**Chlamydia trachomatis** Growth Stimulates Interleukin 8 Production by Human Monocytic U-937 Cells

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**Growth of Chlamydia trachomatis** serotypes L2 and L3 in a human monocytic cell line, U-937, increased the rate of interleukin 8 (IL-8) release 100-fold. Heat-killed chlamydiae induced a 10-fold-lower level of production of IL-8. IL-8 may play an important role in the inflammatory reaction to chlamydial infection.

*Chlamydia trachomatis* is an obligate intracellular pathogen with a primary tropism toward mucosal epithelium. It is known to be the causative agent of trachoma in developing countries and of sexually transmitted diseases worldwide. Recent progress in the study of host immunity strongly suggests that an immunopathologic component is involved in the pathogenesis of chlamydial diseases and in the development of fibrosis and sequelae such as blindness or tubal infertility (13). Few authors have investigated the in vivo effects of *Chlamydia* on the production of inflammatory cytokines (1, 3, 9, 10, 21, 22). A local T-lymphocyte Th-1-like protective response has been observed after intravaginal infection of mice with the mouse pneumonitis biovar of *C. trachomatis* (2). In previous in vitro studies, *C. trachomatis* was found to induce the production of interleukin 1 (IL-1) by human blood monocytes (18), the release of tumor necrosis factor alpha (TNF-α) from monocyte-derived macrophages (11), and the production of IL-1 and TNF-α by human peripheral blood mononuclear cells (7, 18). Another important cytokine is the chemokine IL-8, which plays a major role in the inflammatory response to several microorganisms mostly by activating polymorphonuclear leukocytes (10). This activation may contribute to both microbial clearance and tissue damage (12, 15, 17). To our knowledge no previous study has reported a role for *C. trachomatis* in the production of IL-8. The purpose of the present investigation was to examine the ability of *C. trachomatis* to induce and/or regulate the production of IL-8 in infected cells.

We first tried to establish a reproducible in vitro model of chronic infection of human monocytic U-937 (ATCC CRL 1593) (19) cells with two strains of the *C. trachomatis* lymphogranuloma venereum biovar: L2 (ATCC VR 902B) and L3 (ATCC VR 903). U-937 cells were seeded at 5 × 10⁴/ml in RPMI 1640 medium (Biowhittaker, Verviers, Belgium) containing 10% heat-inactivated fetal calf serum (Gibco BRL, Prisley, Scotland), glutamine, streptomycin, and vancomycin. Cell viability, assessed by trypan blue exclusion, was greater than 95% prior to all experiments. Since some mycoplasmas can induce cytokine production by monocytic cells (4, 6, 16), cultures were periodically checked for contamination by culture and DNA staining (Hoechest 33258) (20). U-937 cells were infected with *C. trachomatis* by centrifugation at 3,500 × g for 30 min at 37°C in 24-well flat-bottomed tissue culture plates (Falcon; Becton Dickinson Labware, Lincoln Park, N.J.) at a multiplicity of infection of 100 inclusion-forming units per cell, determined by titrations with HeLa 229 cells (ATCC CCL 21). After centrifugation, the bacterial inoculum was replaced by 1 ml of supplemented RPMI 1640 medium per well. Uninfected control cells were treated with sucrose phosphate buffer instead of the infectious inoculum. Fourteen serial passages were performed every 2 or 3 days for 1 month. Intracellular chlamydial growth was monitored by direct fluorescence assay (DFA) with an anti-lipopolysaccharide (LPS) fluorochrome-labeled monoclonal antibody (*Chlamydia* direct IF; BioMérieux, Marcy l’Etoile, France) after cytocentrifugation at 100 × g for 3 min (Cytospin 3; Shandon). The release of *C. trachomatis* antigens in the culture supernatants was measured by an enzyme immunoassay (EIA) (*Chlamydia*zyme; Abbott Laboratories, North Chicago, Ill.). Each assay was performed in two independent experiments. U-937 cells sustained the intracellular growth of serotypes L2 and L3 of *C. trachomatis*. The reproducible and chronic *C. trachomatis* infection established in U-937 cells with serotype L2 yielded typical intracytoplasmic inclusions, as detected by DFA and high-level chlamydial-antigen production in the culture supernatants (Fig. 1 and 2). In contrast, serotype L3 induced only few and nontypical intracytoplasmic inclusions and little or no antigen production in the culture supernatants (Fig. 2).

To measure the amount of IL-8 present in the cell culture supernatants, frozen stocks of U-937 cells, infected with *C. trachomatis* serotype L2 or L3, were used to infect U-937 cells as described above. The multiplicity of infection was 1:25 for serotype L2 and 1:100 for serotype L3. Cultures were incubated for 8 days in three different assays. Every 1 or 2 days, cells were smeared by cytocentrifugation, growth of *C. trachomatis* was visualized by DFA, and culture supernatants were collected and aliquoted at −70°C until the cytokine assay. Supernatants were tested by EIA for IL-8 (Quantitine; R & D Systems, Minneapolis, Minn.) and for TNF-α, IL-1β, and IL-6 (all with EASIA; Medgenix, Fleurus, Belgium). The minimum detectable levels are estimated to be 2 pg/ml for IL-1β, 3 pg/ml for IL-6, and 5 pg/ml for TNF-α. Each sample was tested in duplicate in each assay. Supernatants of control cells were negative for TNF-α, IL-1β, and IL-6 but contained low concentrations of IL-8, ranging from 0 to 224 pg/ml (Fig. 3). To our knowledge, no previous study has reported spontaneous IL-8 secretion by U-937 cells. Results obtained after infection with *C. trachomatis* serotypes L2 and L3 confirmed most previous studies showing amounts of TNF-α ranging from 10 to 12 pg/ml (L2 serotype) and 14 to 78 pg/ml (L3 serotype), showing...
amounts of IL-1β ranging from 50 to 130 pg/ml (L2 serotype) or no detection (L3 serotype) and showing no detection of IL-6 during the course of infection (7, 11, 18). Infection of cells with the L2 serotype of *C. trachomatis* dramatically increased IL-8 concentrations in the supernatants of infected cells from 0.7 ng/ml at day 1 to 3.5 ng/ml at day 8 (Fig. 3). The L3 serotype of *C. trachomatis*, which has less ability to replicate than the L2 serotype, induced lower levels of IL-8 production, which ranged from 0.1 ng/ml at day 1 to 2.2 ng/ml at day 8 (Fig. 3). The early release of IL-8 (within 1 day) with both L2 and L3 suggested that the interaction of cells with the bacteria could be the triggering stimulus. To confirm this hypothesis, heat-killed chlamydiae or heterologous LPS was also tested for cytokine induction in control experiments. The serotype L2 inoculum was heat killed at 100°C for 1 h and used to test for a homologous LPS effect. For heterologous LPS stimulation, cells were grown in the presence of 100 ng of LPS from the Re mutant of *Escherichia coli* J5 (Sigma Chemical Co., St. Louis, Missouri) (Fig. 3). The early release of IL-8 (within 1 day) with both L2 and L3 suggested that the interaction of cells with the bacteria could...
Mo.) per ml. Supernatants of cells stimulated with heat-killed chlamydiae or LPS from E. coli were negative for TNF-α, IL-1β, and IL-6. Heat-killed chlamydiae induced lower levels of IL-8 production than live bacteria during the 8 days of the experiment (0.4 to 0.8 ng/ml) (Fig. 3). When cell cultures were stimulated with heterologous LPS, IL-8 production was equivalent to that of nonstimulated control cells (Fig. 3). These data suggested that intracellular replication of the bacteria was required for enhancing IL-8 production by the infected cells.

In vivo studies suggest that IL-8 may be an important component of local immunity in response to bacterial infections (5, 14). IL-8 production by cells infected with C. trachomatis is probably a complex process and may be associated with the production of growth factors, such as transforming growth factor β, could be important effectors of fibrosis and ultimately tubal obstruction. However, the extrapolation of these results to chlamydial infection calls for further studies, and this hypothesis clearly has to be investigated in animal models as well under clinical conditions.

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