued to rise up to 96 h p.i. and then remained steady at 120 h p.i. Infection of monocyte-derived macrophages with either *C. psittaci* or *C. trachomatis* at an input MOI of 1 per cell resulted in a decrease in the number of infectious particles at 24 h p.i. followed by a rise in the number of infectious particles at 72 h p.i. At 120 h p.i. no additional significant rise in the yield was detected. The yield of both *C. trachomatis* and *C. psittaci* in control (MA-104) cells was higher by about 2 logs than in monocyte-derived macrophages and by about 3.5 logs than in *C. psittaci*-infected monocytes at 72 h p.i. The source of the monocytes and monocyte-derived macrophages, whether they came from a chlamydia-seropositive (immunoperoxidase immunoglobulin G titer of ≥8) or -seronegative (immunoperoxidase immunoglobulin G titer of <8) donor did not affect the replication pattern of *C. trachomatis* and *C. psittaci* in these cells.

Inverted light-microscopic observation of the monocyte and monocyte-derived macrophage cultures infected with either *C. trachomatis* or *C. psittaci* at an MOI of 1 at 48 h p.i. showed the following. (i) In monocytes infected with *C. trachomatis*, no distinct inclusion could be seen, whereas with *C. psittaci*, about 20 to 30% of the monocytes had large inclusions inside the infected cells. (ii) With *C. trachomatis* and *C. psittaci* about 40 to 50% of the monocyte-derived macrophages had inclusion bodies. Infection at a higher MOI (5 to 10 IFU per cell) resulted in a cytolytic effect on the cells.

TEM studies of *C. trachomatis* in peripheral human monocytes and monocyte-derived macrophages. At 1 h p.i., EBs could be seen attached to and internalized in human monocyte cells (Fig. 2A and B). Two kinds of phagosome containing only one chlamydia were observed: one in which the chlamydia was tightly surrounded by the vacuole membrane (Fig. 2C) and another in which the chlamydia was present in a large vacuole (Fig. 2D). At a high MOI (5 to 10 IFU per cell), one or more particles were seen in each phagosome (Fig. 2E).

In general, the most prominent difference between uninfected and infected monocytes, other than the presence of inclusion bodies, was the presence of many enlarged mitochondria in the cytoplasm of the infected monocytes. At later times (24 and 48 h p.i.), some phagosomes contained EB-like particles (Fig. 2F), some contained abnormal-appearing reticulate bodies, and some contained damaged particles. These observations may explain the decrease in infectious chlamydia obtained in the one-step growth curve (Fig. 1). Monocyte-derived macrophages infected with *C. trachomatis* at an input MOI of 1 and observed by TEM at 72 h p.i. held two kinds of inclusions: typical and atypical (Fig. 3). The typical ones were similar to the inclusions observed in control (MA-104) cells and contained all of the different stages of the life cycle of chlamydia (EB, reticulate body, etc.). The atypical ones were pleomorphic and contained a few normal-appearing EBs and reticulated bodies and abnor-

mal or damaged forms. The inclusions, furthermore, often contained cytoplasmlike areas. These "channels" may be associated with the pleomorphism of the atypical inclusions, perhaps indicating the presence of a relatively weaker (more fluid?) membrane than is seen defining the typical vacuoles (Fig. 3). Lysosomes were often prominent in the cell cytoplasm in the vicinity of atypical vacuoles. Examination of complete serial sections of 300 cells showed that 53% were not infected, 25% were infected and had typical *C. trachomatis*-containing inclusion vacuoles, and 22% were infected but had atypical *C. trachomatis*-containing inclusion vacuoles. In contrast, 90% of control MA-104 cells were infected and all had only typical inclusions.

DISCUSSION

Many previous studies have demonstrated that cellular components of the immune system, especially macrophages and lymphoid cells, play a decisive role in acquired resistance of the host to facultative and obligate intracellular organisms (19).

It has been shown that, in herpes simplex virus (19) and *Leishmania tropica* (2, 15) infection of different animal strains, a significant correlation exists between the ability of macrophages of a given strain to support replication in vitro and the severity of the disease in vivo in that strain. With respect to *C. psittaci* infection in mice, no such correlation was found between the severity of the disease in vivo and macrophagic replication of the agent in vitro (22).

The present study provides data on the fate of the L_2 serovar of *C. trachomatis* in human peripheral blood monocytes and monocyte-derived macrophages obtained from healthy donors. By TEM we observed EB particles attached to and internalyzed in monocytes at 1 h p.i. (Fig. 1). The two different phagosomes, tight and loose, which we observed at 1 to 5 h p.i. (Fig. 2C and D) may be analogous to the two types of vacuoles observed by Rikihisa and Ito (23) in the case of rickettsia in human polymorphonuclear leukocytes and by Silva et al. (27) in the case of toxoplasma in mouse macrophages. Both groups suggested that their findings could be related to a dual mechanism of entry of the parasites. EB of chlamydiae can enter phagocytes such as mouse macrophages by two different endocytic pathways. Infectious EBs enter via a parasite-specific pathway, whereas heat-inactivated EBs are taken in by a host-specific route (7, 30). The involvement of two mechanisms in the entry of *C. trachomatis* EBs into human monocytes is a subject for further investigation.

The one-step growth experiments described in this study showed that infection of human monocytes by *C. trachomatis* resulted in a decrease of infectious chlamydiae, whereas infection of the monocytes by *C. psittaci* resulted in productive infection with, however, a 3.5-log lower yield than in control MA-104 cells. The mechanism by which human monocytes inhibit *C. trachomatis* replication but support limited replication of *C. psittaci* is unknown. Differences in metabolite requirements between *C. trachomatis* and *C. psittaci* may be an explanation for the results obtained. Further studies are required to clarify this observation.

We showed that, in contrast to the abortive replication of *C. trachomatis* in human monocytes, monocyte-derived macrophages support productive replication (Fig. 1 and 3A) with, however, a 2-log lower yield than that obtained in MA-104 cells. The fact that under conditions of MOI = 1 only 47% of monocyte-derived macrophages contained inclusions (versus 90% of MA-104 cells) cannot fully account
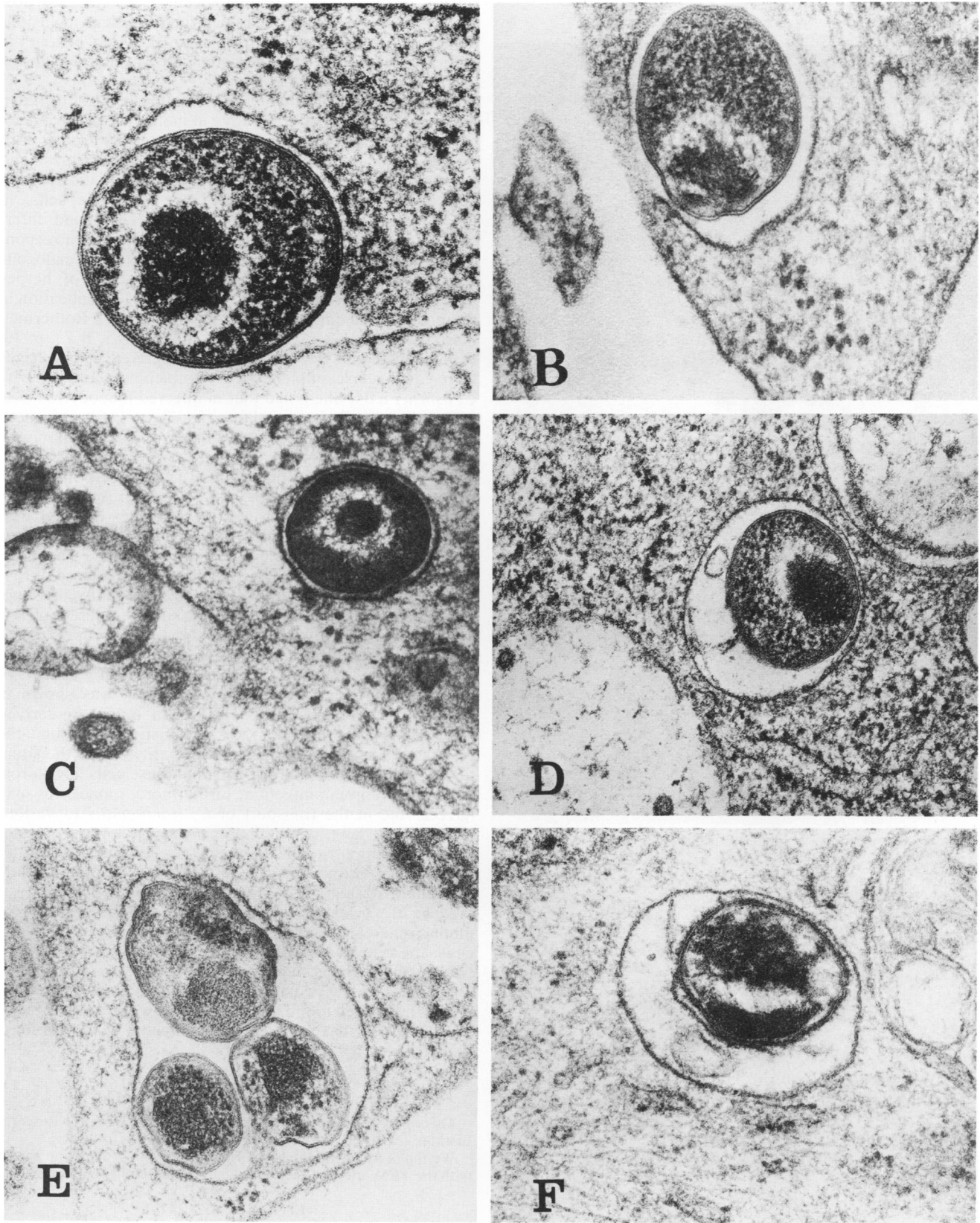
FIG. 2. Electron micrographs of monocytes infected with *C. trachomatis* at an input MOI of 1 at 1 h p.i. except where otherwise noted. Panels: A, Cell-particle contact; B, enclosure of EB in cellular phagocytic vacuole; C, EB tightly surrounded by phagosomal membrane; D, EB loosely surrounded by phagosomal membrane; E, at a high MOI (5 to 10 IFU per cell), more than one particle in a vacuole; F, an EB-like particle at 24 h p.i. Magnifications: A and B, ×100,000; C, D, and F, ×70,000; E, ×45,000.
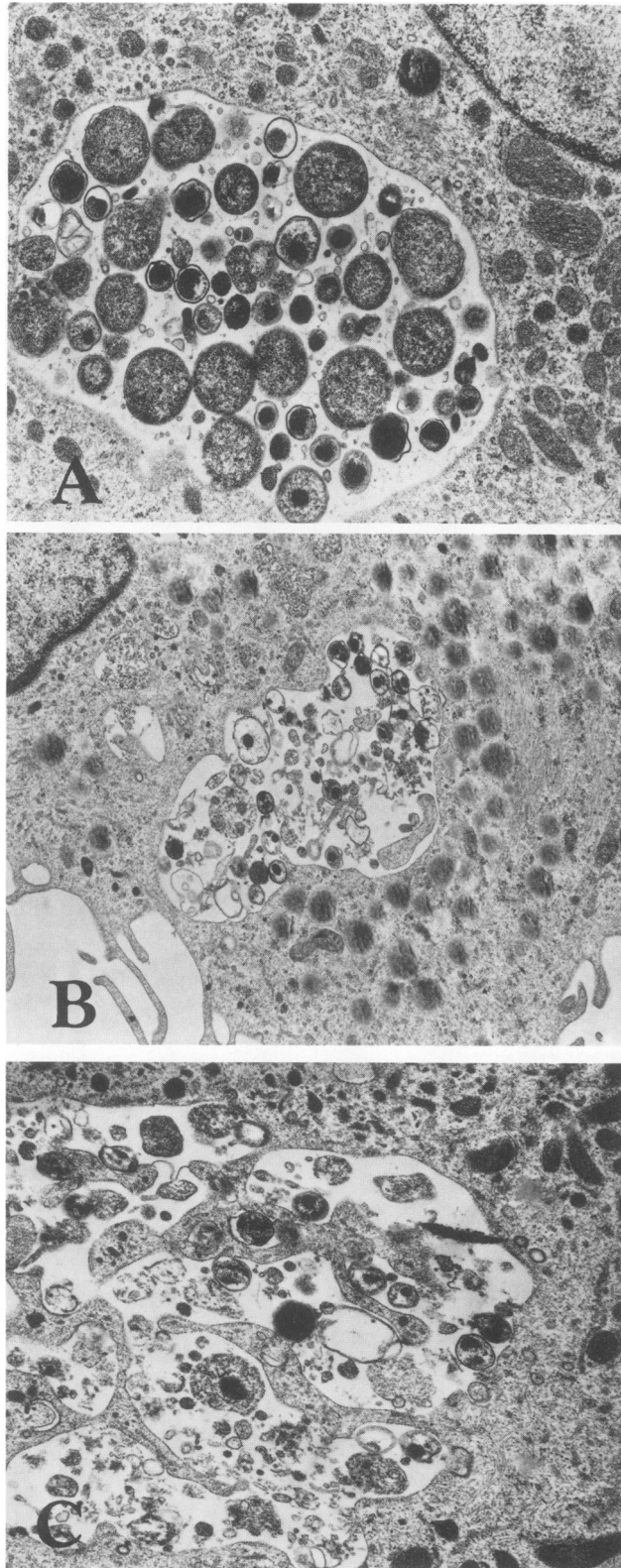
FIG. 3. Electron micrographs of monocyte-derived macrophages infected with *C. trachomatis* at an MOI of 1.0 at 72 h p.i. Panels: A, typical inclusion; B, atypical inclusion; C, atypical inclusion containing channels. Magnifications: A and C, ×15,000; B, ×10,000.

for this phenomenon; perhaps relevant to the relatively low yield is the fact that both typical and atypical inclusions appeared in macrophages (Fig. 3A and B). It may be that the different responses to infection are made by different subsets of the macrophage population.

The reason for *C. trachomatis* infection being abortive in human monocytes but productive in monocyte-derived macrophages is unknown. Adherent monocytes cultured in vitro for 7 days undergo a series of morphologic, biochemical, and functional changes that resemble their differentiation in vivo into mature macrophages (12). Thus, the same cells, in different metabolic or differentiation states, respond differently to infection. Although the precise mechanism responsible for such differences has not been identified, analogous observations have been reported in the case of herpes simplex virus (10) and varicella zoster virus (1) replication in these cells. Recently, it was reported (C. D. Rothermel, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, B 75, p. 30) that blood monocytes and monocyte-derived macrophages were equally susceptible in vitro to attachment and entry by *C. psittaci* EBs. She noted that in infected monocytes a rise in inclusion scores appeared from 5 to 8 days after infection, correlated to the display of typical macrophage morphology. She postulated that "this association between parasite replication and host cell differentiation may be relevant to the persistence of chlamydiae in vivo." In the present study we showed that monocyte-derived macrophages infected with *C. psittaci* give higher yields than monocytes; however, since monocytes do yield infectious particles, one must show that differentiation of a given cell has permitted further *C. psittaci* replication and that the rise in inclusion score observed with differentiation is not simply a result of late infection of initially uninfected monocytes which have meanwhile differentiated to macrophages.

In summary, infection of human monocytes by *C. trachomatis* serovar lymphogranuloma venereum was shown in this study to be abortive, whereas in monocyte-derived macrophages the infection was productive. Although the results obtained in vitro do not necessarily reflect the situation in vivo, the possibility exists that these cells have a role in latent or chronic infection; furthermore, monocytes may have a role in the transport of *C. trachomatis* to various organs of the host, where they may mature to macrophages which can support replication. The possibility that the latter can occur in vitro in the case of *C. trachomatis* is presently being investigated.

Since *C. trachomatis* biovars other than lymphogranuloma venereum are thought not to replicate in lymphoid cells, the relevance of the data reported here to infections with such biovars is so far unknown. However, it should be noted that persistent infiltrates of lymphocytes and macrophages have been found in the conjunctiva and cornea during trachoma (18). The interaction of lymphoid cells with these biovars deserves further investigation.

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