Infection of Human Monocyte-Derived Macrophages with *Chlamydia trachomatis* Induces Apoptosis of T Cells: a Potential Mechanism for Persistent Infection

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Viruses can escape T-cell surveillance by infecting macrophages and thereby induce apoptosis of noninfected T cells. This ability had not been demonstrated for bacteria. We investigated whether infection of macrophages with the important human pathogen *Chlamydia trachomatis* can induce T-cell apoptosis. Because *Chlamydia*-Mycoplasma coinfection is a frequent event, the ability of *Mycoplasma fermentans*-infected macrophages to induce T-cell apoptosis was also studied. Infected macrophages were cocultivated with autologous T cells in different activation states. Propidium iodide-based fluorescence-activated cell sorter analysis demonstrated that macrophages infected with viable chlamydiae induced T-cell death. Apoptosis was identified as the mode of death induction by using a terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling assay. Induction of T-cell death was macrophage dependent. Incubation of T cells with infectious chlamydiae in the absence of macrophages did not lead to T-cell apoptosis. UV irradiation of chlamydiae diminished the ability to induce death. T-cell death was induced by a cell-free supernatant of infected macrophages. Not only phagocytosis but also non-mitogen-activated T cells were susceptible to *C. trachomatis*-induced apoptosis. In contrast, *M. fermentans* infection of macrophages did not induce T-cell death. Coinfection had no additional effect. In summary, intracellular chlamydial infection of macrophages can induce T-cell apoptosis. Apoptosis induction by chlamydiae possibly explains how persistently infected macrophages escape T-cell surveillance and why the *Chlamydia*-specific T-cell response is diminished during persistent chlamydial infection.

The progress of apoptosis-related research has prompted great interest in the mechanisms of cell death (41). Apoptosis (programmed cell death) occurs when a normally functioning cell receives a variety of different cell signals (25, 27). Microbe-induced apoptosis was first identified for viral infections and has subsequently been reported in cases of infections with any of a large number of pathogenic bacteria and parasites. The ability of pathogens to induce apoptosis may play a role in the initiation of the infection, the survival of the pathogens, and their escape from the host immune response (4, 49, 57). Infection of cells not only changes the susceptibility of host cells to apoptosis-inducing mechanisms but also can mediate apoptosis of noninfected cells. It was demonstrated that human immuno-deficiency virus infection of macrophages leads to up-regulation of Fas ligand on infected host cells and, consequently, to the induction of apoptosis of noninfected T cells (5, 35). The induction of apoptosis of cytotoxic T cells is an attractive model to explain the persistence of intracellular pathogens in host cells. However, this mechanism had not been demonstrated for bacterial infections.

*Chlamydia trachomatis* is an obligate intracellular bacterial pathogen known to be the etiological agent responsible for several diseases, such as blinding trachoma, nongonococcal urethritis, and reactive arthritis (8, 51). Using molecular biology methods, we and others have frequently detected *C. trachomatis* nucleic acids in synovial tissues of patients with re-active arthritis or undifferentiated oligoarthritis (9, 15, 22, 32, 43, 46). It was further shown that synovial chlamydiae are viable and metabolically active (14). They reside predominantly within macrophages below the synovial lining (9, 32). There is evidence that antibiotic treatment of *Chlamydia*-induced arthritis can modify the course of the disease (7, 26). The eradication of chlamydiae in the synovial tissue seems to be a reasonable goal in amelioration of the synovitis. Therefore, for the improvement of treatment, it is vital to understand how chlamydia can persist in the synovial membrane.

Other bacteria recently linked with apoptosis induction are members of the genus *Mycoplasma* (36, 39). Paddenberg et al. showed that Mycoplasma-infected fibroblasts undergo apoptosis after treatment with cycloheximide (36). They have demonstrated that some *Mycoplasma* strains possess an endonuclease. This nuclease can cleave DNA in a manner similar to the endogenous endonucleases that become activated as apoptosis progresses. The activities of both nucleases lead to DNA ladder formation, the hallmark of apoptosis. *Mycoplasma fermentans* has become an object of interest as a human pathogen. It probably serves as a cofactor in AIDS (10, 39). *M. fermentans* is a facultative intracellular gram-negative bacterium which can survive in macrophages (28, 40).

We have developed a human macrophage model to study persistent chlamydial infection (13, 24). Macrophages are the primary host cells for *C. trachomatis* in synovial tissue (32). Despite the presence of a detectable T-cell response in joints, *C. trachomatis*-infected macrophages are not eliminated (9). Su and Caldwell have reported that adding infected macro-
phages to T cells in a mouse model can induce T-cell death (45). We hypothesized that Chlamydia-induced T-cell death can be mediated by the induction of apoptosis. We used the in vitro macrophage model for persistent chlamydial infection, added autologous T cells, and assayed this cell system for T-cell apoptosis. In addition, we investigated whether infection with the facultative intracellular bacterium *M. fermentans* can also induce T-cell apoptosis. The rationale for studying *M. fermentans* was that it can persistently infect macrophages, and coinfection with both bacteria occurs in vivo and in vitro (19, 28, 29). A large percentage of *Chlamydia pneumoniae* strains used for experimental studies were coinfected with *Mycoplasma* (23, 30). It was therefore possible that previous observations were attributable to *Mycoplasma* coinfection.

**MATERIALS AND METHODS**

**Organisms.** *C. trachomatis* elementary bodies (EBs) were prepared as previously described (42). In brief, infectious EBs of *C. trachomatis* serovar K (UW/31/Cc; Washington Research Foundation, Seattle, Wash.) were grown in HEP-2 cells. Serovar K was chosen because of its potential to induce arthritis. Chlamydia-infected EBs were collected from the culture supernatants (Scherer AG, Berlin, Germany) density gradient centrifugation. Purified EBs were resuspended in 1 ml of sucrose phosphate buffer (0.01 M sodium phosphate, 0.25 M sucrose, 5 mM L-glutamic acid, pH 7.2 [all chemicals were purchased by Sigma, Deisenhofen, Germany]). EBs were washed in 2 ml small aliquots at –80°C. Inclusion-forming units were quantitated by titration on HEP-2 cells and subsequent indirect immunoperoxidase assay. To visualize inclusion bodies in HEP-2 cells, cells were incubated with a fluorescein isothiocyanate (FITC)-labeled, mouse-derived anti-*MOMP* antibody (Boehringer, Mannheim, Germany) density gradient centrifugation. Purified EBs were resuspended in 1 ml 3% bovine serum albumin, 500 μM EDTA, and washed and resuspended at 10^7 EBs/ml in PBS containing 1% FCS and 60 IU of rIL-2/ml. EBs were stored in small aliquots at -20°C. The antichlamydial activity of the patient serum was tested by a commercially available enzyme-linked immunosorbent assay (ELISA) (Medac, Hamburg, Germany). The titer for immunoglobulin G antibodies was 1:200,000. After addition of the substrate 4-chloro-1-naphtol, inclusion bodies appeared as black dots and were counted by light microscopy. The method was controlled by application of a fluorescein isothiocyanate (FITC)-labeled, mouse-derived anti-major outer membrane protein (anti-MOMP) antibody (Boehringer, Mannheim, Germany). Incubation of *C. trachomatis*-infected HEP-2 cells with this antibody and detection by fluorescence microscopy led to similar results. *C. trachomatis* cultures were routinely screened for mycoplasma infection by culture and PCR techniques followed by restriction enzyme digestion as described previously (23). The protocol used for purification of *C. trachomatis* after coinfection with *M. fermentans* was recently published (23). *M. fermentans* strain PG 18 was kindly provided by G. Gerlach, Department of Microbiology, University of Heidelberg School Hannover, Germany. Mycoplasma expanded in glass tubes containing mycoplasma broth for 4 days at 37°C in an atmosphere of 5% CO2 and tested for viability by a microtiter dilution method as described elsewhere (1). Mycoplasma suspension was serially diluted in sterile microtiter plates (Nunc, Wiesbaden, Germany). After dilution, a 10-μl volume from each well of the dilution assay was inoculated on the surface of mycoplasma agar plates. Plates were incubated at 37°C in 5% CO2 until colonies could be detected by inverted light microscopy at 40× magnification, usually after 7 to 10 days (1). Colonies were quantitated by multiplying the microtiter dilution by the average number of colonies counted.

**Purification and stimulation of PBMCs and monocytes.** Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized buffy coats obtained from normal blood donors of our blood bank by Ficoll-Hypaque density gradient centrifugation. PBMCs were resuspended in RPMI medium containing 10% FCS, 2% bovine serum albumin, and 50 μM EDTA. After washing and resuspension in PBS containing 1% FCS and 60 IU of rIL-2/ml, PBMCs were resuspended in phosphate-buffered saline (PBS) and fixed in 75% ethanol for 1 h at 4°C. They were then washed and resuspended in PBS containing PI (50 μg/ml Sigma) and RNase A (250 μg/ml of type I-A; Sigma). The PI fluorescence of viable cells was determined by a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). We restricted our analysis to viable cells with at least diploid DNA content due to the limitations of the method in clearly distinguishing between necrotic and apoptotic cells. We therefore analyzed only viable cells. The propidium iodide (PI) channel was set to the 10th percentile to exclude the gate for viable cells was set according to the PI content of control populations. Control populations included viable T cells and apoptotic T cells. T-cell apoptosis was induced according to the method of Seki et al. by irradiation of T cells with 2,000 GY using a 137Cs source irradiator (Division of Molecular Pharmacology, Medical School Hannover) (44). Cells were resuspended in RPMI medium and cultured for an additional 24 h at 37°C and 5% CO2 before undergoing PI staining. Cells with less than diploid DNA content were excluded from analysis as nonviable or nonviable nuclei. Analysis of unfixed cells from coculture experiments with an FITC-labeled anti-C3d antibody revealed that 80% of the analyzed cells were T cells. Macrophages comprised a minor population of PI-stained cells. Only a few macrophages were resuspended as assayed by light microscopy analysis of the emptied plates.

To discriminate between T cells and macrophages and to analyze CD4+ and CD8+ T cells separately, double staining with FITC-labeled anti-CD3, anti-CD4, or anti-CD8 antibodies and PE- or FITC-conjugated anti-CD14 antibodies (Becton Dickinson, Heidelberg, Germany) was performed. Analysis of unfixed cells from coculture experiments with an FITC-labeled anti-C3d antibody revealed that 86 to 93% of the analyzed cells were T cells. Macrophages comprised a minor population of PI-stained cells. Only a few macrophages were resuspended as assayed by light microscopy analysis of the emptied plates.

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**Flow cytometry.** Flow cytometry analysis with propidium iodide (PI) was applied for quantification of viable cells in coculture assays or of T cells incubated with cell-free supernatants. PI staining and FACS analysis were performed as described elsewhere (33, 35). In brief, cells were resuspended in phosphate-buffered saline (PBS) and fixed in 75% ethanol for 1 h at 4°C. They were then washed and resuspended in PBS containing PI (50 μg/ml Sigma) and RNase A (250 μg/ml of type I-A; Sigma). The PI fluorescence of viable cells was determined by a FACScan flow cytometer (Becton Dickinson, Heidelberg, Germany). We restricted our analysis to viable cells with at least diploid DNA content due to the limitations of the method in clearly distinguishing between necrotic and apoptotic cells. We therefore analyzed only viable cells. The propidium iodide (PI) channel was set to the 10th percentile to exclude the gate for viable cells was set according to the PI content of control populations. Control populations included viable T cells and apoptotic T cells. T-cell apoptosis was induced according to the method of Seki et al. by irradiation of T cells with 2,000 GY using a 137Cs source irradiator (Division of Molecular Pharmacology, Medical School Hannover) (44). Cells were resuspended in RPMI medium and cultured for an additional 24 h at 37°C and 5% CO2 before undergoing PI staining. Cells with less than diploid DNA content were excluded from analysis as nonviable or nonviable nuclei. Analysis of unfixed cells from coculture experiments with an FITC-labeled anti-C3d antibody revealed that 86 to 93% of the analyzed cells were T cells. Macrophages comprised a minor population of PI-stained cells. Only a few macrophages were resuspended as assayed by light microscopy analysis of the emptied plates.

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Infection of macrophages with *C. trachomatis* induced apoptosis of PHA-preactivated T cells. A *C. trachomatis* serovar K strain was used to address the question of whether infection of macrophages with *C. trachomatis* induces T-cell apoptosis. T cells were preactivated with PHA and, after 6 days of individual culture were cocultivated with autologous *C. trachomatis*-infected macrophages. Cocultured cells were tested for viability of cells under different culture conditions compared to that of cells in noninfected cultures.

| Cultivation of PHA-stimulated T cells plus: | % of viable cells | p
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Noninfected macrophages</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Noninfected macrophages plus PHA</td>
<td>93 ± 19</td>
<td>1.0</td>
</tr>
<tr>
<td><em>C. trachomatis</em> (MOI = 5) without macrophages</td>
<td>90 ± 7</td>
<td>0.24</td>
</tr>
<tr>
<td>Macrophages infected with <em>C. trachomatis</em> at an MOI of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>86 ± 5</td>
<td>0.27</td>
</tr>
<tr>
<td>5</td>
<td>67 ± 10</td>
<td>0.007</td>
</tr>
<tr>
<td>10</td>
<td>69 ± 11</td>
<td>0.006</td>
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</tbody>
</table>

a Values, quantified by FACS analysis of PI-stained cells after 4 days of cocultivation, are means ± standard deviations of data from four independent experiments, each performed in duplicate. Percentages represent the viability of cells under different culture conditions compared to that of cells in noninfected cultures.

b Values of the different culture conditions versus cultures with noninfected macrophages, as determined by one-way ANOVA accounting for multiple comparisons by using the Bonferroni test. *P* in boldface are significant.

| T-cell apoptosis was induced by cell-free supernatant of *C. trachomatis*-infected macrophages. To investigate whether humoral mechanisms or cell-cell contact between *C. trachomatis*-infected macrophages and T cells was necessary for apoptosis induction, supernatants from infected and noninfected macrophages were removed after 6 days of culture. PHA-preactivated autologous T cells were incubated with supernatant for 6 days, and viability was determined by FACS analysis of PI-stained cells. In addition, coculture of T cells and macrophages was performed (Table 2).

Significant T-cell death in comparison to noninfected cultures was induced by cell-free supernatant of infected macrophages. Apoptosis induction was dependent on the MOI of *C. trachomatis*. The results were consistent with the data presented in Table 1. Due to the larger number of experiments, the decrease of viability was statistically significant for macrophages infected with *C. trachomatis* at an MOI of 1. Apoptosis was induced by a humoral death mechanism. However, in comparison to cell-cell contact, apoptosis induction by supernatant was less efficient (*P* = 0.001).

Nonmitogen-preactivated T cells were also susceptible to *C. trachomatis*-induced apoptosis. The susceptibility of T cells to apoptosis is dependent on their functional state (2, 25). Having demonstrated that activated T cells undergo *C. trachomatis*-induced apoptosis, we investigated the susceptibility of nonprestimulated peripheral T cells. Blood from the same individual was drawn twice: first for the isolation and infection of macrophages, and 6 days later for isolation of nonactivated peripheral lymphocytes. After 6 days of coculture, the number of apoptotic cells was quantified by microscopic analysis of TUNEL assays. A twofold increase in number of apoptotic cells was observed when PHA-preactivated and non-activated peripheral T cells were cultured with *C. trachomatis*-infected macrophages (Fig. 3). Under these culture conditions, including the addition of rIL-2, the susceptibility of nonmitogen-preactivated T cells to *C. trachomatis*-induced apoptosis was similar to that of PHA-preactivated T cells.

Infection of macrophages with *M. fermentans* did not induce apoptosis of activated T cells. *M. fermentans* is a facultative intracellular bacterium which can be detected in peripheral blood monocytes and has recently been linked with apoptosis induction (28, 39, 40). Experiments were performed to investigate whether *Mycoplasma* infection also primes macrophages for apoptosis induction. We infected macrophages with *M. fermentans* at an MOI of 0.05 or 0.5 and, after 6 days of individual culture, cocultured them with PHA-preactivated T cells. These MOIs were chosen because infection with higher concentrations of *M. fermentans* led to macrophage death. At these MOIs, 75% of all macrophages were viable after 6 days of individual culture.

FACS analysis of PI-stained cells showed that there was no decrease in number of viable cells when preactivated T cells were incubated with *M. fermentans* alone or with *M. fermentans*-infected macrophages for either MOI (*n* = 4) (one typical
experiment is shown in Fig. 4). In contrast, infection of macrophages in parallel with *C. trachomatis* at an MOI of 5 led to a significant reduction in the number of viable cells. Because *Mycoplasma-Chlamydia* coinfection occurred in vivo and in vitro, we investigated whether coinfection might have a synergistic effect on apoptosis induction (19, 30, 50). FACS analysis of preactivated T cells cocultured with doubly infected macrophages did not show an increase in apoptosis beyond that induced by infection with *C. trachomatis* alone. *Mycoplasma* coinfection had no effect on *Chlamydia*-induced macrophage-dependent T-cell apoptosis.

**UV attenuation of *C. trachomatis* EBs decreased the ability to induce apoptosis.** To test whether infection of macrophages with viable chlamydia was important for death induction, macrophages were incubated with UV-attenuated EBs coinfected with *M. fermentans*. Viability was quantified by FACS analysis of PI-stained cells (Table 3). Irradiation of bacteria with UV light resulted in minor, nonsignificant decreases in numbers of viable cells (4% after 3 days and 14% after 6 days). A significant increase in number of dead cells was seen when activated T cells were exposed to infected macrophages.

**DISCUSSION**

In this article we report that intracellular bacterial infection of macrophages can induce T-cell apoptosis. Macrophages not only can stimulate T cells but also can induce cell death (3, 29, 31, 53). This ability was recognized for certain viral infections, like that of human immunodeficiency virus, but is now newly demonstrated for bacteria. However, infection with the facultative intracellular bacterium *M. fermentans* failed to induce T-cell apoptosis. Coinfection of *C. trachomatis* and *M. fermentans* had no additional effect. The ability to induce apoptosis is not shared by *M. fermentans*. It remains to be elucidated which intracellular bacteria have this property.

For apoptosis induction, *Chlamydia* viability was essential. UV irradiation of *Chlamydia* strongly diminished apoptosis induction. This underlines the fact that macrophages have to be infected with viable bacteria. For many chlamydial diseases, the key feature is the persistent infection of human cells (8, 51). Especially with regard to *Chlamydia*-induced arthritis, we and others have demonstrated that chlamydia persist in viable, aberrant forms in macrophages (13, 24). During cultivation in human macrophages, intracellular chlamydiae change and, after 1 week, strongly resemble these aberrant forms in terms of protein synthesis and morphological features (8, 13, 24, 32). It is possible that persistently infected macrophages have the ability to induce T-cell apoptosis in vivo.

The susceptibilities of PHA-preactivated and nonpreactivated peripheral T cells to apoptosis induction were not different. PHA stimulation allowed the study of properties of preactivated T cells. T cells in this activation state are sensitive
FIG. 2. Detection of apoptotic cells by TUNEL assay: light microscopy analysis of PHA-activated T cells cocultivated with *C. trachomatis*-infected macrophages (MOI = 5). TUNEL staining was performed after 6 days of coculture. (A and C) Specificity controls; assays were performed without the addition of TdT; (B and D) TdT was added. Apoptotic nuclei appeared only after the addition of the enzyme. In the figure, apoptotic cells can be distinguished by their black staining. Cytoplasm was visualized by staining with haemalum. The mean percentage ± standard deviation of apoptotic cells in these assays after coculture with *C. trachomatis*-infected macrophages (MOI = 5) was 56% ± 11% (n = 3 experiments); the frequency of apoptotic cells after coculture with noninfected macrophages was 28% ± 7% (n = 3 experiments; P = 0.001). Magnifications, ×30 (A and B) and ×75 (C and D).
TABLE 2. Induction of T-cell death by supernatant and cell-cell contact of C. trachomatis-infected macrophages

<table>
<thead>
<tr>
<th>Cultivation of PHA-activated T cells plus:</th>
<th>% of viable cells (P) after induction with:</th>
<th>Supernatant</th>
<th>Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Noninfected macrophages</td>
<td></td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Macrophages infected with C. trachomatis at an MOI of:</td>
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<td></td>
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<tr>
<td>1</td>
<td>87 ± 9 (0.005)</td>
<td>84 ± 12 (0.0001)</td>
<td></td>
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<tr>
<td>5</td>
<td>78 ± 8 (0.0001)</td>
<td>61 ± 9 (0.0001)</td>
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</table>

* Percentages represent the viability of PI-stained cells under different culture conditions compared to the viability of noninfected cultures. Values are means ± standard deviations (six independent experiments, four performed in duplicate and two performed in triplicate). Supernatants were derived from macrophages after 6 days of culture prior to the addition of T cells. FACS analysis for PI-stained cells was performed after 6 days of culture.

All values were highly significantly different (P < 0.01) from value for preactivated T cells cocultivated with supernatant from uninfected macrophages or from value for preactivated T cells cocultivated with noninfected macrophages, as determined by one-way ANOVA.

pneumonitis agent, it was demonstrated that resolution mostly depends on the presence of CD4+ Th-1-type T cells (38). However, observations of human chlamydial infection indicate that host T-cell immunity is impaired during an ongoing infection (6, 16, 17, 54). Bailey et al. showed, by measuring the T-cell immune response to chlamydial antigens with a lymphocyte proliferation assay, that patients who recovered from trachoma exhibited greater T-cell proliferation than patients with active cases of trachoma (6). Wilkinson et al. observed that patients had only minor synovial T-cell responses to chlamydial antigens despite having PCR-detectable levels of C. trachomatis in their joints (54). In contrast, patients with Chlamydia-induced arthritis who were negative for synovial chlamydial DNA by PCR testing demonstrated a high-level synovial T-cell response to chlamydial antigens. The insufficient T-cell response against C. trachomatis, which can contribute to the persistence of chlamydia in the joints, may be caused by induction of T-cell apoptosis. However, the mechanism we have elucidated so far is not dependent on the recognition of chlamydial antigens by T cells. Because the T cells used in the present study were derived from normal donors, only a minority of them were Chlamydia specific. We do not know whether preferentially Chlamydia-specific T cells are eliminated by this mechanism.

Macrophages induce T-cell apoptosis by Fas-Fas ligand interaction, tumor necrosis factor alpha (TNF-α) production, or secretion of reactive oxygen species (3, 31, 35, 53, 56). Experiments showed that CD4+ T cells are preferentially eliminated by Fas ligand whereas CD8+ T cells may be more susceptible to TNF-α signaling (56). Mainly CD4+ T cells were isolated from the synovial fluid of patients with Chlamydia-induced arthritis; only a few activated CD8+ T cells were identified (16, 18). Ingalls et al. showed that infection of macrophages with C. trachomatis stimulates TNF-α production (20). We have revealed that T-cell apoptosis can be induced by a cell-free supernatant, demonstrating that humoral death mechanisms are involved in C. trachomatis-induced apoptosis. However, the higher efficacy of cell-cell contact between T cells and infected macrophages might be due to sustained production of cytokines (macrophages were allowed to produce TNF-α or oxygen species for an additional 6 days) or to a contribution of Fas-Fas ligand interaction, raising the possibility that the execution of T-cell death is the result of different apoptosis pathways. In our experiments we did not observe a significant preference for apoptosis of CD8+ T cells.

A recent report by Perfettini et al. strongly supports the important role for TNF-α in Chlamydia-induced apoptosis (37). They observed that apoptosis of noninfected cells—mainly epithelial—was induced by genital infection of mice with the mouse pneumonitis strain of C. trachomatis. By ap-

FIG. 3. Apoptosis of nonpreactivated T cells induced by macrophages (MO) infected with C. trachomatis at an MOI of 5. After 6 days of coculture, the number of apoptotic cells was quantified by microscopic analysis of TUNEL assays (n = 3 experiments). Mean percentages of apoptotic cells were as follows: for cultures of nonpreactivated T cells, 15% ± 5% (noninfected) and 35% ± 5% (C. trachomatis infected); for cultures of PHA-preactivated T cells, 28% ± 7% (non-infected) and 56% ± 11% (C. trachomatis infected). P values of the indicated comparisons were determined by one-way ANOVA accounting for multiple comparisons by using the Tamhane test. This analysis revealed that for both T-cell populations a highly significant increase in numbers of apoptotic cells occurred in the presence of C. trachomatis-infected macrophages (P = 0.001). Abbreviations: MO, macrophage; TC, T cell; Ctr., C. trachomatis.
PHA-preactivated T cells plus

A) Non-infected macrophages

B) C. trachomatis-infected macrophages

C) M. fermentans-infected macrophages

D) C. trachomatis/M. fermentans coinfect macrophages

E) M. fermentans without macrophages

F) C. trachomatis/M. fermentans without macrophages

FIG. 4. Analysis of viable cells in coculture experiments with M. fermentans-infected macrophages by flow cytometric analysis of PI-stained cells. PHA-preactivated T cells were cocultivated with macrophages. Staining was performed after 6 days of coculture. Histograms of PI-stained cells are depicted. Gated cells (marker M1) have at least a diploid DNA content. The percentages refer to the proportion of viable cells in comparison to that of the cultures of T cells with noninfected macrophages of this characteristic experiment. A total of four experiments were performed. P values refer to the different culture conditions in comparison to cultures with noninfected macrophages as determined by one-way ANOVA accounting for multiple comparisons by using the Bonferroni test. T cells were cocultivated with noninfected macrophages (A), C. trachomatis-infected (MOI = 5) macrophages (72% ± 8%; P = 0.001) (B), M. fermentans-infected (1.5 × 10⁴ CFU/ml, MOI = 0.05) macrophages (105% ± 11%; P = 1) (C), macrophages infected with C. trachomatis (MOI = 5) and M. fermentans (1.5 × 10⁴ CFU/ml, MOI = 0.05) 76% ± 5%; P = 0.005) (D), M. fermentans (1.5 × 10⁴ CFU/ml, MOI = 0.05) and C. trachomatis EBs (MOI = 5) without macrophages (95% ± 8%; P = 1) (F).

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