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Cross-Reactive Cytotoxic T-Lymphocyte-Mediated Lysis of *Chlamydia trachomatis*- and *Chlamydia psittaci*-Infected Cells

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Cells infected with *Chlamydia trachomatis* are lysed by $CD8^+$ T cells in vitro. The ability of *C. trachomatis*-elicited spleen cells to lyse target cells infected with other chlamydial strains was determined by measuring lysis by immune spleen cells of targets infected with three strains of *C. trachomatis* and two strains of *C. psittaci*. *C. trachomatis* (lymphogranuloma venereum [LGV])-elicited immune murine spleen cells lysed target cells infected with other *C. trachomatis* serovars, although with lower sensitivity than they lysed LGV-infected target cells. Additionally, target cells infected with *C. psittaci* were lysed by *C. trachomatis*-elicited immune spleen cells. Notably, *C. psittaci*-infected cells were lysed with greater efficiency than were cells infected with the *C. trachomatis* strain used to elicit the immune spleen cells. The lysis of *C. psittaci*-infected cells was characterized further and could be only partially accounted for by $CD8^+$ T-cell-mediated lysis, the remaining lysis being due to an antigen-nonspecific component. These results indicate that mechanisms of immunologically mediated lysis differ between *C. trachomatis*- and *C. psittaci*-infected cells. This has important implications for the interpretation of results obtained with *C. psittaci* models of infection and immune resolution, particularly as they may be extrapolated to *C. trachomatis*.

Chlamydiae are eubacteria characterized by an intracellular biphasic developmental cycle, during which growth is confined to a membrane-bound vacuole known as an inclusion. The genus *Chlamydia* consists of four species, two of which, *C. trachomatis* and *C. pneumoniae*, are primarily human pathogens and the remaining two of which, *C. psittaci* and *C. pecorum*, are pathogens of birds or animals. *C. psittaci* includes a diverse group of strains which predominantly infect birds and animals but which also can be transmitted to humans. *C. psittaci* as a species has been less well characterized than *C. trachomatis*, due to the genetic and serological diversity between strains. Although phenotypically similar, *C. trachomatis* and *C. psittaci* have only 10% DNA homology (8) and can be differentiated from each other by inclusion morphology, accumulation of glycogen, synthesis of folates, and host range (14). *C. trachomatis* can be separated into three biovars based on host range and disease spectrum (14). The 12 serovars of the trachoma biovar (A, B, Ba, and C to K) infect the conjunctiva and genital tract, leading to immunologically mediated scarring. They are closely related to the three serovars of the more virulent lymphogranuloma venereum (LGV) biovar that infect regional lymph nodes. The third biovar contains only one strain, mouse pneumonitis (MoPn), which may be a natural pathogen of mice.

The ability of the immune system to lyse bacterially infected cells has been established as an important surveillance mechanism (7). *Chlamydia* remains sequestered from the host cell cytoplasm throughout its growth cycle; however, infected cells are efficiently lysed by *Chlamydia*-specific cytotoxic T lymphocytes (CTLs), as was demonstrated recently in vitro (2, 19). The ability of *Chlamydia*-specific CTLs to kill infected target cells in vitro is potentially important for in vivo resolution of active infection and for immune protection. Studies of murine models have supported the role of $CD8^+$ T cells in immunity to chlamydial infection. Adoptive transfer of $CD8^+$ T cells can protect mice from challenge (4), and a $CD8^+$ T-cell line promotes resolution of infection in nude mice (6). Beta-2-microglobulin-deficient mice that are defective in $CD8^+$ T-cell function are unable to resolve chlamydial infection as efficiently as wild-type mice and display greater mortality (10).

Although $CD8^+$ T cells have been shown to lyse *C. trachomatis*-infected cells in vitro, the antigens that are processed and presented on the surfaces of infected cells in association with major histocompatibility complex (MHC) class I have not been identified. This study compares immune spleen cell-mediated lysis of *C. trachomatis*- and *C. psittaci*-infected target cells to determine if antigens recognized by *C. trachomatis*-elicited $CD8^+$ T cells are common to the genus or limited in specificity to the strain or species. The finding that *C. psittaci*-infected cells were more susceptible to lysis than were *C. trachomatis*-infected cells suggested fundamental differences in either the mechanism of lysis or the immune recognition of cells infected with the two chlamydial species, and thus the mechanism of lysis was further characterized.

MATERIALS AND METHODS

Bacterial strains. *C. trachomatis* strains were L2/434/Bu, C-TW-3/OT, and MoPn. *C. psittaci* strains were guinea pig inclusion conjunctivitis (GPIC) and avian psittacosis (AP1). The MoPn and GPIC strains were obtained from J. Schachter (University of California, San Francisco), and AP1 was obtained from P. Timms (Queensland University of Technology, Brisbane, Australia). All strains were propagated in cultures of L929 or McCoy cells. After 48 to 72 h, the infected cells were sonicated and centrifuged to obtain infectious organisms, which were used for infecting monolayers of L cells. All *Chlamydia* strains used were titrated on L-cell monolayers in 24-well plates and stained with a fluorescein isothiocyanate-conjugated, genus-specific monoclonal antibody (Meridian Diagnostics, Cincinnati, Ohio) to determine the number of inclusion forming

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units (IFU). For mouse inoculations, L2 elementary bodies were partially purified by centrifugation over 30% Renografin (Squibb Diagnostics, New Brunswick, N.J.). *Listeria monocytogenes* 85E01167 was obtained from K. Grant (University of California, Berkeley). For mouse inoculations, *Listeria* was grown to log phase and then optical density was measured at 600 nm.

Effector cells. C3H mice (Jackson Laboratory, Bar Harbor, Maine), 6 to 8 weeks of age, were intravenously inoculated on three different occasions with viable *C. trachomatis* serovar L2. At day 0, mice were inoculated with 1×10^7 IFU followed by a booster injection at day 7 with 2×10^8 IFU and a final booster injection at day 14 with 7.5×10^8 IFU. Spleen cells were obtained 18 to 21 days following the primary infection for use in the in vitro CTL assay. A separate set of C3H mice were inoculated with 10^5 *L. monocytogenes* organisms on day 0 and with 10^6 *Listeria* organisms on day 7. Immune spleen cells were obtained from *Listeria*-infected mice on day 14. Single-cell suspensions of spleen cells were prepared as previously described (2). The spleen cells from all mice were used at a concentration of 10^7 cells/ml.

Infection and cytotoxicity assay. Fibroblastic mouse L cells transfected with ICAM-1 cDNA (LICAM cells) were obtained from Rene de Waal Malefyt (DNAX Research Institute, Palo Alto, Calif.). Subconfluent monolayers of LICAM cells (approximately 10^7) were infected with 5.7×10^7 IFU of each *Chlamydia* strain diluted in 2 ml of sucrose phosphate glucose buffer (200 mM sucrose, 4 mM KH_2PO_4, 8.5 mM Na_2HPO_4, 5 mM L-glutamic acid). The infection and ^{51}Cr labeling protocol has been described previously (2). For the cytotoxicity ^{51}Cr release assay, all experiments were performed in triplicate at a 100:1 effector/target cell ratio. Effector cells (10^6) were incubated with 10^4 labeled target cells in a volume of 200 μl of medium in 96-well tissue culture plates. In addition, 10^4 target cells were incubated in six wells containing medium alone for measurement of spontaneous release and in six wells for determination of maximum release after lysis with 2% Triton X-100. The plates were centrifuged for 15 min at 1,000 × *g* and incubated at 37°C for 6 to 8 h. After incubation, the supernatants were absorbed onto filter strips (Skatron Instruments, Oslo, Norway) and counted with a gamma counter (Packard, Meriden, Conn.). Results were reported as percent specific cytolysis = (experimental ^{51}Cr counts per minute − spontaneous ^{51}Cr release counts per minute)/(maximum ^{51}Cr release counts per minute − spontaneous ^{51}Cr release counts per minute). All experimental assessments were repeated a minimum of three times with different mice as donors of immune spleen cells and had a spontaneous release of less than 20% of the total counts per minute.

Metabolic inhibitors. The following compounds were all obtained from Sigma Chemical Co. (St. Louis, Mo.). Chloramphenicol (20 μg/ml) was added to the growth medium of the LICAM cells immediately following the 2-h *Chlamydia* infection and removed prior to the 6-h incubation with effector cells. Brefeldin A (10 μg/ml) or cycloheximide (2 μg/ml) was added to the infected target cells 1 h prior to the cytotoxicity assay and maintained throughout the experimental incubation time. These concentrations of brefeldin A and cycloheximide had no effect on chlamydial growth or subsequent infectivity (data not shown).

Antibodies and cytokines. Monoclonal anti-CD8.1 (clone 3.155) and anti-CD4 (clone GK1.5) were kind gifts of J. P. Allison (University of California, Berkeley). The antibodies against CD4 and CD8 were partially purified by ammonium sulfate precipitation from ascites and used at 10 μg/ml with immune spleen cells and complement. Rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada) was titrated for use with the monoclonal antibodies at a 1:30 dilution. Purified monoclonal antibody blocking the activity of gamma interferon (IFN-γ) (clone XMG1.2) was obtained from Pharmingen (San Diego, Calif.) and used at concentrations of up to 20 μg/ml. Recombinant mouse IFN-γ (Genzyme, Cambridge, Mass.) was used at concentrations of up to 100 U/ml.

To determine the efficiency of depletion, spleen cells (approximately 10^6) were washed twice in Hanks balanced salt solution and incubated for 30 min on ice with 1 μg of either clone CTCD8a (CalTag, Burlingame, Calif.) directed against murine CD8 or clone CTCD4 (CalTag) directed against murine CD4. Cells were washed twice with Hanks balanced salt solution and analyzed with a FACScan (Becton Dickinson, Bedford, Mass.).

RESULTS

Target cell lysis by *C. trachomatis*-specific immune spleen cells. *Chlamydia*-specific immune spleen cells were generated by inoculating C3H mice with *C. trachomatis* LGV biovar (serovar L2) organisms. The target cells were LICAM cells, as it has been shown that ICAM-1 is underrepresented on L cells and that it is required for the efficient measurement of in vitro CTL-mediated lysis of LGV-infected cells (2). The strength of this approach is that it enables measurement of CTL killing with T cells that have not been stimulated or selected in vitro prior to use, thus reflecting dominant responses elicited in vivo. LICAM cells were infected with the various *Chlamydia* strains at a multiplicity of infection (MOI) of 6 and assayed 24 h after infection. The LGV-specific immune spleen cells lysed the trachoma biovar (serovar C)- and MoPn biovar-infected target cells (Fig. 1). Lysis of the target cells infected with trachoma biovar (serovar C) was approximately 30% of that of cells infected with the LGV biovar. Similarly, the lysis obtained with MoPn-infected cells was 50% of the lysis obtained with the L2-infected cells. The ability of the L2-elicited effector cells to lyse target cells infected with other *C. trachomatis* strains suggests that some T-cell antigens are shared between these biovars.

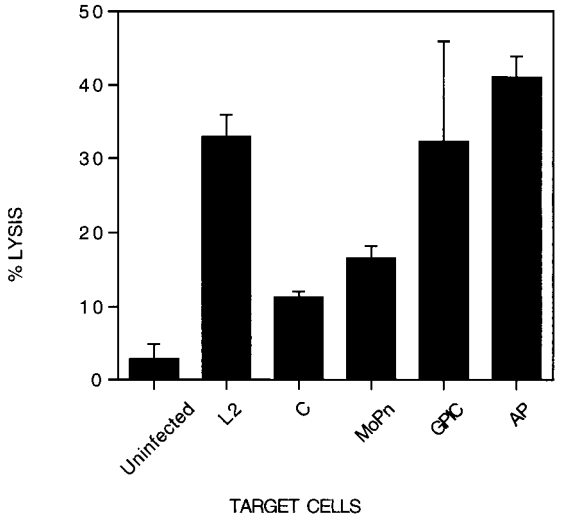

FIG. 1. Comparison of cytolysis with *C. trachomatis*-specific immune spleen cells of target cells infected with different strains of *Chlamydia*. Amounts of lysis were compared by incubating ^{51}Cr-labeled infected target cells with effector spleen cells for 6 to 8 h at 37°C, after which supernatants were harvested and release of ^{51}Cr was determined. Percent lysis was calculated as (experimental counts per minute − spontaneous counts per minute)/(maximum counts per minute − spontaneous counts per minute) × 100. Results shown are the means (± standard errors of the means) of triplicate wells from a representative experiment of three performed. The effector-to-target cell ratio was 100:1.

To determine if lysis was species specific or more broadly reactive, LICAM cells were infected with two *C. psittaci* strains, either GPIC or AP1, and tested for lysis by immune spleen cells elicited by *C. trachomatis*. Cells infected with either of the *C. psittaci* strains were also susceptible to L2-elicited spleen cell-mediated lysis (Fig. 1). However, despite the fact that the same T-cell preparation and the same MOI were used for all strains tested, *C. psittaci*-infected cells were typically lysed with greater efficiency than were LGV-infected cells. As shown in Fig. 1, which includes a simultaneous assessment of each strain, lysis of *C. psittaci*-infected targets was only marginally greater than that of *C. trachomatis* (L2)-infected cells, but in 80% of experiments the lysis of *C. psittaci*-infected cells was significantly greater than that of *C. trachomatis*-infected cells, as illustrated in Fig. 2. Because this result was unexpected, the lysis of *C. psittaci*-infected cells was further characterized to better define the basis for this increased lysis.

$CD8^+$ T-cell depletion. Initially, we evaluated whether $CD8^+$ cells were responsible for the lysis observed with cells infected with *C. psittaci*. The lysis of cells infected with *C. trachomatis* was previously attributed predominantly to $CD8^+$ T cells (2). The LGV-elicited spleen cells were depleted of CD8- or CD4-bearing cells to evaluate the effect on lysis of *C. psittaci* (AP1)-infected target cells. Analysis with a fluorescence-activated cell sorter confirmed that >95% of $CD8^+$ or $CD4^+$ cells were eliminated (data not shown). Lysis of the LGV-infected target cells was decreased by 64% with the removal of $CD8^+$

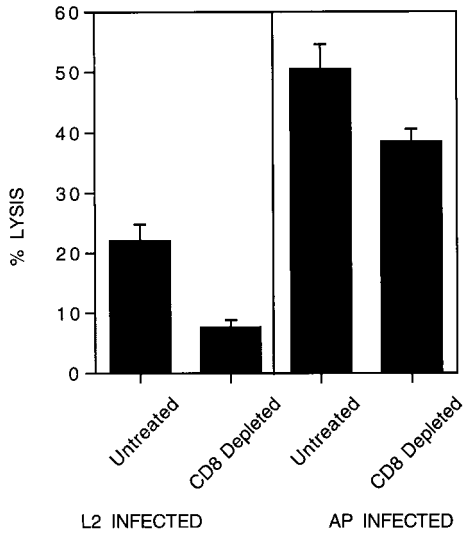

FIG. 2. Effect of $CD8^+$ cell depletion on immune spleen cell cytolysis of *C. trachomatis*- and *C. psittaci*-infected and uninfected target cells. *C. trachomatis*-specific spleen cells were depleted of $CD8^+$ cells by treatment with an anti-CD8 monoclonal antibody and rabbit complement. Cells were then assayed for lysis of infected target cells in a ^{51}Cr release assay as described in the legend to Fig. 1.

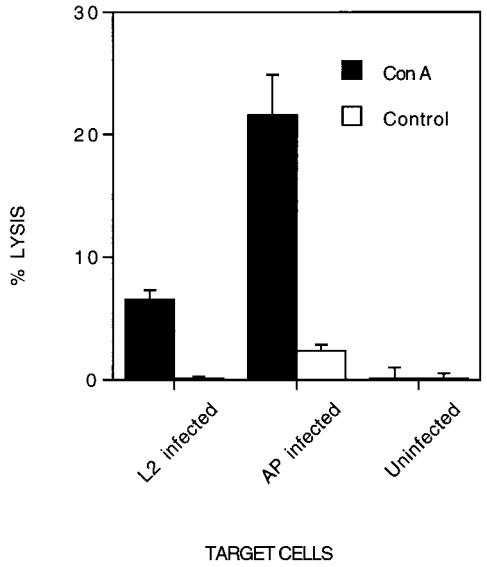

FIG. 3. Measurement of cytolysis by ConA-treated spleen cells. Spleen cells (4×10^6/ml) from naive mice were incubated for 18 h with 5 μg of ConA/ml or in medium alone with no ConA added. Both sets of cells were used as effector cells in a ^{51}Cr assay to test for lysis of *C. trachomatis*-infected, *C. psittaci*-infected, and uninfected LICAM cells as described in the legend to Fig. 1. The effector-to-target cell ratio was 100:1.

cells; however, lysis of the AP1-infected target cells was decreased only by 27% (Fig. 2). In contrast, the elimination of $CD4^+$ cells did not result in decreased activity against either target population. Thus, a significant portion of the lysis cannot be attributed to $CD8^+$ cells and may be due to nonspecific killing by other activated spleen cells. The $CD8^+$ T-cell-mediated component of lysis of *C. psittaci*-infected cells may be due to recognition of cross-reactive or genus-specific antigens.

Lysis with *Listeria*-specific immune spleen cells. In order to determine if the lysis that could not be attributed to $CD8^+$ cells was due to *Chlamydia*-specific immune cells or bacterially activated immune cells in general, we tested immune spleen cells obtained from mice inoculated with *Listeria*. Mice were inoculated twice with *Listeria*, and their spleen cells were tested for their abilities to lyse *C. trachomatis*- and *C. psittaci*-infected target cells.

The results shown in Table 1 demonstrate that many of the *C. psittaci*-infected target cells were lysed when they were incubated with the immune spleen cells elicited following infection with *Listeria* whereas the *C. trachomatis*-infected and uninfected target cells were not lysed. Thus, *Listeria*-elicited immune spleen cells had the ability to lyse the *C. psittaci*-infected target cells, in an undefined manner that nevertheless required immune spleen cells, as spleen cells from naive mice did not lyse either type of infected target cell. These results imply that the lysis of *C. psittaci*-infected target cells by *C. trachomatis*-elicited immune spleen cells has two components: one that leads to *Chlamydia*-specific cross-reactive lysis and a non-*Chlamydia*-specific component. It appears that this second component is antigen independent, as *C. trachomatis*-infected cells were not lysed by *Listeria*-elicited cells. However, we cannot discount the possibility that the second component is due to cross-reactive antigens that are common to several bacterial species but that have greater access to the processing pathway in *C. psittaci*-infected cells than they do in *C. trachomatis*-infected cells.

TABLE 1. Target cell lysis by *Chlamydia*-specific and *Listeria*-specific effector cells

Effector cells	% Lysis of target cells[a]		
	C. trachomatis	*C. psittaci*	Uninfected
Chlamydia elicited	22	44	4
Listeria elicited	0	16	0

[a] Percent lysis of target cells infected with *C. trachomatis* L2 or *C. psittaci* AP1.

ConA-stimulated spleen cells. To test whether the observed lysis of *C. psittaci*-infected cells by *Listeria*-immune spleen cells was antigen dependent or antigen independent, the T-cell mitogen concanavalin A (ConA) was used to activate spleen cells in the absence of antigen. Spleen cells from naive mice were treated with ConA for 18 h and then tested for lytic activity on the *Chlamydia*-infected target cells. Half of the spleen cells were incubated for 18 h without ConA to control for nonspecific activity generated during overnight culture. The *C. psittaci*-infected cells were lysed much more efficiently by cells activated with ConA than were the *C. trachomatis*-infected or the uninfected target cells (Fig. 3). No lysis of infected target cells was observed with cell-free supernatants obtained from ConA-stimulated and control cultures (data not shown). To determine if the activated cells responsible for lysis were $CD8^+$, ConA-treated cells were depleted of CD8-bearing cells and assayed for their ability to kill *C. psittaci*-infected target cells. Although fluorescence-activated cell sorter analysis showed that >95% of the $CD8^+$ T cells were eliminated (data not shown), elimination of $CD8^+$ cells did not decrease the activity of ConA-stimulated cells against *C. psittaci*-infected cells (Fig. 4).

Because prolonged incubation with IFN-γ has lytic effects on *Chlamydia*-infected cells (5), we tested whether IFN-γ could be directly responsible for the effects observed. However, the lysis measured with ConA-stimulated spleen cells was unchanged when an antibody blocking the activity of IFN-γ or IFN-γ itself was added (data not shown). The addition of IFN-γ alone without spleen cells did not elicit measurable lytic

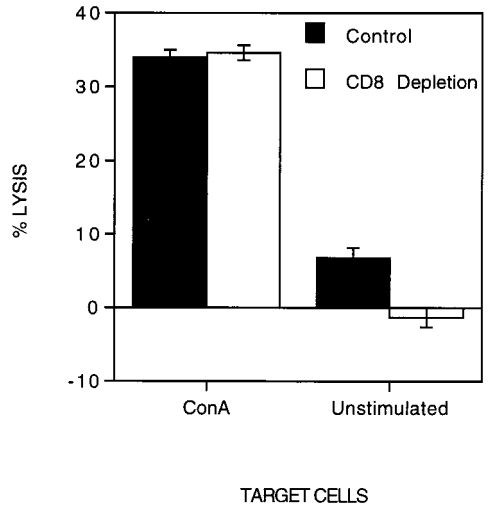

FIG. 4. Effect of depletion of $CD8^+$ cells from ConA-stimulated spleen cells on cytolysis of *C. psittaci*-infected target cells. Spleen cells (4×10^6/ml) from naive mice were incubated for 18 h with 5 μg of ConA/ml or in medium alone with no ConA added. Stimulated cells were depleted of $CD8^+$ cells by treatment with an anti-CD8 monoclonal antibody and rabbit complement. Depletion was >95% successful. Cells were then assayed for lysis of infected target cells in a ^{51}Cr release assay as described in the legend to Fig. 1.

activity (data not shown). These results demonstrate that lysis of the *C. psittaci*-infected target cells required the presence of activated, but not antigen-specific, spleen cells. This immunologically activated lysis could not be directly attributed to the presence of IFN-γ during the short incubation time of the cytotoxicity assay.

Effect of inhibitors on *C. psittaci*-infected target cell lysis. The use of ConA-stimulated spleen cells as effector cells demonstrated that, in contrast to CTL killing of cells infected with *C. trachomatis*, much of the cell lysis of cells infected with *C. psittaci* could not be attributed to antigen-specific killing. To distinguish between increased susceptibility to lysis elicited by the entry into the host cells versus lysis requiring growth of bacteria inside the cell, the antibiotic chloramphenicol was used to inhibit growth of *C. psittaci* yet allow attachment and entry into the target cell. No lysis was seen when either *C. trachomatis*- or *C. psittaci*-infected target cells were treated with chloramphenicol prior to being tested in the CTL assay (Table 2).

Cycloheximide was added to *Chlamydia*-infected target cells to examine the effect of inhibition of presentation of endogenously synthesized antigens by class I MHC molecules (21) on lysis of infected cells. Cycloheximide decreased the lysis of *C. trachomatis*-infected targets by 50%, as previously reported (2). Similarly, there was a 70% reduction in the lysis of *C. psittaci*-infected targets when they were treated with cycloheximide (Table 2). Thus, as similar reductions in lysis were obtained with both species, the effect of cycloheximide on *C. psittaci*-infected cells does not indicate a major difference in the requirement for host cell protein synthesis in the lysis of infected target cells compared with that for cells infected with *C. trachomatis.*

Brefeldin A, an inhibitor of class I MHC processing, completely abrogated lysis of *C. trachomatis*-infected cells. However the lysis of *C. psittaci*-infected cells was decreased by only 61% (Table 2). The reduction in lysis of *C. trachomatis*-infected cells with brefeldin A treatment may be attributed to a disruption in the processing of antigens into endosomes for presentation with class I MHC. The lysis of *C. psittaci*-infected cells observed to be independent of brefeldin A may be attributed to the antigen-independent mechanism of lysis that apparently does not require $CD8^+$ T cells or presentation of antigens by class I MHC.

DISCUSSION

C. trachomatis and *C. psittaci* display similar cell culture and infection characteristics; however, they can be readily differentiated based upon their inclusion morphologies and host ranges (14). Despite the low level of DNA homology (8) between these two species of *Chlamydia*, the major structural proteins are present in both species and there is considerable similarity in the amino acid sequences of the proteins that have been examined. The 16S rRNA sequences of *C. psittaci* and *C. trachomatis* are 95% homologous (22), and their abundant major outer membrane proteins (MOMP) display 68% amino acid homology (24). Several antigenic epitopes are also shared between the species. Antibodies that recognize genus-specific antigenic determinants have been described for MOMP (11) and lipopolysaccharide (3). While the two species of chlamydiae display many similarities, experimental results are often conflicting, indicating that caution must be exercised in extrapolating conclusions from one species to the other (12).

We have recently demonstrated $CD8^+$ T-cell-mediated lysis of *Chlamydia*-infected target cells (2). Starnbach et al. (19) confirmed these findings using $CD8^+$ T-cell lines and additionally demonstrated that adoptive transfer of these cells conferred immune protection in mice. However, neither of these studies addressed the issue of potential cross-reactivity or determined whether killing was species specific. More recently, *C. trachomatis*-specific T-cell lines were shown to lyse target cells infected with multiple serovars, implicating species-specific, rather than serovar-specific, antigens in presentation to $CD8^+$ cells (18). Our present study more broadly characterized the CTL response by examining the specificity of unselected immune spleen cells obtained from *C. trachomatis*-infected mice for lysis of target cells infected with different strains from two chlamydial species. Target cells infected with other *C. trachomatis* biovars and two strains of *C. psittaci* were lysed with *C. trachomatis* (L2)-elicited immune spleen cells. The ability of the LGV biovar-elicited immune spleen cells to lyse cells infected with either trachoma biovar or murine biovar organisms suggests the presence of antigens recognized by CTLs that are common to all *C. trachomatis* serovars. Moreover, the fraction of lysis of the *C. psittaci*-infected cells attributable to *C. trachomatis*-specific spleen cells suggests that predominant antigens recognized by CTLs are conserved across the chlamydial genus. This is consistent with the finding of a $CD8^+$ T-cell clone that proliferates in response to an antigen apparently common to the chlamydial genus (6).

While cross-reactive genus-specific antigens may be impor-

TABLE 2. Percent reduction of lysis by *C. trachomatis* (L2)-specific effector cells with inhibitors added to *C. psittaci* (AP1)- or *C. trachomatis* (L2)-infected target cells

Inhibitor	% Reduction of lysis by effector cells of target cells infected with:	
	C. psittaci	*C. trachomatis*
Chloramphenicol	100	96
Cycloheximide	70	50
Brefeldin A	61	100

tant targets for recognition by $CD8^+$ T cells, cross-reactivity alone is insufficient to explain the finding that the amount of lysis of *C. psittaci*-infected target cells by *C. trachomatis*-specific spleen cells was consistently greater than the amount of lysis of *C. trachomatis*-infected cells. Lysis of cells infected with *C. psittaci*, mediated by *Listeria*-elicited immune cells, suggested recognition of antigens common to these bacteria or, alternatively, a mechanism of lysis that could be stimulated by bacterial infection but was antigen independent. The possibility of antigen-independent lysis was further supported by the ability of ConA-stimulated spleen cells to lyse *C. psittaci*-infected cells. We conclude from these data that cells infected with *C. psittaci* are significantly more susceptible to lysis by activated spleen cells than are cells infected with *C. trachomatis* at the same MOI. This observation raises two questions: (i) what is the mechanism of antigen-independent lysis? and (ii) what implications do these results have for interpreting existing data and for designing future studies?

Regardless of the method of stimulation, activated spleen cells had greater effects on *C. psittaci*-infected cells than on *C. trachomatis*-infected cells. This indicates that the increase in lysis is due to an inherent property of *C. psittaci*-infected cells and may be related to differences between species in inclusion biology or interaction with the host cell. Although one might consider the possibility of immediate toxicity due to *C. psittaci* infection at high MOIs (13, 23), we discount this because no cytotoxicity was observed when infected cells were incubated with naive spleen cells. It is unlikely that the increased lysis was a result of *C. psittaci* entry damaging the host cell membrane, because no measurable lysis could be demonstrated after treatment of infected cells with chloramphenicol, which blocks growth but not entry. Although the lack of lysis by supernatants from stimulated cells does not rule out a soluble factor which may increase lysis, it strongly implicates a requirement for cell-cell interaction even for the antigen-independent lysis of the *C. psittaci*-infected target cells.

Cycloheximide treatment affected equally the lysis of *C. psittaci*- and that of *C. trachomatis*-infected cells, while brefeldin A treatment completely abrogated the lysis of *C. trachomatis*-infected cells and reduced the lysis of *C. psittaci*-infected cells by 61%. Thus, while lysis of *C. trachomatis*-infected cells required intracellular processing of antigens through the class I pathway, only a portion of lysis of *C. psittaci*-infected cells could be attributed to processing through this pathway and subsequent killing by $CD8^+$ cells. The lysis of *C. psittaci*-infected cells rendered incapable of antigen presentation by brefeldin A or cycloheximide treatment indicates that a component of lysis is initiated by activated spleen cells which specifically lyse *C. psittaci*-infected cells but by a mechanism that does not require recognition of peptides in association with class I MHC. It is important that depletion of $CD8^+$ cells from ConA-activated spleen cells did not result in decreased lysis of *C. psittaci*-infected cells. This finding indicates that the $CD8^+$ T-cell activity of *C. trachomatis*-immune spleen cells directed against *C. psittaci*-infected cells is antigen specific and not due to nonspecific activation of $CD8^+$ cells.

Morphologically, the *C. psittaci* inclusion differs significantly from the *C. trachomatis* inclusion, since it is more pleomorphic and expands to fill the host cytoplasm. The *C. psittaci* inclusion membrane thus appears to be more fluid and perhaps has more surface area in contact with the cytoplasm. Furthermore, it appears that there is at least one *C. psittaci* protein that is localized to the inclusion membrane and that may also be found in the host cytoplasm (17). Thus, through either an active or a passive mechanism, it is possible that the amount of *C. psittaci* proteins or peptides that enters the host cytoplasm is greater than the amount of *C. trachomatis* proteins or peptides. Such proteins would presumably be available for processing by the endogenous pathway, leading to more efficient recognition by CTLs. However, this rationale accounts only for an increase in antigen-dependent lysis. The observed increase in antigen-independent lysis is more difficult to explain. It has been observed previously that *C. psittaci* inhibits host-cell DNA and protein synthesis (1), which may disturb the cellular homeostasis, resulting in an increased susceptibility to lysis. *C. psittaci* infection may increase the expression of apoptosis triggering-surface receptors, such as Fas (15), which would result in an increased susceptibility to antigen-independent lysis by activated T cells. Other possibilities are that *C. psittaci* infection prevents host metabolism, recycling of receptors, or presentation of self peptides, any of which may lead to recognition of the infected cell as being damaged. Alternatively, factor-mediated induction of host cell activity, such as inducible nitric oxide synthase, may result in increased susceptibility to lysis. Additionally, it is possible that *C. psittaci* interferes with the integrity of internal membranes of the host cell (20), which may render the cell more susceptible to lysis. Studies characterizing the cell biology, particularly the inclusion membrane composition, of these two chlamydial species may clarify the variability observed here in the interactions between infected host cells and activated immune cells.

The finding that *C. psittaci*- and *C. trachomatis*-infected cells are lysed by different mechanisms may account for some of the differences between results obtained in previous immunological studies of the two species. Early studies analyzing $CD8^+$ cell-mediated cytotoxicity of *Chlamydia*-infected cells had inconclusive results. Lammert (9) found that *C. psittaci*-infected target cells were lysed in the presence of immune spleen cells but did not show specificities for infectious organisms. This is in contrast to a study by Pavia and Schachter (16), who found no cytolytic activity using *C. trachomatis*-infected target cells. The results presented here demonstrating antigen-independent immune lysis of *C. psittaci*-infected target cells and only antigen-specific lysis of *C. trachomatis*-infected cells apparently resolve the ambiguity of the earlier conflicting reports (9, 16).

Our results have important implications for animal protection models, a large number of which have utilized *C. psittaci* infections. It has previously been suggested that different immune mechanisms may be important in *C. trachomatis* and *C. psittaci* infections (25). A search for MOMP antigenic variants between GPIC strains of *C. psittaci* showed no variation in MOMP sequences, suggesting that the immune interactions of *C. psittaci* with its natural hosts are significantly different than those for *C. trachomatis*, at least in terms of selective pressure by antibody recognition. The differences in cytolytic mechanisms for *C. trachomatis* and *C. psittaci* described here suggest that cellular responses or antigen-independent mechanisms may play a more significant role in immunologically mediated resolution of *C. psittaci* infections than in resolution of *C. trachomatis* infections. If this is true, a consequence could be less selective pressure due to antibody-mediated neutralization, which would presumably account for the lack of surface antigen variation in *C. psittaci* (25). Thus, it is critical that vaccine models assessing the importance of individual epitopes, and the relative roles of humoral versus cell-mediated immunity, utilize the relevant chlamydial species and that caution is taken in extrapolating protection results between species. Finally, our data points to fundamental differences not only in the immune surveillance and clearance of *C. psittaci* and *C. trachomatis* but also in the cell biology and interactions of these species with their host cells.

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