BACTERIAL PATHOGENICITY

Studies of persistent infection by *Chlamydia trachomatis* serovar K in TPA-differentiated U937 cells and the role of IFN-γ

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Inoculation of phorbol ester-differentiated U937 cells as a model for human macrophages with *Chlamydia trachomatis* of the urogenital serovar K resulted in a persistent infection, with maximal growth at day 7, until day 10 post-infection. At these times inclusion bodies were present in 0.5–2% of the cells. Typical inclusion bodies containing elementary bodies and reticulate bodies were observed by electron microscopy. Furthermore, single chlamydial particles resembling atypical elementary or intermediate bodies were identified in the cytoplasm in >80% of the host cells. IFN-γ exerts antichlamydial activity in epithelial and fibroblastic cells, but the infection of U937 cells by *C. trachomatis* was not affected by IFN-γ. The activity of the tryptophan-degrading enzyme indoleamine 2,3-dioxygenase (IDO) was not detected in untreated or IFN-γ-treated or chlamydia-infected or mock-infected U937 cells. The presence of atypical persisting chlamydiae and the lack of IDO expression in U937 cells indicates that the development of these atypical bacteria is independent from IFN-γ-mediated tryptophan deprivation and other IFN-γ-mediated effects. Evaluation of persistently infected cells revealed that the expression of the chlamydial major outer-membrane protein, heat-shock protein (hsp60) and lipopolysaccharide (LPS) antigens was not significantly altered in the course of the culture. An intense staining of the LPS on the surface of the host cells was demonstrated by immunofluorescence. The data show that phorbol ester-differentiated U937 cells restrict chlamydial growth strongly but not completely through a mechanism distinct from IDO-mediated tryptophan deprivation. The mechanisms of persistence of chlamydiae in monocytes, which differ considerably from those described for other cells, require further investigation.

Introduction

The obligate intracellular bacterium *Chlamydia trachomatis* exhibits a complex developmental cycle involving an infectious metabolically inactive form (elementary body: EB) and a non-infectious metabolically active form (reticulate body: RB) [1]. One characteristic of the biovar trachoma is its capacity to induce chronic diseases such as blinding trachoma or – after urogenital infection – complications like infertility or ectopic pregnancy [2–4]. Moreover, *C. trachomatis* of the serovars D–K have also been implicated in Reiter's disease and chlamydia-induced arthritis [5, 6].

The pathogenesis of the extra-urogenital chronic sequelae of chlamydial infections is not well understood. One important observation is that *C. trachomatis* persists at the site of inflammation in a viable but culture-negative state [7, 8]. Chlamydia antigen has been detected in specimens from patients with active trachoma, even though chlamydiae could not be isolated by culture [9]. Chlamydia major outer membrane protein (MOMP), lipopolysaccharide (LPS) and rRNA have been identified in culture-negative specimens from inflamed joints of patients with chlamydia-induced arthritis and Reiter's syndrome [5, 10–12]. Although the target cells for the trachoma biovar are preferentially mucosal epithelial cells, published data show the persistence of chlamydiae in monocytic cells [13–15]. Recently, Nanagara et al. demonstrated chlamydiae in both macrophages and fibroblasts from synovial membranes from patients.
with Reiter's disease some 12 years after the onset of the disease [8]. These observations suggest that persistent infection of monocytes may be relevant for the pathogenic process leading to chronic chlamydial-induced inflammatory disease.

An earlier study showed that morphologically altered but metabolically active C. trachomatis serotype K persists in human peripheral blood monocytes for 14 days post-infection in vitro [13, 16]. The in-vitro infection of monocytes possibly reflects one basic mechanism of chronic chlamydial inflammation at the cellular level. However, the use of these cells to investigate the basic intracellular events in chlamydial-mediated persistence has several limitations. The methods of preparation and separation do not exclude contamination with lymphocytes, only a limited number of cells can be obtained from one blood donor, and monocytes may vary according to the biological variability of the blood donors. The establishment of an in-vitro infection model with monocytic phorbol ester-treated U937 cells could therefore serve as an alternative tool to study the mechanisms of chlamydial persistence [17].

The aim of this study was to investigate the fate of C. trachomatis serovar K in differentiated U937 cells and the influence of IFN-γ. This cytokine induces persistence of chlamydiae in fibroblasts and epithelial cells [18–20]. Because persistent chlamydial infections have been proposed as a source of antigens that induces or maintains the chronic inflammatory diseases or both, the levels of chlamydial immunogens were evaluated during the course of chlamydial infection of U937 cells [21, 22].

Materials and methods

Preparation of bacteria

C. trachomatis serovar K (UW/31/Cx; Washington Research Foundation, Seattle, USA) was grown in HEp-2 cells in complete medium RPMI 1640 supplemented with heat-inactivated fetal calf serum (Gibco) 10%, 2 mM L-glutamine, vancomycin 25 mg/L, gentamicin 5 mg/L, glucose 1% w/v and cycloheximide 1 μg/ml (i.e. infection medium). Infected cells were harvested after culture for 48 h at 37°C. EB were purified in a discontinuous gradient of Urographin [23] and stored in 100 μl volumes at −80°C.

Culture of cells and infection with C. trachomatis

U937 cells from the American Type Culture Collection (Rockville, MD, USA) were cultured in RPMI 1640 medium with fetal calf serum 10%, 2 mM L-glutamine, vancomycin 25 mg/L and gentamicin 5 mg/L (i.e. culture medium) at 37°C in a humidified atmosphere with CO2 5%.

U937 cells were plated at a density of 1 × 10⁶/cells per well in six-well culture plates and differentiation was induced by adding the phorbol ester 12-O-tetradecanoyl phorbol-13-acetate (TPA) (5 × 10⁻⁹ m) to the culture medium. After 72 h, the differentiated cells were washed once and inoculated with C. trachomatis at a multiplicity of infection (MOI) of 0.5. Non-infected controls were run in parallel. After centrifugation (20 min, 450 g), the cells were incubated for 2 h at 37°C in CO2 5% and then washed three times with Hank's Balanced Salts Solution (HBSS) to remove non-ingested bacteria. At various times, cells were harvested by scraping with a rubber scraper and frozen at −80°C for determinations of infectivity and chlamydial rRNA; preparations for immunofluorescence microscopy were made on the day of harvest. As positive controls for chlamydial infectivity, cultures of HEp-2 cells (5 × 10⁵/ml) were infected and harvested as described above.

IFN-γ treatment of infected U937 culture

Differentiated U937 cells were incubated with IFN-γ at 25 (1.25 ng) and 400 (20 ng) U/ml (1 × 10⁶ U ≃ 50 μg, Biogen) for 24 h. Thereafter, the cells were infected with C. trachomatis and harvested at day 2 post-infection to determine infectivity and the number of inclusions. Treatment of HEp-2 cells with IFN-γ at 0.1-400 U/ml and subsequent infection with C. trachomatis (MOI 0.5) was performed as a control.

Assay for intracellular IDO activity

TPA-differentiated U937 cells were treated with or without IFN-γ and with or without C. trachomatis and harvested 2, 3 and 5 days after infection. After washing in PBS, the pellets (1 × 10⁶ cells) were frozen at −70°C. The cell pellets were then thawed and the assay for indoleamine 2,3-deoxygenase (IDO) was performed as described previously [24]. The assay is based on the determination of the decyclisation product of L-tryptophan, kynurenine, that is measured at an absorbance of 490 nm after addition of Ehrlich's reagent (0.4% p-dimethylaminobenzaldehyde/acetic acid). As a positive control IFN-γ-stimulated 86HG39 cells (glioblastoma cell line) were used. A strong IDO activation was found in these cells. They produced > 30 μg of kynurenine/ 1 × 10⁶ cells.

Determination of the infectious yield of C. trachomatis

The infectious yield of C. trachomatis was determined by titration on HEp-2 cells [25]. Frozen samples of infected TPA-differentiated U937 cells were thawed in an ice-bath and subsequently sonicated for 2 min (Transsonic 460; Merck, Germany); 50-μl volumes of 10-fold dilutions in infection medium were added to the HEp-2 cells growing in 96-well microtitration
plates. After incubation for 48 h at 37°C, the cultures were fixed with absolute ethanol. Inclusion bodies were stained by an immunoperoxidase assay, with the serum of a C. trachomatis-positive patient having an IgG specific titre of > 256 (Ipazymtest, Medac, Hamburg, Germany), and counted by light microscopy. The results are expressed as infection forming units (IFU)/ml.

**Immunofluorescence microscopy**

For the direct immunofluorescence microscopy, infected and mock-infected U937 cells (10^5/100 μl) were centrifuged on to slides and fixed with methanol. Chlamydial inclusion bodies and single bacteria were visualised with an anti-MOMP FITC-conjugated monoclonal antibody (MAb) (Syva, Palo Alto, CA, USA). Brightly shining green inclusions were counted and the results were expressed as inclusion bodies/10^5 cells. For the indirect immunofluorescence assay, TPA-differentiation of U937 cells was performed on coverslips and cells were infected as described. At different times cultures were air-dried or fixed with methanol and subsequently treated with either the anti-hsp60 MAb GP 51-19 (a generous gift from Dr Morrison, Hamilton, MT, USA) or the anti-LPS MAb S 25-23 (a generous gift from Dr Brade, Borstel, Germany). Bound antibodies were visualised with a second FITC-labelled goat anti-mouse F(ab’)2 fragment (Dianova, Hamburg, Germany). All samples were screened with an epifluorescence microscope (Leitz, Wetzlar, Germany).

**Determination of chlamydial rRNA synthesis**

Chlamydial rRNA was determined by an in-vitro hybridisation assay with a single-stranded chemiluminescent-labelled cDNA probe (PACE 2; Gen-probe, San Diego, CA, USA). Samples of infected U937 cells (1–3 × 10^5) were resuspended in 100 μl of lysis buffer (Gen-probe) and the test was performed according to the manufacturer’s instructions. Concentrations of chlamydial rRNA are expressed as relative light units (RLU). In our experience, the test has a detection limit of rRNA corresponding to 50 IFU [12].

**Transmission electron microscopy**

Pellets of TPA-differentiated U937 cells (2 × 10⁶ cells/pellet) infected with C. trachomatis were harvested at day 7 after infection and fixed in glutaraldehyde 3% in 0.1 M Na-cacodylate HCl buffer, pH 7.3, for 1 h, post-fixed in OsO₄ 2% in the same buffer, dehydrated in a graded series of ethanol and embedded in Agar-Araldite. Thin sections were stained with uranyl acetate and lead citrate and examined in a Siemens Elmiskop IA electron microscope at 80 KV. Mock-infected U937 cells served as negative controls.

**Immunoblot assay**

TPA-differentiated U937 cells were infected in six-well culture plates and harvested at the times indicated, centrifuged and resuspended in 100 μl of PBS. After sonication, the concentration of protein in the samples was determined by a micro-Bradford assay with bovine serum albumin as a standard protein. Equal amounts of protein were denatured in SDS 6%, glycerol 30% and β-mercaptoethanol 15% in 0.0625 M Tris and resolved by SDS-PAGE (acylamide 10% gel) as described by Laemmli [26]. Lysates of isolated EBs served as the positive control. After electrophoresis, the separated proteins were transferred to PVDF membranes (Immobilon-P, Millipore, Bedford, MA, USA). Non-specific binding sites were blocked by incubation in blocking buffer (PBS, Tween 20 0.05%, milk 5%). The blot was probed with either the murine GP5-19 MAb directed against the chlamydial hsp60 or the mouse anti-MOMP MAB LV 22 (Washington Research Foundation) or the mouse anti-LPS MAB [27, 28]. Immunopositive proteins were visualised with alkaline phophatase-conjugated rabbit anti-mouse IgG (Dianova) followed by staining with 5-bromo-4-chloro-3-indolyl phosphate (BCIP) and nitrobluetetrazolium (NBT; both from Sigma). Lanes were analysed with a scanning densitometer (Pharmacia, Freiburg, Germany).

**Statistical analysis**

Statistical analysis was performed on the mean and SD of independent experiments. The significance of the values was calculated with the non-paired two-tailed Student’s t test. A p value < 0.05 was considered to indicate a significant difference between two sets of data.

**Results**

**Infection of U937 cells with C. trachomatis**

The fate of C. trachomatis in TPA-differentiated U937 cells was investigated over 14 days. The infection was followed by determination of chlamydial inclusion bodies by immunofluorescence, by titration of cell lysates from infected U937 cells on HEp-2 cells and by measurement of chlamydial rRNA. Cells inoculated with C. trachomatis at an MOI of 0.5 permitted low grade replication over the whole cultivation period without destroying the cell culture (Fig. 1a–c). The number of fluorescent inclusion bodies stained with the anti-MOMP MAB increased gradually from the day of infection to day 7 after infection (Fig. 1a). An additional significant rise in the yield of inclusions was found between day 7 and day 10 after infection (p < 0.001). At that time, 0.5–2% of the TPA-differentiated U937 cells contained fluorescent inclusions as shown in Fig. 2. Single fluorescent chlamydial particles were also observed in cells containing no inclusion bodies. From day 10 to day 14 after infection,
The number of inclusion bodies as determined by direct immunofluorescence in TPA-differentiated U937 cells at the indicated time points following infection with *C. trachomatis* serovar K at a MOI of 0.5. Data are the means and SD of four separate experiments, each performed in duplicate. (b) Infectious yield of *C. trachomatis*. Titration of the lysates from TPA-differentiated U937 cells at the indicated time points after infection with *C. trachomatis* at a MOI of 0.5 on HEp-2 cells. Data are the means and SD of four separate experiments, each performed in duplicate. In control experiments (insert A) the growth in HEp-2 cells infected with *C. trachomatis* at a MOI of 0.5 was determined. The yield of infective chlamydia is expressed as IFU/ml. (c) The kinetics of chlamydial rRNA synthesis in TPA-differentiated U937 cells after infection with *C. trachomatis* at a MOI of 0.5. One representative experiment of four is shown.

The number of inclusions decreased considerably (p < 0.003). Coincident with the increase of inclusion bodies, a rise in the infectious yield of *C. trachomatis* was observed from day 1 to day 7 after infection (p < 0.004) and a decrease of infectivity was apparent from day 7 to day 14 after infection (p < 0.002) as determined by titration of the lysates from infected TPA-differentiated U937 cells on HEp-2 cells (Fig. 1b). In control (HEp-2) cells infected at an MOI of 0.5, the yield of *C. trachomatis* was c. 2.3 log10 higher than in infected U937 cells (Fig. 1b). In contrast to the U937 cells, the monolayer of HEp-2 cells was destroyed after culture for 3 days as observed by immunofluorescence and light microscopy. The amount of chlamydial rRNA that was determined in infected cultures of TPA-differentiated U937 cells followed a similar time course to the infectivity (Fig. 1c).

**Effect of IFN-γ**

Pretreatment of chlamydiae-infected Hep-2 cells with IFN-γ concentrations > 25 U/ml inhibited the development of infective elementary bodies as shown by titration of the cell lysates on HEp-2 cells, whereas lower concentrations of IFN-γ still permitted infectivity (Table 1). 25 U of the cytokine/ml were incubated with TPA-differentiated U937 cell 24 h before infection with *C. trachomatis*. As shown in Table 1, the
Table 1. The effect of human IFN-γ on the growth of C. trachomatis serovar K in HEp-2 cells or U937 cells

<table>
<thead>
<tr>
<th>Cells and treatment</th>
<th>Mean (SD) number of inclusions/10^5 cells</th>
<th>Mean (SD) number of IFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>HEp-2 Medium</td>
<td>5000 (430)</td>
<td>800 000 (35 000)</td>
</tr>
<tr>
<td>+ IFN-γ (0.1 U/ml)</td>
<td>1230 (120)</td>
<td>230 000 (10 000)</td>
</tr>
<tr>
<td>+ IFN-γ (10 U/ml)</td>
<td>70 (10)</td>
<td>216 (22)</td>
</tr>
<tr>
<td>+ IFN-γ (25 U/ml)</td>
<td>12 (4)</td>
<td>0</td>
</tr>
<tr>
<td>+ IFN-γ (100 U/ml)</td>
<td>7 (3)</td>
<td>0</td>
</tr>
<tr>
<td>+ IFN-γ (400 U/ml)</td>
<td>10 (4)</td>
<td>0</td>
</tr>
<tr>
<td>U937</td>
<td>20 (15)</td>
<td>260 (30)</td>
</tr>
<tr>
<td>+ IFN-γ (25 U/ml)</td>
<td>35 (18)</td>
<td>300 (50)</td>
</tr>
<tr>
<td>+ IFN-γ (400 U/ml)</td>
<td>23 (12)</td>
<td>250 (45)</td>
</tr>
</tbody>
</table>

HEp-2 cells and TPA-differentiated U937 cells were pretreated with or without IFN-γ at concentrations indicated and infected with C. trachomatis serovar K for 2 days. The numbers of inclusions/10^5 cells and IFU/ml were the mean (SD) of three experiments.

IFN-γ treatment has been shown to induce the expression of IDO, which converts l-tryptophan to kynurenine. It has been proposed that IFN-mediated chlamydial persistence arises from enhanced IDO expression and subsequent tryptophan depletion. However, this enzyme was not found to be induced in U937 cells by either IFN-γ or chlamydiae or both together (data not shown), whereas in the positive control (IFN-γ-treated 86HG39 cells), strong IDO activity was measured.

Electron microscopy

Only few (1–2%) of the TPA-differentiated U937 cells that were examined 7 days after incubation with C. trachomatis at a MOI of 0.5 contained a typical inclusion body with numerous EB, RB and intermediate forms (Fig. 3a). However, > 80% of the U937 cells contained vacuoles in which one or two chlamydiae were present. The bacteria within the vacuoles often had an enlarged shape containing electron-dense material and resembled EB or intermediate forms but not RB (Fig. 3b). In mock-infected U937 cells, no such structures were observed.

Expression of chlamydial antigens

To determine levels of chlamydial antigens during persistent infection of U937 cells, three were selected for evaluation: the MOMP antigen, a target of neutralising antibodies (the major vaccine antigen); LPS, a major antigen on the chlamydial surface; and the hsp60 antigen, chosen because the immune response to this protein may be important in the immunopathogenesis of persistent inflammation [22, 29–31]. Immunoblotting and immunofluorescence were used to compare the amounts and localisation of MOMP, LPS and hsp60 in infected U937 cells. Densitometer tracings of immunoblots of cells 48 h after infection indicated a 6.5:1 ratio of hsp60 to MOMP (Fig. 4, lane A) and a nearly 8.6:1 ratio of hsp60 to MOMP (Fig. 4, lanes C, D, E) in cells 7, 10 and 14 days after infection. The 5.7:1 ratio of hsp60 to MOMP in isolated elementary bodies corresponds to the ratio in infected cells 48 h after infection. The level of the chlamydial LPS slightly increased during infection of U937 cells.

Immunofluorescence with the anti-LPS MAb gave an intense staining on the surface of the air-dried U937 cells at every time-point of the culture, whereas the anti-hsp60 MAb stained only the inclusions in the air-dried cells (Fig. 5a, b). In infected U937 cells that were fixed with methanol, only chlamydial inclusions were stained with the anti-LPS MAb or with the anti-hsp60 MAb (not shown). No staining was observed with either mock-infected U937 cells or the second antibody alone.

Discussion

Persistent infection by C. trachomatis has been studied in a limited number of cell culture systems and most experiments have been performed in fibroblasts or epithelial cells [20, 21, 32]. However, these cells are not the only important host cells in vivo, because increasing evidence shows that ‘professional’ phagocytes play a decisive role in the persistence of chronic chlamydial infections [13, 14, 33]. It has been proposed that monocytes act as vehicles for chlamydial dissemination in the infected host and serve as reservoirs of antigens that trigger the inflammatory response [34–36]. A persistent infection of C. trachomatis was established in the monocytic cell line U937 under non-limiting growth conditions, i.e., in the absence of inhibitors or deficiencies. This cell culture system differs from the established host-parasite system with biphasic cycles of McCoy cells and C. trachomatis introduced by Lee and Moulder [32]. The present study showed that TPA-differentiated U937 cells became persistently infected after inoculation at a low and non-destructive MOI with the chlamydial urogenital strain serovar K. Clearly the transition from infected host cells to new host cells was low and, therefore, over a period of 14 days a co-existence or balance between chlamydial development and intact live cell culture was maintained. It is important to note that only 0.5–2% of the U937 cells contained inclusion bodies at the time when chlamydial infectivity and replication were at a maximum, i.e. day 7 until day 10 after infection. However, > 80% of these cells contained chlamydiae differing in ultrastructure from the typical EB and RB present in inclusion bodies. This observation may explain the observation of single fluorescent particles by immunofluorescence microscopy. The atypical EB-
Fig. 3. Transmission electron micrographs of TPA-differentiated U937 cells 7 days after infection with C. trachomatis. 

a: U937 cell with an inclusion body containing typical elementary bodies, reticulate bodies and intermediate forms; arrows point to a single C. trachomatis in the cytoplasm (magnification \( \times 10000 \)). 

b: Magnification of an intracellular phagosome containing atypical chlamydiae (magnification \( \times 60000 \)).
CHLAMYDIAL PERSISTENCE IN U937 CELLS

Fig. 4. Immunoblot analysis of TPA-differentiated U937 cells infected at a MOI of 1 with C. trachomatis serovar K. Blots were treated with anti-hsp60, anti-MOMP and anti-LPS antibodies. Lanes, A–E represent day 2 (A), day 5 (B), day 7 (C), day 10 (D) and day 14 (E). Lane F shows reactivity for isolated elementary bodies as control.

Significant differences were noted in the ability of C. trachomatis serovar K to generate infectious EB in inclusion bodies in various cell types. Whereas inclusion bodies developed in only very few TPA-treated U937 cells they occurred in virtually all cells of HEp-2 cultures infected in parallel with the same preparation of chlamydiae and did not develop at all in human peripheral blood monocytes [13]. These-like or intermediate bodies resemble those described in human monocytes infected with serovar L2 [34]. Furthermore, a recent electron microscopy and immuno-electronmicroscopy study showed that atypical chlamydiae of serovar K are present in human monocytes during non-replicative infection [16]. These organisms are in a metabolically active state as demonstrated by molecular analyses of primary chlamydial rRNA transcripts [16] and look very similar to those found in U937 cells harvested at day 7 after infection.

Fig. 5. TPA-differentiated U937 cells 7 days after infection with C. trachomatis. Cells were air-dried and stained by indirect immunofluorescence. LPS antibody (A) yielded a surface staining pattern of the U937 cells (arrow), whereas the hsp60 antibody (B) stained the inclusion in the host cell (arrow).
observation indicates that, depending on the host cell, the replication of chlamydiae is permitted, retarded or inhibited.

In vivo, the intracellular pathogen is sheltered from direct immune attack, but it remains susceptible to the cytokine-mediated immune response. IFN-γ has been proposed as a physiological mediator of chlamydial persistence, because it induces atypical, non-infectious chlamydiae in fibroblasts and epithelial cells in vitro. This persistent state is caused by IFN-γ-mediated induction of IDO [19, 25, 37–39]. This enzyme catalyses the decyclusion of tryptophan in human cells. The depletion of this essential amino acid correlates with the growth inhibition of C. trachomatis in epithelial or fibroblastoid cells and addition of tryptophan was shown to reverse the inhibitory effect of IFN-γ [39, 40]. No IFN-γ mediated growth inhibition was observed in infected U937 cells although the cells were susceptible to the cytokine as shown previously. Exogenously added IFN-γ specifically mediates the release of IL-1β [41]. The phenomenon of chlamydial growth in spite of IFN-γ treatment can be explained by the fact that IDO is not active in these cells [42]. Even though the enzyme is not expressed, atypical organisms were detected in 80% of the U937 cells examined, indicating that tryptophan deficiency was not the cause of the development of these atypical forms. Moreover, in peripheral blood monocytes infected with serovar K, the addition of tryptophan or antibodies against IFN-γ did not result in the generation of replicative/infective organisms [16]. Thus, an unknown mechanism(s) exists that inhibits the growth cycle of phagocytosed chlamydiae in monocytic cells.

The intracellular persistence of C. trachomatis may possibly initiate and contribute to the maintenance of chronic inflammation. The chlamydial hsp60 antigen is considered to be a strong candidate responsible for the stimulation of the immune response [22]. IFN-γ-mediated persistent infection in HeLa cells is characterised by a decreased expression of chlamydial MOMP and LPS, whereas the chlamydial hsp60 expression remains unchanged [21, 43]. In lysates of persistently infected U937 cells an increase of hsp60 and LPS was observed whereas the level of MOMP did not alter from day 2 to day 14. The different antigen expression in these two persistence models may be explained by the developmental stage of chlamydiae in both cell lines. Whereas in IFN-γ-treated HeLa cells no infectious bacteria developed, in U937 cells low infectivity and the enhanced appearance of atypical bacteria resulted in the synthesis of hsp60 and LPS.

By immunofluorescence, the LPS antigen was not only shown in association with chlamydial development stages in inclusions, but was also stained on the surface of the infected U937 cells. These findings are in accordance with previous studies that have demonstrated extrachlamydial LPS on the surface of host cells [44, 45]. Several investigators have reported that isolated LPS stimulates monocytes to produce the pro-inflammatory cytokines IL-1β and TNF-α [46, 47]. The observation of chlamydial LPS on the surface of the host cell and the well known induction of inflammatory cytokines in response to endotoxin suggest that LPS may play a profound role in the pathogenesis of chlamydial infections.

Chlamydial hsp60 antibody was kindly provided by Dr Morrison (National Institute of Allergy and Infectious Diseases, Hamilton, MT, USA) and the chlamydial LPS antibody by Dr Brade (Department of Biochemical Microbiology, Borstel, Germany). The data were presented in part at the Eighth International Symposium on Human Chlamydial Infections in June 1994, Couvieux Chantilly, France: Abstract number 198. This work was supported by the Deutsche Forschungsgemeinschaft, grant Ko 949/2-1 and grant Ko 949/2-2.

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