Chlamydiapneumoniae Inhibits Apoptosis in Human Peripheral Blood Mononuclear Cells Through Induction of IL-10

Yuemei Geng, Ryan B. Shane, Klara Berencsi, Eva Gonczol, Mohamed H. Zaki, David J. Margolis, Giorgio Trinchieri and Alain H. Rook

*J Immunol* 2000; 164:5522-5529; doi: 10.4049/jimmunol.164.10.5522
http://www.jimmunol.org/content/164/10/5522

References
This article cites 48 articles, 29 of which you can access for free at:
http://www.jimmunol.org/content/164/10/5522.full#ref-list-1

Subscription
Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
**Chlamydia pneumoniae** Inhibits Apoptosis in Human Peripheral Blood Mononuclear Cells Through Induction of IL-10

Yuemei Gao,* Ryan B. Shane,* Klara Berenesi, † Eva Gonczol, ‡ Mohamed H. Zaki, § David J. Margolis,* Giorgio Trinchieri, † and Alain H. Rook 2 *

**Chlamydia pneumoniae** is a common cause of pulmonary infection, with serum positivity in at least 50% of the general population. In this study, we report that human PBMCs exposed to **C. pneumoniae** are resistant to apoptosis induced by the potent photoactivated chemotherapeutic agents 8-methoxypsoralen and hypericin. In contrast, PBMCs treated with a heat-inactivated inoculum exhibit normal susceptibility to apoptosis. We also observed that human PBMCs are responsive to **C. pneumoniae** infection by secretion of key immune regulatory cytokines, including IL-12 and IL-10. While IL-12 may play an important role in limiting **C. pneumoniae** proliferation within cells, IL-10 serves an anti-inflammatory function by down-regulating proinflammatory cytokines such as IL-12 and TNF-α. Depletion of endogenous IL-10, but not of IL-12, abolished the apoptosis resistance of **C. pneumoniae**-infected PBMCs. Furthermore, addition of exogenous IL-10, but not IL-12, significantly increased the resistance of control inoculum-treated PBMCs to photoactivated 8-methoxypsoralen- and hypericin-induced apoptosis. Therefore, we conclude that **C. pneumoniae** possesses an antiapoptotic mechanism. The resistance to apoptosis observed in PBMCs exposed to **C. pneumoniae** is due, at least partially, to the IL-10 induced during **C. pneumoniae** infection. *The Journal of Immunology, 2000, 164: 5522–5529.*

A poptosis is an active process of cell death that is important for cell development and tissue homeostasis. Moreover, other biological processes such as host defense against viral (1–3) and bacterial (4, 5) infections may depend critically upon apoptotic events for the normal processing of microbes (6, 7). Many intracellular organisms rely on host cells to survive and propagate; therefore, it is advantageous for them to develop strategies to inhibit host cell apoptosis. A number of virus and viral factors have been described as antiapoptotic (8–10), including caspase inhibitor CrmA in the cowpox virus and p35 in baculovirus, viral Bcl-2 homologues, viral products that can modulate p53 activity, viral homologues of mammalian death receptors, and viral Fas-associated death domain-like IL-1-converting enzyme-inhibitory proteins. Reports of bacterial antiapoptotic activity, however, have been scarce. Recently, Fan et al. (10) reported that host cells infected with *Chlamydia trachomatis* are profoundly resistant to apoptosis induced by a wide spectrum of proapoptotic stimuli, including the kinase inhibitor staurosporine, the DNA-damaging agent etoposide, and several immunological apoptosis-inducing molecules such as TNF-α, Fas Ab, and granzyme B/perforin, through blockage of mitochondrial cytochrome c release and caspase activation. The role that cytokines play in infection-mediated antiapoptosis and whether this antiapoptotic effect is unique to *C. trachomatis*, however, were not addressed. Furthermore, the situation of infected cells treated with other clinically relevant apoptotic inducers such as 8-methoxypsoralen (8-MOP) (11) and hypericin (12), two light-activated photochemotherapeutic reagents that have been used in the treatment of lymphoproliferative diseases and that cause a high level of apoptosis, is also unknown.

**Chlamydia pneumoniae**, the newest member of the chlamydial family, has been established as a common cause of acute and chronic respiratory disease worldwide and implicated in the pathogenesis of coronary artery disease (13, 14). Monocytes/PBMCs have been postulated to serve as a vehicle for systemic dissemination of the infection (15, 16). Like *C. trachomatis*, *C. pneumoniae* has a unique intracellular biphasic life cycle. The spore-like elementary bodies facilitate transit between cells. The metabolically active reticulate bodies are responsible for intracellular replication (13, 14). During chlamydial intracellular growth, maintenance of the host cell’s integrity is essential not only for supplying nutrients, but also for shielding the intracellular organisms from host phagocytosis. Therefore, *C. pneumoniae* may also have evolved mechanisms that allow it to actively interrupt host apoptotic process.

Researchers have shown that **C. pneumoniae** is a potent inducer of the proinflammatory cytokines TNF-α, IL-1β, and IL-6 in human monocyteic cells as well as freshly isolated human PBMCs (17, 18). However, the ability of **C. pneumoniae** to induce PBMC secretion of key regulatory factors in the immune response such as IL-12 and IL-10 has never been addressed. In this communication, we report that **C. pneumoniae** is able to infect human PBMCs and that human PBMCs respond to infection by secreting the critical immunoregulatory cytokines IL-12 and IL-10. Furthermore, we demonstrate that PBMCs treated with **C. pneumoniae** have

---

1 Abbreviations used in this paper: 8-MOP, 8-methoxypsoralen; HI, heat-inactivated; IF, immunofluorescence; ifu, inclusion-forming unit; RPA, RNase protection assay.

Received for publication September 3, 1999. Accepted for publication March 9, 2000.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

This work is supported in part by grants from the Leukemia Society of America and the W. W. Smith Charitable Trust, and by Public Health Service Grants CA 80108, CA 10815, and CA 32898. Y.G. was supported by National Institutes of Health Training Grant T32CA09140.

Address correspondence and reprint requests to Dr. Alain H. Rook, Department of Dermatology, University of Pennsylvania, 415 Curie Boulevard, Philadelphia, PA 19104. E-mail address: arook@mail.med.upenn.edu

Copyright © 2000 by The American Association of Immunologists 0022-1767/00/$02.00
increased resistance to the high level of apoptosis induced by the photovacinated DNA intercalator, 8-MOP, or hypericin, a naturally occurring photodynamic compound, as well as to soluble Fas ligand-mediated apoptosis. Importantly, C. pneumoniae infection-induced IL-10, but not IL-12, is at least partially responsible for this increased resistance to apoptosis.

Materials and Methods

Bacterial culture and inoculum preparation

C. pneumoniae strain TW 183 was purchased from the American Type Culture Collection (ATCC, Rockville, MD) and was propagated in McCoy cells (ATCC) in supplemented DMEM (Life Technologies, Grand Island, NY) in a 35°C, 5% CO2-saturated humidified incubator, as described previously (16). Infected cells were harvested on day 3 or 4 and disrupted by two cycles of freezing/thawing and ultrasonification, and different harvests were pooled. After centrifugation at 1000 rpm for 5 min to remove cell debris, bacteria were concentrated by high speed centrifugation at 25,000 × g for 25 min. Pellets were resuspended in PBS, pH 7.4, mixed with an equal volume of sucrose-phosphate-glutamic acid buffer, aliquoted, and frozen at −70°C until use. A control inoculum was prepared according to the same procedure with uninfected McCoy cells.

Chlamydia titers were determined by immune-fluorescence (IF) assay. Briefly, McCoy cells were infected with serial dilutions of bacterial stock, incubated overnight, fixed with methanol-acetone (1:1), stained with an antianimal outer membrane protein Ab (IgG3 mouse mAb anti-C. pneumoniae; Dako, Cambridgeshire, U.K.), and followed by FITC-labeled secondary Ab (goat F(ab)2; anti-mouse IgG, Sigma, St. Louis, MO) to identify chlamydial inclusions. After counting inclusions under a fluorescence microscope and correcting for dilution factors, bacterial titers were expressed as inclusion-forming units per ml (ifu/ml). C. pneumoniae infection of human PBMCs/macrophages was also examined by IF assay.

All cultures were free of mycoplasma contamination, as determined by PCR and Hoechst staining (Cell Center, The University of Pennsylvania, Philadelphia, PA).

Human PBMC and monocyte preparations

PBMCs were prepared essentially as previously described (20). First, PBMCs were isolated from blood of healthy human donors through centrifugation on a standard Ficoll gradient (Pharmacia, Piscataway, NJ). The interface containing the mononuclear cell fraction was washed with Dulbecco’s PBS through centrifugation and resuspended in complete medium (RPMI 1640 medium, supplemented with 10% heat-inactivated fetal bovine serum [FBS; GIBCO, Grand Island, NY]). Terminal deoxynucleotide transferase and nucleotide labeling solution (FITC-dUTP) into the free ends of these apoptotic DNA fragments. Briefly, cells cultured as described above were fixed in 4% paraformaldehyde for 30 min at 25°C and permeabilized in 0.1% Triton-containing 0.1% sodium citrate for 2 min on ice. Terminal deoxynucleotidyl transferase and nucleotide mixture were then added to the cells, and the mixture was incubated for 60 min at 37°C. Then, using a FACSCan flow cytometer (Becton Dickinson, Mountain View, CA), all reagents used in this study were free of endotoxin contamination, as determined by the Limulus amebocyte assay.

Human PBMC infection and cytokine measurement

PBMCs or monocytes were inoculated with various concentrations of C. pneumoniae (viable or HI) and then cultured at 37°C. Supernatants were collected at various times during the culture and stored at −80°C for the cytokine assays. In some experiments, cells were inoculated with the bacteria in the presence or absence of certain cytokine-neutralizing Abs or were treated with certain cytokines before their exposure to the inoculum. In all cases, samples were centrifuged for 1 h at 550 × g immediately after Chlamydia inoculation to facilitate contact between cells and the bacteria.

RIAs for human IL-12 p40, IL-12 p70, TNF-α, and IL-10 were performed as previously described (21–24), using mAb pairs C11.79/C8.6, 12H4/C8.6, B154/9/B154.7, and 9D7/12G8, respectively.

RNase protection assay (RPA)

RPA kits were purchased from PharMingen (San Diego, CA). 32P was used for riboprobe labeling. RNAs were extracted with Ultraspec (Biotecx, Houston, TX), and RNA samples were hybridized with an excess amount of [32P]UTP (New England Nuclear-DuPont, Boston, MA)-labeled probes for 12–16 h at 56°C, according to the suggestions of the manufacturer. The protected fragments were fractionated on 5% polyacrylamide/urea-sequencing gels and detected by phosphor imaging.

Cell culture and photoactivation

Purified mononuclear cells were diluted to a concentration of 2 × 106 cells/ml with complete medium and aliquoted into each well of a 24-well plate. Vehicle and predetermined concentrations of hypericin or 8-MOP were added into their respective sets of wells in a darkened tissue culture room. Control plates receiving no exposure to photoactivating wavelengths of light were placed immediately in a 37°C incubator. The remaining plates were exposed to either white light from four fluorescent T15SCW 15-W bulbs under the tissue culture plates or to UVA light with UVA illumination, as described previously (11, 12). Photoactivation with white light lasted for 30 min and delivered the equivalent of 2 J/cm2; UVA light lasted for 4 min and delivered the equivalent of 2 J/cm2. The plates were then incubated at 37°C for 24–72 h, depending on the assay to be performed. The quantity of light delivered to the medium-containing wells of a tissue culture plate was measured with an IL-700 A research radiometer (International Light, Newburyport, MA).

Apoptosis assay

Apoptosis was assessed using a modification of the TUNEL method previously described by Sgonc et al. (25), as supplied in kit form by Boehringer Mannheim (Indianapolis, IN). Apoptotic cell death is characterized by internucleosomal cleavage of DNA into fragments of ~180 bp. The TUNEL assay allows for the incorporation of fluorescein-isothiocyanate-labeled dUTP (FITC-dUTP) into the free ends of these apoptotic DNA fragments. Briefly, cells cultured as described above were fixed in 4% paraformaldehyde for 30 min at 25°C and permeabilized in 0.1% Triton-containing 0.1% sodium citrate for 2 min on ice. Terminal deoxynucleotidyl transferase and nucleotide mixture were then added to the cells, and the mixture was incubated for 60 min at 37°C. Then, using a FACScan flow cytometer (Becton Dickinson, Mountain View, CA), the percentage of cells (of the 10,000 total acquired) undergoing apoptosis was determined as: [(number of cells incorporating FITC-dUTP into fragmented DNA)/(total number of cells)] × 100.

Cytokines, Abs, and other reagents

Chinese hamster ovary cell-derived recombinant NK-stimulatory factor IL-12 was a gift from Dr. S. Wolf (Genetics Institute, Boston, MA). Anti-C. pneumoniae, C. pneumoniae, and a stock solution of 462.5 μM was prepared by dissolving 100 μg hypericin in 40 ml 2% benzyl alcohol (vehicle) to yield a 2 mM solution, then kept in the dark at 4°C.

Results

The data on cytokine production are presented as means ± SD. An unpaired, two-tailed Student’s t test was performed to determine the statistical significance of the data, and p values < 0.05 were considered significant. The data on cell survival are presented as the percentage of cells that survived under various experimental conditions. Percentages are not normally distributed. Therefore, the percentage data were transformed to the arcsine of the square root of the proportion. The transformed data were then analyzed using an analysis of variance. To ensure that the overall probability of detecting a significant difference between two pairs was maintained at a constant type I error of 0.05, comparisons between treatments were made using the Tukey-Kramer multiple comparison test.

C. pneumoniae-treated human PBMCs have increased resistance to apoptosis induced by photovacinated 8-MOP or hypericin

It has been reported that certain C. trachomatis-infected host cells (HeLa, L929, and U937) are profoundly resistant to apoptosis induced by proapoptotic stimuli such as the kinase inhibitor staurosporine, the DNA-damaging agent etoposide, and immunological...
molecules including TNF-α, Fas Ab, and granzyme B/perforin (10). To understand whether this antiapoptotic effect is unique to *C. trachomatis* and to explore how *Chlamydia*-exposed and non-exposed human PBMCs responded differently following clinically relevant apoptotic inducer treatment, we tested another species of *Chlamydia*, *C. pneumoniae*, using photoactivated 8-MOP or hypericin (18). With IF assays, we observed that *C. pneumoniae* is able to infect PBMCs, as evidenced by the positive immunofluorescent signals detected in bacteria-inoculated cells (data not shown). We also observed that predetermined suboptimal doses of 8-MOP (100 ng/ml and 2 J/cm2 UVA) or hypericin (1 μM and 2 J/cm2 white light) induce 70–80% apoptosis of PBMCs 60 h following initial photoactivation (Fig. 1, b and h). But cells treated with *C. pneumoniae* (2000–4000 ifu/5 × 10⁶ host cells) before photopexposure had 18% and 40% reduction in apoptosis (Fig. 1, c and h). Furthermore, there is a dose-dependent relationship between the amount of *C. pneumoniae* inoculated and the magnitude of apoptosis resistance. For instance, with 1 μM of hypericin (2 J/cm² white light), 2000 ifu of *C. pneumoniae* caused a 40% reduction in host cell apoptosis, as shown in Fig. 1h, while 200 ifu did not cause any significant changes (data not shown). Cells treated with *C. pneumoniae* alone did not significantly affect the rate of apoptosis (Fig. 1d).

Cytokine production and regulation during *C. pneumoniae* infection

To understand the mechanism of *C. pneumoniae* antiapoptotic activity, we investigated whether PBMCs are able to respond to *C. pneumoniae* infection by inducing cytokine production. Fig. 2 shows that the first cytokine detected following *Chlamydia* infection, among cytokines measured, was TNF-α. TNF-α peaked at about 8 h postinoculation (Fig. 2). IL-12 p40 (Fig. 2) and IL-10 (Fig. 2) peaked between 15–48 and 24–48 h after inoculation, respectively. Optimal cytokine production was measured at 10,000 ifu of *C. pneumoniae* per ml, a maximum dose used in our study. Below 100 ifu/ml, *C. pneumoniae* was unable to induce any statistically significant cytokine production (Table I).

Replication of *C. pneumoniae* appeared to be necessary for the optimal induction of cytokine production. Table I shows that compared with the viable *C. pneumoniae*, HI bacteria are not a strong inducer of the cytokines measured. With 10,000 ifu, viable *C. pneumoniae* induced the production of 10.7 ± 1.2 ng of IL-12 p40, 18.8 ± 4.5 ng of IL-10, and 22.9 ± 4.1 ng of TNF-α; HI bacteria, however, induced only 0.3 ± 0.1 ng of IL-12-p40, 1.7 ± 0.5 of IL-10, and 2.8 ± 0.4 ng of TNF-α.

To examine the relationship between different cytokines and to facilitate our understanding about the functions of those cytokines produced during the infection, a number of cultures were set up in the presence or absence of certain cytokines or cytokine-neutralizing Abs. Fig. 3A demonstrates the results from a representative RPA experiment in which PBMCs had been optimally precultured for 16 h in the presence or absence of IFN-γ, then stimulated with *C. pneumoniae* for 8 h in the presence or absence of IL-10-neutralizing Ab. In Fig. 3B, lane 1 shows that *C. pneumoniae* was able
to induce IL-12 p40 and IL-10 mRNA expression. If cells had been pretreated with IFN-γ, Chlamydia-induced IL-12 p40 mRNA expression was enhanced (Fig. 3B, lane 2). Chlamydia were also able to induce IFN-γ-pretreated cells to express IL-12 p35 mRNA (Fig. 3B, lane 2). Chlamydia-induced IL-10 mRNA expression, however, was decreased by such a treatment (Fig. 3B, lane 2). Interestingly, the presence of IL-10-neutralizing Ab in the culture greatly enhanced IL-12 p40 mRNA expression and induced the expression of IL-10 p35 mRNA (Fig. 3B, lane 3). Furthermore, the presence of IL-10-neutralizing Ab enhanced the expression of IL-10 mRNA (Fig. 3B, lane 3).

These results were further corroborated by data from protein studies: pretreatment of cells with IFN-γ enhanced the C. pneumoniae-induced production of IL-12 p40, p70, and TNF-α, but inhibited the production of IL-10 protein (Fig. 4A). The presence of IL-10-neutralizing Ab along with Chlamydia also enhanced the production of IL-12 p40, p70, and TNF-α (Fig. 4B). Addition of exogenous IL-10, on the other hand, caused a significant reduction in this infection-induced production of IL-12 and TNF-α (Fig. 4B). C. pneumoniae infection mediated PBMC production of IFN-γ as well. But under no circumstance was IL-4 production detectable (data not shown).

**IL-10, but not IL-12, contributes to the resistance of C. pneumoniae-infected PBMCs to apoptosis**

To explore the role of cytokines in the antiapoptotic activity of C. pneumoniae-treated PBMCs, we performed depletion experiments. As shown in Fig. 5, in comparison with control Ab treatment, anti-IL-10 treatment before C. pneumoniae inoculation significantly decreased the resistance of the cells to photoactivated 8-MOP- or hypericin-induced apoptosis (Fig. 5, Ae, Ak, and B). The presence of IL-12-neutralizing Ab, on the other hand, did not significantly affect the apoptotic activity of these cells (Fig. 5, Ad, Aj, and B). Furthermore, supplement of exogenous IL-10, but not IL-12, in the absence of C. pneumoniae, significantly decreased 8-MOP- or hypericin-induced apoptosis (Fig. 5, Af, Al, and B). Similar results were obtained in the system of soluble Fas ligand-mediated cell death (Fig. 6). Following culture of C. pneumoniae-infected cells with soluble recombinant Fas ligand, the level of apoptosis was markedly augmented by addition of anti-IL-10-neutralizing Ab to the culture system (Fig. 6). Together, these results strongly indicated that C. pneumoniae infection-mediated secretion of IL-10, but not IL-12, is at least partially responsible for the antiapoptotic activity of C. pneumoniae. Similar

**FIGURE 3. Effects of IFN-γ and anti-IL-10 on C. pneumoniae infection-induced IL-12 (p35 and p40) and IL-10 mRNA expression.** Cells were preincubated for 16 h in the presence or absence of IFN-γ (100 ng/ml), then stimulated with C. pneumoniae in the presence of anti-IL-10 or control Ab (10 μg/ml). Eight hours later, cells were harvested and RNAs were extracted, and RPA was conducted to detect the expression of IL-12 p35, IL-12 p40, and IL-10 mRNA, as described in Materials and Methods. A. Normalized arbitrary units of detected mRNA. B, The mRNA detected on a 5% polyacrylamide/urea sequencing gel. This result is representative of three independent experiments with two donors in each experiment.

### Table I. IL-12 p40, IL-10, and TNF-α production by human PBMCs following stimulation with viable or HI C. pneumoniae*

<table>
<thead>
<tr>
<th>Stimulus</th>
<th>IL-12 p40</th>
<th>IL-10</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viable C. pneumoniae (ifu)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>138 ± 44</td>
<td>159 ± 56</td>
<td>150 ± 56</td>
</tr>
<tr>
<td>10,000</td>
<td>10,680 ± 1,236</td>
<td>18,828 ± 4,500</td>
<td>22,890 ± 4,145</td>
</tr>
<tr>
<td>1,000</td>
<td>3,958 ± 251</td>
<td>1,327 ± 375</td>
<td>1,311 ± 351</td>
</tr>
<tr>
<td>100</td>
<td>1,178 ± 179</td>
<td>987 ± 279</td>
<td>800 ± 268</td>
</tr>
<tr>
<td>Heat-inactivated (organism equivalent)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>145 ± 31</td>
<td>95 ± 34</td>
<td>106 ± 29</td>
</tr>
<tr>
<td>10,000</td>
<td>302 ± 101</td>
<td>1,701 ± 456</td>
<td>2,872 ± 441</td>
</tr>
<tr>
<td>1,000</td>
<td>212 ± 98</td>
<td>299 ± 223</td>
<td>1,615 ± 231</td>
</tr>
<tr>
<td>100</td>
<td>103 ± 61</td>
<td>179 ± 91</td>
<td>301 ± 99</td>
</tr>
</tbody>
</table>

* Viable but not the heat-inactivated C. pneumoniae is a potent inducer of IL-12 p40, TNF-α, and IL-10. Cells (5 × 10⁶) were isolated and stimulated for 48 h (IL-12 p40), 24 h (IL-10), or 15 h (TNF-α) with viable C. pneumoniae (10,000, 1,000, or 100 ifu) or equivalent amount of heat-killed organisms. Cytokines were measured by RIA as described in Materials and Methods. Results are presented as mean ± SD of three separate experiments with three donors in each experiment.
Cytokines and Antiapoptosis in Chlamydia pneumoniae Infection

FIGURE 4. A, Effects of IFN-γ on C. pneumoniae-induced IL-12 and IL-10 production. Cells (2 × 10^6/ml) were isolated and cultured overnight (12–16 h) in the presence of IFN-γ (100 ng/ml), followed by C. pneumoniae stimulation (48 h). Culture supernatants were collected, and IL-12 p40, p70, and IL-10 productions were measured, as described in Materials and Methods. The result here is representative of three experiments with two donors in each experiment. B, Effects of IL-10 on IL-12 and TNF-α production. Freshly isolated PBMCs (2 × 10^6) were inoculated with C. pneumoniae for 24 h in the presence of IL-10 or anti-IL-10. Culture supernatants were collected, and IL-12 p40, p70, and TNF-α productions were measured. The result is representative of three independent experiments with two donors in each experiment.

Discussion

It is known that host cells are able to respond to intracellular bacterial invasion with apoptosis (4, 5). Organisms that induce this response include Shigella flexneri (27), Listeria monocytogenes (28), Mycobacterium tuberculosis (29), and Salmonella typhimurium (30). Because host apoptotic responses can facilitate the killing of intracellular bacteria, it is advantageous for intracellular organisms to evolve strategies against apoptosis to ensure the continuous supply of nutrients and protection from the host. There has been no report, however, of bacterial antiapoptotic activity until very recently. Fan et al. (10) reported for the first time that C. trachomatis-infected host cells (HeLa, L929, and U937) become resistant to a number of apoptotic stimuli. But issues such as whether this antiapoptotic effect is unique to C. trachomatis and what is the role of cytokines in this infection-mediated resistance to apoptosis were not addressed. Furthermore, responses of infected cells to potent photochemotherapeutic reagents such as 8-MOP and hypericin, which have been used in the clinic to treat skin-related lymphoproliferative disorders such as cutaneous T cell lymphoma and Sezary syndrome, were not studied. In this communication, we demonstrated for the first time that human PBMCs from C. pneumoniae-inoculated cell cultures are profoundly resistant to apoptosis induced by 8-MOP and hypericin, which have been used in the clinic to treat skin-related lymphoproliferative disorders such as cutaneous T cell lymphoma and Sezary syndrome, were not studied. In this communication, we demonstrated for the first time that human PBMCs from C. pneumoniae-inoculated cell cultures are profoundly resistant to apoptosis induced by 8-MOP and hypericin. Furthermore, IL-10 produced during C. pneumoniae treatment contributes, at least partially, to the infection-mediated resistance to apoptosis. A similar protective effect against apoptosis of C. pneumoniae-induced IL-10 was also demonstrated using Fas ligand-treated PBMC.

The ability of C. pneumoniae to infect human PBMCs and monocytes has been reported previously. In our study, we observed that there are only a small percentage of PBMCs or purified monocytes that by IF assay appear infected. This is consistent with partial resistance to 8-MOP- or hypericin-induced apoptosis. These results may be due to the small amount of inoculum used in our studies. It is also possible, however, that C. pneumoniae infects and protects only specific subpopulations of PBMCs. As to whether those cells that were positive for C. pneumoniae by IF staining are the same cells that have increased resistance to apoptosis induction is an issue currently under investigation.

Photochemotherapy is a unique type of antitumor therapy. It has been used in the treatment of cutaneous T cell lymphoma and Sezary syndrome. The therapeutic efficacy of psoralen in combination with UVA has been linked to the potent induction of apoptosis (11). Hypericin, although still in clinical trial, has also been reported to induce a high rate of apoptotic death of normal, transformed, and malignant T lymphocytes and, thus, has promise for the treatment of cutaneous lymphoproliferative and inflammatory disorders (12). Recently, Abrams et al. (31) described a C. pneumoniae-associated peptide identified in the blood of Sezary syndrome patients that is able to sustain the growth of malignant Sezary T cells and to prevent apoptotic death. This observation is relevant to our in vitro findings. Clearly, understanding the mechanisms of apoptotic resistance induced by C. pneumoniae infection is relevant to enhancing our immunotherapeutic approaches to lymphoproliferative disorders in general and to the use of photochemotherapeutic agents in particular.

Cytokines participate in many physiologic processes, including the regulation of immune and inflammatory responses. Our previous studies (19) with murine infection models (BALB/c and 129 mice) have shown that host immune systems are able to respond to C. pneumoniae infection by producing a number of cytokines, including IL-12, TNF-α, and IL-10. Although IL-12 appears to play an important role in early stage host defense against this infection, the function of IL-10 was not addressed. We observed that depletion of IL-10 by addition of IL-10-neutralizing Ab to the cell culture significantly increased the infection-mediated production of IL-12 and TNF-α, and supplementation with exogenous IL-10 at the time of infection decreased such proinflammatory cytokine production. Together, these results strongly suggest an anti-inflammatory role of IL-10 produced during C. pneumoniae infection. Interestingly, the presence of IL-10-neutralizing Ab in the culture significantly reduced IL-10 protein levels, yet it increased IL-10 mRNA expression, confirming IL-10 as an autocrine regulatory cytokine, as reported previously by Masood et al. (32).

To further explore the role of IL-12 and IL-10 produced during C. pneumoniae infection in host cell resistance to apoptosis, we performed blockade experiments with specific mAbs to IL-12 and
IL-10. We observed that the presence of anti-IL-10-neutralizing Ab in the infected PBMC culture significantly increased the percentage of apoptotic cells following 8-MOP or hypericin exposure. The presence of anti-IL-12, however, did not change the apoptotic rate. Furthermore, we determined that exogenous IL-10 was able to rescue human PBMCs from photoactivated 8-MOP- or hypericin-induced apoptosis. Exogenous IL-12, however, did not affect the apoptosis-inducing efficacy of those reagents. These findings clearly indicate that the resistance of *C. pneumoniae*-infected human PBMCs to apoptosis is, at least partially, mediated through the IL-10 produced following infection. The ineffectiveness of HI *C. pneumoniae* in inducing production of cytokines has been reported previously with an in vivo system (19). In this current study, we observed that HI *C. pneumoniae* is unable to elicit significant cytokine responses in vitro in comparison with viable organisms. Furthermore, human PBMCs inoculated with HI *C. pneumoniae* did not show resistance to apoptosis (data not shown). These observations indicated a relationship between cytokines produced during *C. pneumoniae* infection and the antiapoptotic activity of these bacterium-exposed cells.

The effect of cytokines on cell survival has been studied previously. It has been shown, in different experimental systems, that signals transduced through TNFR1 (p55) can induce an activation of proteases, including caspases. Caspases mediate apoptosis by proteolytic cleavage of the death substrates (26, 33–38). TNF-α also increases synthesis of NO in different cells, and this molecule has been extensively associated with induction of DNA damage and apoptosis (39–42). Additionally, TNF-α may activate sphingomyelin breakdown into ceramide, which has a recognized role in apoptosis (43). IL-10, on the other hand, has been reported to have variable effects on apoptosis, depending upon the cell type and model systems used (44–46). IL-10 has been observed to prevent lymphocyte activation-induced apoptosis and spontaneous death of germinal center B cells (47, 48). This effect of IL-10 has been...
shown to be associated with increased expression of the antiapoptotic factor Bcl-2. Furthermore, treatment of cells with anti-IL-10 diminished cell viability and Bcl-2 expression, while increasing caspase 1 activation and p53 expression. Balcewicz-Sablinska et al. (49) reported that IL-10 down-regulates apoptosis in human alveolar macrophages infected with *M. tuberculosis* by inducing the release of TNFR2, leading to the formation of nonactive TNF-α-TNFRF2 complexes. More recently, Rojas et al. (50) reported that the TNF-α/IL-10 ratio might account for the balance between apoptosis and survival of virulent *M. tuberculosis*-infected murine macrophages. In our system, the time course for optimal IL-10 production following *C. pneumoniae* infection appeared to occur at a point when TNF-α production had fallen to baseline. Thus, induction of IL-10 most likely played a role in the decline of TNF-α levels. As to whether C. pneumoniae infection-mediated production of IL-10 up-regulates antiapoptotic factors such as Bcl-2 and Fas-associated death domain-like IL-1-converting enzyme-inhibitory protein expression and decreases caspase activation are questions deserving further study.

### Acknowledgments

We thank Kati Hugdus for technical assistance in *C. pneumoniae* culture and titration, and Dr. Jonni Moore for helpful discussions in flow-cytometric analysis.

### References


### Figure 6

**Role of IL-10 in resistance to Fas ligand-mediated apoptosis.** *C. pneumoniae* infected or noninfected cells were cultured in the presence of IL-10 (10 ng/ml) or anti-IL-10 (10 μg/ml) from 36 h before exposure to Fas ligand (100 ng/ml) and Fas ligand enhancer (1 μg/ml). Twelve hours later, cells were harvested for TUNEL assay, as described in Materials and Methods. Data demonstrate representative histograms of FITC-dUTP incorporation, and the percentages of FITC-dUTP-positive cells are the numbers above histograms.


