Role of CD8 T Cells in Primary *Chlamydia* Infection

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Received 14 April 1994/Returned for modification 8 August 1994/Accepted 15 November 1994

The role of CD4 and CD8 T cells in primary *Chlamydia trachomatis* pneumonia was investigated by using in vivo depletion techniques to eliminate T-cell populations. Reduction of either CD4 T cells or CD8 T cells caused a significant increase in organism burden in the lungs, as measured by both quantitative culture and detection of chlamydial antigen on day 14 postinfection. *Chlamydia*-specific antibody levels in plasma or antigen-induced gamma interferon (IFN- γ) production by spleen cells was dramatically reduced by depletion of CD4 cells. The reduction in IFN- γ achieved by depletion of CD8 cells did not reach statistical significance. In the survival studies, depletion of CD4 cells led to a significant increase in mortality. Although there was a trend toward higher mortality, depletion of CD8 cells did not significantly increase mortality. The role of CD8 T cells in host defense was clarified in studies using beta 2-microglobulin-deficient (major histocompatibility class I antigen-deficient, C1D) mice which are defective in CD8 T-cell function. In this model, a significant increase in organism burden was seen during infection in C1D mice compared with that in C57BL/6 controls and a significant increase in mortality was observed as well. However, surviving C1D mice were able to clear the infection by day 34. C1D mice had increased numbers of CD4 T cells in both the spleen and the lungs during infection compared with those of C57BL/6 controls. IFN- γ in C57BL/6 mice was produced by both CD4 and CD8 cells. Thus, there is a protective role for both CD4 and CD8 cells in host defense against *Chlamydia* infection, but the former appear to be dominant.

T cells have been shown to play a critical role in protective immunity against *Chlamydia* infection in studies using a variety of animal models (18, 29, 40, 41). A number of studies from our laboratory and others have shown that *Chlamydia* infection does not resolve in the absence of alpha/beta T cells (athymic mouse model) and that CD4 T cells are important components in host defense in this infection (15, 18, 20, 29, 38, 40–42). The latter studies include our demonstration of adoptive transfer of protection to T-cell-deficient mice by a *Chlamydia*-specific CD4 T-cell clone (15, 20). Although the role of CD8 T cells against *Chlamydia* spp. is much less well defined, their participation in host defenses is beginning to be assessed. Studies by Patton et al. (25) with the primate model of genital infection due to *Chlamydia trachomatis* showed that CD8 T cells are a very important component of the inflammatory response to the infection. Recently, Buzoni-Gatel et al. (2), using a model of secondary infection with *Chlamydia psittaci* given intravenously, found that CD8 T cells were the principal modality of T-cell-mediated host defense in their model and that CD4 T cells played virtually no role. Also, in our own model of secondary infection of *C. trachomatis*-induced pneumonia due to the mouse pneumonitis agent (MoPn) (murine *C. trachomatis*) delivered by an initial mucosal route (intranasally), both CD4 and CD8 T cells played a role in host defense, with the former being dominant (38). Additionally, we have recently shown that passive transfer of one of two CD8 T-cell clones protects nude mice and allows for clearance of organisms (14). Therefore, the relative roles of CD4 and CD8 T cells in host defense against *Chlamydia* infection need to be more precisely defined.

Most importantly, since data for CD8 T cells in primary infection are limited, this question is of particular importance because of the possibility that CD8 T-cell-dependent mechanisms of resistance may overlap with those usually attributed principally to, or modulated by, CD4 T cells (cytokine generation and antibody production) (3, 37, 39, 40, 45).

We therefore decided to investigate the role of CD8 T cells compared with CD4 T cells in host defense against MoPn pneumonia in a model of primary, mucosally introduced infection. To do this, we employed two models. The first involved depletion of both CD4 and CD8 T cells in vivo during initial MoPn pneumonia. The second involved the use of beta 2-microglobulin-deficient mice (C1D mice), which lack functional major histocompatibility complex class I molecules and major histocompatibility complex class I-directed CD8 T cells (24), as a vehicle for study of primary MoPn infection.

MATERIALS AND METHODS

Mice. Male and female specific-pathogen-free *nu/nu* mice from a BALB/c background were bred and maintained under barrier conditions at the Audie L. Murphy Veterans Administration Hospital. These mice were free of pathogenic bacteria and viruses as assessed by culture or serology. C1D and C2D mice and C57BL/6 controls were purchased from GenPharm, Inc. (Sunnyvale, Calif.). They were also free of pathogenic bacteria and viruses as determined by culture and serology and were housed as per the *nu/nu* mice. Mice were housed in rooms on a 12-h dark-light cycle and used between 6 and 12 weeks of age. Infected mice were housed in isolator units in separate rooms. Mice were fed rodent chow and water ad libitum.

MoPn. The MoPn biovar of *C. trachomatis* was maintained in embryonated hen's eggs (41). Mice were infected intranasally, after anesthesia with sodium pentobarbital, with 2×10^2 to 5×10^5 inclusion-forming units (IFU) diluted in McCoy's modified 5A medium in a volume of 0.05 ml. Purified elementary bodies (EBs) were prepared by Renografin density gradient centrifugation of cell culture-derived *C. trachomatis* (12). The purified EBs were killed under UV light before use as an antigenic stimulus. Quantitative culture of infected tissue was performed with McCoy cell monolayers and reported as log₁₀ IFU per lung (21).

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Chlamydia antigen levels were determined by an enzyme-linked immunosorbent assay (ELISA) to detect chlamydial lipopolysaccharide (LPS) (Ortho Diagnostic, Inc., Raritan, N.J.) (39). Homogenized lungs were diluted in medium and processed for assay according to the instructions of the manufacturer. A standard curve was run with each determination by using purified MoPn EBs. An EB protein dose response between 10 and 200 ng/ml was detected by a linear increase in the optical density (OD). The data are expressed as a ratio of experimental OD values to the OD values for uninfected lungs.

Hybridomas and monoclonal antibody production. The hybridomas GK 1.5 (anti-L3T4 or -CD4) and TIB 210 (anti-Lyt 2.2 or -CD8) were purchased from the American Type Culture Collection. Hybridomas were injected into pristane-treated BALB/c *nu/nu* mice. Ascites fluid was collected and frozen at -70°C until processing. The immunoglobulins were collected by precipitation with 50% ammonium sulfate. The precipitated antibodies were dialyzed against 0.01 M phosphate-buffered saline (PBS) to remove sulfate ions. Protein determinations were performed by the Bio-Rad protein assay (Bio-Rad, Richmond, Calif.). Purified rat immunoglobulin G (IgG) was purchased (ICN Immunobiologicals, Lisle, Ill.) to use as a control. Mice were treated with 0.5 mg of each antibody on days -4, 0, 7, and 14. This schedule and dosage constituted the optimal regimen. Preliminary experiments indicated that the higher doses of antibody or increased numbers of injections caused some toxic reactions in a small number of recipients. No dose was given on day 14 in experiments ending on that day. Antibodies to natural killer (NK) cells, anti-asialo GM1 antibodies, were purchased from Wako BioProducts (Richmond, Va.). Monoclonal antibody against the murine gamma/delta (γ/δ) T-cell receptor (UC7-13D5) was purchased from PharMingen (San Diego, Calif.).

FMF. Flow microfluorometry (FMF) analysis was used to verify *in vivo* depletions (22). Briefly, whole spleen cell populations (2×10^6 total cells; $>95\%$ viable as determined by trypan blue exclusion) were treated with 1 μg of primary antibodies reactive for either Thy 1.2, CD4, or CD8 antigens for 30 min at 4°C (all primary antibodies were purchased from Becton Dickinson Immunocytometry, Mountain View, Calif.). The cells were washed twice and incubated with fluorescein-labeled goat anti-rat second antibody for 30 min at 4°C (diluted 1:1,000) (Jackson Immunoresearch, West Grove, Pa.). After this incubation, cells were washed twice and resuspended in PBS containing 1% formalin. Cells were analyzed, within 1 week of staining, by gating on lymphocytes, using the forward and right-angle light scatter, by the Department of Microbiology Flow Cytometry Laboratory, University of Texas Health Science Center, San Antonio. We controlled for the presence of antibody that might remain attached to spleen cells as a result of the *in vivo* treatments. This was performed by staining an aliquot of cells with fluoresceinated anti-rat secondary antibody directly without any primary antibody in the first incubation. With this control, we did not detect any antibody bound *in vivo* to the spleen cell populations.

Supernatant production. At various times postinfection, mice were sacrificed and the spleens were removed aseptically. Single-cell suspensions were prepared by grinding the spleens between the frosted ends of glass microscope slides. The spleen cells were washed and resuspended in RPMI 1640 supplemented with 5% fetal bovine serum (HyClone Laboratories, Logan, Utah), 100 μg of vancomycin per ml, and 50 μg of gentamicin sulfate per ml (medium). Spleen cells from individual mice were plated in 24-well plates at $2.5 \times 10^6/\text{ml}$ with or without 5 μg of EB antigen per ml. In some experiments, spleen cells were treated *in vitro* with antibody to CD4 or CD8 and low-toxicity rabbit complement (Cederlane Laboratories, London, Ontario, Canada) or with complement alone (control) prior to incubation with EB antigen. There were three to five mice per group for each time.

IFN- γ ELISA. Supernatants were tested for gamma interferon (IFN- γ) activity by modifying the protocol for a previously reported specific ELISA (28). Immulon-2 plates (Dynatech, Chantilly, Va.) were coated with 50 μl of monoclonal anti-murine IFN- γ (from hybridoma XMG 1.2; 1 $\mu\text{g}/\text{ml}$) and incubated overnight at 4°C in carbonate buffer (pH 9.6). The plates were washed and blocked with 5% bovine serum albumin in PBS-0.05% Tween 20. Samples and recombinant standards (50 μl per well) were added, and the plates were incubated for 2 h at room temperature. The plates were washed, and 50 μl of biotinylated anti-murine IFN- γ (from hybridoma R4-6A2, at 1 $\mu\text{g}/\text{ml}$) was added per well. Following a 1-h incubation at room temperature, the plates were washed and 50 μl of avidin-peroxidase was added (Sigma) (1:2,500 dilution). After a final 1-h incubation, 50 μl of substrate per well was added (*o*-phenylenediamine [1 mg/ml] in 0.003% H_2O_2 in citrate buffer, pH 5.0). The reaction was stopped after 30 min with 4 N H_2SO_4 , and the OD at 492 nm was read. Results are expressed as picograms of IFN per milliliter.

Antibody assay. Plasma samples were collected at the times specified below by cardiac puncture of mice under metophane anesthesia. Plasma IgG levels were determined by an indirect immunofluorescence assay as previously described (44).

Statistical analysis. Groups were compared by Student's *t* test with correction for unequal variance. When specified, the Mann-Whitney U test was used for nonparametric analyses, and Fisher's exact test was used to compare mortality on specific days. All *P* values reported are two-tailed.

TABLE 1. Quantitative culture of *C. trachomatis* on day 14 postinfection in the lungs of BALB/c *nu/nu* mice treated to deplete T-cell subsets^a

Antibody treatment	Mean log ₁₀ IFU/ lung $\pm$ SD ^b	<i>Chlamydia</i> antigen ^c
Rat IgG (control)	3.87 ± 1.04	0.88 ± 0.22
Anti-CD4	4.95 ± 0.75^d	2.10 ± 1.26^d
Anti-CD8	5.15 ± 0.55^d	1.56 ± 0.38^d

^a Lungs were harvested on day 14 postinfection and processed for quantitative culture.

^b Results are for 8 to 10 mice per group.

^c *Chlamydia* antigen was measured by ELISA to detect *Chlamydia* LPS. Data are expressed as mean ODs $\pm$ standard deviations for samples from infected lungs divided by the OD for uninfected lungs at a 1:1,000 dilution. There were seven mice per group.

^d *P* < 0.05 compared with control value.

RESULTS

Effect of *in vivo* depletion of T-cell subsets on quantitative culture and mortality in BALB/c mice. An experiment was performed to compare differences in quantitative culture of MoPn on day 14 postinfection in BALB/c *nu/nu* mice depleted of CD4 or CD8 T cells (Table 1). Depletion of either CD4 or CD8 cells led to a significant increase in the organism burden after challenge with 10^4 IFU of MoPn (*P* < 0.05). These results were confirmed in a second experiment which measured chlamydial antigen levels in the lung tissue by ELISA (Table 1). Measurement of antigen levels allows detection of both phases of chlamydial replication (the EB and the reticulate body) and nonviable organisms, whereas quantitative culture detects only viable EBs (33, 35). Compared with control values, either antibody significantly increased the chlamydial antigen levels in the lungs (*P* < 0.05). An additional experiment measuring antigen levels on day 14 showed similar results (data not shown). Two experiments were performed to assess the chlamydial burden by ELISA on day 28 postinfection. These experiments were performed with a lower-dose challenge (10^3 IFU of MoPn) to allow for survival throughout the time course. Chlamydial antigen was detected in both CD4-depleted and CD8-depleted mice, with antigen levels (means $\pm$ standard deviations) of 3.84 ± 2.1 and 1.84 ± 0.75 log₁₀ IFU per lung, respectively. Compared with control mice, with antigen levels of 1.2 ± 1.2 , the CD4-depleted mice had a statistically significant increase in chlamydial burden (*P* < 0.03).

Three experiments were then performed to assess the ability of treated mice to survive a higher-dose MoPn challenge (5×10^5 IFU). The results of these three experiments were pooled, and the final mortalities at day 27 were compared (Fig. 1). Mice given the control antibodies had a final mortality of 17.6%. Mice depleted of CD4 T cells had a significantly higher mortality of 59.4% (*P* < 0.001; Fisher's exact test). Although there was a trend toward higher mortality in the CD8-depleted mice (29.4%), there was no significant difference for this group compared with the control group (*P* = 0.391; Fisher's exact test). The mortality of the CD8-depleted group was significantly lower than that of the CD4-depleted group (*P* = 0.025; Fisher's exact test). The CD4-depleted group was significantly different from controls by day 16 postinfection (*P* < 0.005; Fisher's exact test).

As markers of the *in vivo* efficacy of our antibody depletions, we measured the T-cell subsets in the spleens of treated mice on day 14 of infection for the same mice for which results are presented in Table 1. Treatment with the control antibody did not alter either the CD4 or the CD8 phenotype compared with that of normal mice (data not shown). At day 14, the anti-CD4-

FIG. 1. Results of three experiments were pooled to generate the curves of percent mortality over time. Mice were treated with either normal rat IgG ($n = 34$), anti-CD4 ($n = 32$), or anti-CD8 ($n = 34$) on days -4 , 0 , 7 , and 14 . Mice were infected intranasally on day 0 and monitored for mortality over the next 27 days.

depleted group had 4.6% CD4-positive cells detected, compared with 15.8% positive in the control group (70.9% depletion). Treatment with anti-CD8 resulted in a depletion from 8.0 to 2.0% (75.0% depletion). FMF analysis in two separate experiments on day 28 indicated that the efficacy of depletion was maintained well for CD4 cells throughout the time course. CD8 depletion with maximally tolerated doses of the antibody, while maintained reasonably well, was not as complete. Treatment with anti-CD4 resulted in 3.9% CD4 T cells at day 28 , compared with 18.9% in the control group (79.3% depletion), while anti-CD8 treatment led to 4.0% CD8 cells remaining, compared with 9.3% in the control group (53.7% depletion).

The efficacy of in vivo depletion of CD4 T cells was also confirmed by assessing the level of *Chlamydia*-specific IgG in the plasma samples from mice from the various groups on day 14 (Table 2). The geometric mean titer of the IgG was $2,435$ for the control mice. Treatment with anti-CD4 reduced those titers to 8 or less. As expected, depletion of CD8 T cells did not reduce the titer. A repeat experiment showed similar results. Additionally, we measured the capacity of spleen cells to produce IFN- γ in response to chlamydial antigen (Table 2). A similar pattern was observed. Depletion of CD4 cells ablated the production of IFN- γ ($P < 0.05$). Although there was a trend toward lower values, depletion of CD8 did not significantly reduce IFN- γ production.

Quantitative culture and mortality in C1D and control mice.

Because of the discrepancy between the culture and antigen

TABLE 2. Parameters of immunity in BALB/c *nu/+* mice after in vivo depletion

Antibody treatment	IgG titer ^a	pg of IFN- γ /ml (mean $\pm$ SD) ^b
Rat IgG (control)	2,435	$2,692 \pm 4,072$
Anti-CD4	8	136 ± 158^c
Anti-CD8	2,048	$1,267 \pm 339$

^a Plasma samples from groups of 8 to 10 mice were collected on day 14 postinfection and assayed for chlamydia-specific IgG by microimmunofluorescence. Data are the geometric mean titers for the groups.

^b IFN- γ levels were measured after incubation of whole spleen cells with $5 \mu\text{g}$ of UV-inactivated *Chlamydia* EBs per ml for 48 h. Data are averaged from supernatants from four or five mice.

^c $P < 0.05$.

TABLE 3. Quantitative culture of *C. trachomatis* in C1D, C2D, and C57BL/6 mice

Expt	Day	<i>C. trachomatis</i> in lungs of mice ^a		
		C57BL/6	C1D	C2D
1	14	1.21 ± 0.36	2.22 ± 0.45^b	ND ^c
2	21	3.93 ± 0.26	5.99 ± 0.81^d	ND
3	14	2.37 ± 0.64	4.84 ± 0.42^e	5.02 ± 0.40^f
	21	$<0.1^g$	3.32 ± 1.07	3.47 ± 1.27
	28	<1.0	<0.1	3.85 ± 0.34^h

^a Quantitative culture of *C. trachomatis* in lungs, with results reported as mean $\log_{10}$ IFU/lung $\pm$ standard deviations.

^b $P < 0.02$ compared with C57BL/6 mice. A challenge dose of 200 IFU was used in experiment 1.

^c ND, not done.

^d $P < 0.08$ compared with C57BL/6 mice. A challenge dose of $1,000$ IFU was used in experiment 2.

^e $P < 0.0001$ compared with C57BL/6 mice. A challenge dose of 400 IFU was used in experiment 3.

^f $P < 0.04$ compared with C57BL/6 mice.

^g The lowest detectable level was 10 IFU, and for statistical purposes, values of 10 IFU were assigned to the values below detectable limits.

^h $P < 0.05$ compared with C57BL/6 and C1D mice.

data at day 14 and the mortality data, in addition to concerns that CD8 depletion may have waned later in the experiment, which might have reduced the effect of this depletion on mortality late in infection, experiments employing the congenitally CD8-deficient C1D mice and C57BL/6 controls were performed. Lung cultures were performed in both a low-dose (200 IFU) and a higher-dose ($1,000$ IFU) experiment. In the low-dose experiment, quantitative culture of lungs from the C57BL/6 control mice showed that they were significantly better able to control *C. trachomatis* infection than C1D mice at day 14 . By day 34 , however, cultures were negative for all four mice in each group (data not shown). Similar results were observed in a higher-dose experiment when assessed on day 21 (Table 3, experiment 2). These results indicate that CD8-deficient mice were more susceptible to MoPn at both the low and the high doses as determined by quantitative culture, but CD8-deficient mice could clear the infection by day 34 , as could controls (data not shown). Thus, CD8 T cells facilitated elimination of MoPn but were not necessary for final clearance. The last experiment compared the *Chlamydia* burdens after infection in C57BL/6 control mice, C1D mice, and C2D mice (functionally CD4 T-cell-deficient mice of the same genetic background obtained from the same supplier) at days 14 , 21 , and 28 postinfection (Table 3, experiment 3). The percentage of CD4 T cells in the spleens of C2D mice was determined to be undetectable by FMF on day 14 postinfection. Both C1D and C2D mice had significantly elevated levels of MoPn in the lungs at day 14 compared with the controls ($P < 0.001$ and 0.04 , respectively, compared with C57BL/6 mice). By day 28 , however, levels in C1D mice were low and not significantly different from those of the controls, while levels in C2D mice were still significantly elevated compared with those of the controls ($P < 0.05$). These data were thus consistent with those reported above showing a role for CD8 T cells in host defense but a more prominent one for CD4 T cells.

Because of reports that beta 2-microglobulin-deficient (C1D) mice might compensate for the defect by stimulation of NK cells (5) or $\gamma\delta$ T cells (31), C1D mice were treated with 0.5 mg of anti-asialo GM1 or $200 \mu\text{g}$ of UC7-13D5 antibody at days -1 , $+4$, $+8$, and $+12$ of infection with MoPn in separate experiments and compared with mice given the same amount of control immunoglobulin. This dose of anti-asialo GM1 is

FIG. 2. Results of two experiments were pooled to generate the curves of percent mortality over time. Mice were infected intranasally on day 0 and monitored for mortality over the next 32 days.

known to abolish NK cell activity in the lung in our model (43), and this dose of UC7-13D5 has been shown by others to significantly reduce murine $\gamma\delta$ T-cell activity (9). Neither antibody had any significant effect on quantitative culture of MoPn in the lungs at day 14 postinfection (four mice per group challenged with 200 IFU) ($P > 0.10$). Values (means $\pm$ standard deviations) were 0.43 ± 0.49 and $0.60 \pm 0.68 \log_{10}$ IFU per lung, respectively, for control C1D mice and C1D mice given anti-asialo GM1 antibody and 2.02 ± 0.60 and $1.96 \pm 0.44 \log_{10}$ IFU per lung, respectively, for control C1D mice and C1D mice given UC7-13D5. Thus, no evidence for a compensatory increase in NK cell or $\gamma\delta$ T-cell function as it related to levels of MoPn in the lungs was seen in the C1D mice at day 14.

To assess mortality, C1D and C57BL/6 mice were challenged with either 5×10^3 or 1×10^5 IFU. In the lower-dose experiment, there was 15% mortality (5 of 34) in the CD8-deficient mice and 0% mortality (0 of 33) in C57BL/6 control mice at day 14 postinfection ($P = 0.053$; Fisher's exact test), at which time the mice were sacrificed for other studies. In the higher-dose experiment, there was 83% mortality (15 of 18) in the C1D mice and 40% mortality (6 of 15) in the control C57BL/6 mice at day 32 postinfection ($P = 0.014$; Fisher's exact test) (Fig. 2). Therefore, the CD8-deficient mice were more susceptible to MoPn than the controls by the criterion of mortality.

Parameters of immunity during infection in C1D mice. The percentages of CD4 and CD8 T cells in the spleens of each of

TABLE 5. IFN- γ generation in C57BL/6 and C1D mice on day 34 postinfection^a

Mouse strain	pg of IFN- γ /ml (mean $\pm$ SD) for treatment group		
	Control	Anti-CD4	Anti-CD8
C57BL/6	1,470 $\pm$ 271	0 ^b	543 $\pm$ 407 ^b
C1D	2,036 $\pm$ 1,357	136 $\pm$ 136 ^b	ND ^c

^a IFN- γ was measured by ELISA in supernatants of whole spleen cells from MoPn-immunized C57BL/6 and C1D mice (four mice per group) after stimulation in vitro for 48 h with 5 μ g of EBs per ml. No IFN- γ was detected in the absence of MoPn antigen. Control whole spleen cells were treated with complement alone while the other groups were treated with anti-CD4 or anti-CD8 antibody and complement prior to stimulation.

^b $P < 0.03$ compared with control values, as determined by Mann-Whitney U test.

^c ND, not done.

three to five control and CD8-deficient mice at days 14, 21, and 34 postinfection with MoPn and those in the lungs combined from three mice at day 34 postinfection were determined (Table 4). At each time, the percentage of CD4 cells in the spleen was significantly higher ($P < 0.02$) for the C1D mice than the controls. The data for the lungs at day 34 were similar.

IFN- γ produced in response to MoPn antigen in the supernatants of whole spleen cells from C57BL/6 and C1D mice at day 34 after survival of MoPn infection was measured (Table 5). C57BL/6 cells were treated with complement alone (control) or with antibody to CD4 or CD8 plus complement in vitro to deplete those cells prior to antigen stimulation. C1D mice were treated either with complement alone or with anti-CD4 antibody and complement. FMF analysis disclosed $>90\%$ depletion of the target cells (data not shown). As opposed to the data for BALB/c mice shown in Table 2, in C57BL/6 mice IFN- γ production was significantly dependent on both CD4 and CD8 T cells, but CD4 cells appeared to be dominant, since ablation of this phenotype completely suppressed IFN- γ production (Table 5). This indicates that production of IFN- γ by CD8 cells may require the presence of CD4 cells. In C1D mice, IFN- γ production was equal to that in C57BL/6 mice and dependent on CD4 cells.

DISCUSSION

This study, which aimed to define a role for CD8 T cells in host defense against primary infection with *C. trachomatis*, has led to the following conclusions. (i) CD8 T cells play a role in host defense in our model of mucosally induced pulmonary infection in both the in vivo antibody-depleted *nu/+* model and the beta 2-microglobulin-deficient model. (ii) The role of CD8

TABLE 4. Percentages of T-cell subsets in C1D and control mice as determined by FMF

Organ	Day	% Positive cells (total) ^a			
		CD4		CD8	
		C57BL/6	C1D	C57BL/6	C1D
Spleen	14	17 $\pm$ 4 ^b (5.7 $\pm$ 4.3)	36 $\pm$ 5 (8.9 $\pm$ 3.4)	9 $\pm$ 1 ^b (2.9 $\pm$ 2.0 ^b)	0 (0)
	21	13 $\pm$ 4 ^b (3.7 $\pm$ 3.3 ^c)	29 $\pm$ 3 (8.3 $\pm$ 3.5)	5 $\pm$ 2 ^b (1.2 $\pm$ 0.9 ^b)	0 (0)
	34	16 $\pm$ 7 ^b (13.4 $\pm$ 1.9 ^b)	28 $\pm$ 2 (24.3 $\pm$ 2.0)	10 $\pm$ 1 ^b (7.4 $\pm$ 1.4 ^b)	0 (0)
Lung	34	40 (12)	70 (38)	18 (5)	0 (0)

^a Data are presented as the mean percent positivity for the specific cell marker and the total number of cells per spleen (calculated by multiplying the percent positivity for that marker by the total cell yield per spleen; reported as 10^6 cells) $\pm$ standard deviations.

^b $P < 0.02$ compared with C1D value.

^c $P < 0.07$ compared with C1D value.

T cells is apparently modest and less than that of CD4 T cells in the mortality model in *nu/+* mice; while CD8 depletion is only partially maintained over the course of infection in that model, similar data were obtained in a comparison of C1D and C2D mice. (iii) The infection can resolve in beta 2-microglobulin-deficient mice without the presence of functional CD8 T cells. (iv) In beta 2-microglobulin-deficient mice, there was an increase in CD4 T-cell counts. (v) As supported in this study, one potential mechanism of action for CD8 T cells is the production of IFN- γ (16, 19), a role shared with CD4 T cells. The fact that CD8 T cells play a larger role in host defense, as determined by quantitative culture and antigen determination, in *nu/+* mice than in mortality is not completely explained. However, this observation is reproducible and probably due to the fact that data from day 14 are a snapshot of a period when T-cell activity is likely to be high and CD8 depletion is maximal, whereas the mortality data are averages of various host defense mechanisms over time, during which CD8 depletion apparently wanes. It was impossible to achieve total CD8 T-cell depletion and maintain it over the entire experiment, since in our experience the antibody becomes toxic to mice if given more frequently. It is also quite possible that CD8 T cells are maximally important early in infection in our model, while CD4 T cells are important in host defense over a longer period. This is supported by preliminary data showing that although total clearance of organisms is delayed in C2D mice compared with that in C1D mice, infection resolves in both strains of mice by day 50 postinfection (36a). Moreover, rechallenge after recovery from infection reveals that C2D mice are as susceptible as nonimmune mice. In contrast, recovered C1D or C57BL/6 mice are protected against rechallenge. Together, these results suggest that CD8 or CD4 T cells contribute to protection but that the predominant role resides in the CD4 cell population.

The *in vivo* model of T-cell depletion has been of value in examining the roles of CD4 and CD8 T cells in a variety of other models, including infections with *Toxoplasma gondii* (7), *Mycobacterium bovis* (26), *Listeria monocytogenes* (32), *Cryptococcus neoformans* (8, 13), and *Histoplasma capsulatum* (4). While the percent T-cell depletion in some studies which based the calculation on percent reduction compared with total spleen cells rather than splenic T cells, as is reported here, has appeared higher than in our study, the actual depletion was comparable and always less than complete. This provided a rationale for including the beta 2-microglobulin-deficient mouse studies. In addition, since the immunobiology of acquired T-cell deficiency may be different from that of congenital T-cell deficiency (such as in the C1D mouse), it is important to include both models.

The beta 2-microglobulin-deficient mouse has been useful in studies of a variety of infections, with an interesting variety of results. For example, in the intracellular *Leishmania* infection, the course of disease did not vary in beta 2-microglobulin-deficient animals (24). However, infection with *L. monocytogenes* (31) was contained but not resolved in the C1D mouse, with γ/δ T cells serving as a compensatory mechanism of host defense. In another study, C1D mice failed to clear Theiler's virus (6) and were more susceptible to *Trypanosoma cruzi* infection than controls (34) despite increased production of lymphokines. Interestingly, in vaccinated mice, NK cells were able to compensate for the lack of CD8 T cells in *T. cruzi* infection (5). In the case of malaria, treatment *in vivo* with antibody to CD8 exacerbated infection (36) but infection resolved in beta 2-microglobulin-deficient animals, again suggesting a compensatory mechanism in the latter. Although not investigated extensively, in our model neither the γ/δ T-cell- nor the NK

cell-dependent compensatory mechanism described above appeared to be important, in that depletion of γ/δ or NK cells did not exacerbate infection, at least at day 14. Perhaps surprisingly, in a model of tumor rejection, beta 2-microglobulin-deficient mice actually developed CD8 T cells (1, 17). We found no evidence of this in our model by FMF analysis of splenic or lung mononuclear cells. In the case of Sendai virus infection in beta 2-microglobulin-deficient mice, viral clearance was slower (10) but, as in our model, CD4 T cells seemed able to compensate in part for the lack of CD8 T cells (10, 11). For a model of lymphocytic choriomeningitis virus infection in beta 2-microglobulin-deficient mice, a similar CD4 T-cell-mediated compensatory mechanism has been described (23). Thus, the results with our C1D model are most consistent with those for the virus mouse models described above (10, 11, 23), with increased susceptibility but a blunted effect due to an apparent compensatory CD4 response. In those virus models, some of the CD4 cells appeared to have cytotoxic properties (11, 23). This remains to be investigated with our model, in which the most likely mechanism for CD4 compensation to date is CD4 cytokine generation.

The reason the host defense response that we have demonstrated in our model is heavily CD4 dependent while others have found a dominant CD8-dependent mechanism (2) is not clear. One reason may be the difference in infecting organisms (*C. psittaci* versus *C. trachomatis*), but a more important difference may be that our model is one of mucosally introduced infection while the other was intravenous (2). The balance of the CD4 versus CD8 response is likely to be influenced by the route of infection. Ramsey and Rank, using a model of secondary mucosal genital infection with MoPn in which protection was conferred by CD4- and CD8-enriched T-cell lines, came to a conclusion consistent with the data taken as a whole from this study (30). In their study, protection conferred by CD4 T cells was more efficient than that conferred by the CD8 line, but both played a role. These findings have been confirmed in models of primary genital infection using cloned CD4 or CD8 T cells (14, 15).

The fact that CD8 T cells play a role in host defense against MoPn is important in pathogenesis. Recently, it has become apparent that CD8 T cells can function by producing a variety of cytokines (16). Cytotoxicity is also an important function of CD8 T cells (reviewed in reference 16). CD4 cells might compensate for CD8 deficiency by this mechanism. In this regard, perforin, a cytolytic pore-forming protein that can perforate membranes in the presence of calcium ions, is expressed not only in all activated CD8 cytotoxic T cells but also in many CD4 killer T cells (27). CD8 cells may play an important immunologic role by being able to recognize *Chlamydia* antigen presented by major histocompatibility complex class I-bearing cells. The exact mechanisms by which CD8 cells function in our model are the subject of further investigation.

ACKNOWLEDGMENTS

This work was supported by the Edna McConnell Clark Foundation, by grants AI 22380 and AI 26328, and by the General Medical Research Service of the Veterans Administration.

We thank Elizabeth Pack for excellent technical assistance.

REFERENCES

1. Apasov, S. G., and M. V. Sitkovsky. 1994. Development and antigen specificity of CD8⁺ cytotoxic T lymphocytes in β_2 -microglobulin-negative, MHC class I-deficient mice in response to immunization with tumor cells. *J. Immunol.* **152**:2087–2097.
2. Buzoni-Gatel, D., L. Guillebeau, S. Bernard, T. Chardes, and A. Rocca. 1992. Protection against *Chlamydia psittaci* in mice conferred by LYT-2⁺ cells. *Immunology* **77**:284–288.

3. Byrne, G. I., B. Grubbs, T. J. Marshall, J. Schachter, and D. M. Williams. 1988. Gamma interferon-mediated cytotoxicity related to murine *Chlamydia trachomatis* infection. *Infect. Immun.* **56**:2023–2027.
4. Deepe, G. S. 1994. Role of CD8⁺ T cells in host resistance to systemic infection with *Histoplasma capsulatum* in mice. *J. Immunol.* **152**:3491–3500.
5. Denkers, E. Y., R. T. Gazzinelli, D. Martin, and A. Sher. 1993. Emergence of NK1.1⁺ cells as effectors of IFN- γ dependent immunity to *Toxoplasma gondii* in MHC class I-deficient mice. *J. Exp. Med.* **178**:1465–1472.
6. Fiette, L., C. Aubert, M. Brahic, and C. P. Rossi. 1993. Theiler's virus infection of β_2 -microglobulin-deficient mice. *J. Virol.* **67**:589–592.
7. Gazzinelli, R. T., F. T. Hakim, S. Hieny, G. M. Shearer, and A. Sher. 1991. Synergistic role of CD4⁺ and CD8⁺ T lymphocytes in IFN- γ production and protective immunity induced by an attenuated *Toxoplasma gondii* vaccine. *J. Immunol.* **146**:286–292.
8. Hill, J. O., and A. G. Harmsen. 1991. Intrapulmonary growth and dissemination of an avirulent strain of *Cryptococcus neoformans* in mice depleted of CD4⁺ or CD8⁺ T cells. *J. Exp. Med.* **173**:755–758.
9. Hiromatsu, K., Y. Yoshikai, G. Matsuzaki, S. Ohga, K. Muramori, K. Matsumoto, J. A. Bluestone, and K. Nomoto. 1992. A protective role of γ/δ T cells in primary infection with *Listeria monocytogenes*. *J. Exp. Med.* **175**:49–56.
10. Hou, S., P. Doherty, M. Zijlstra, R. Jaenisch, and J. M. Katz. 1992. Delayed clearance of Sendai virus in mice lacking class I MHC-restricted CD8⁺ T cells. *J. Immunol.* **149**:1319–1325.
11. Hou, S., M. Fishman, K. G. Murti, and P. C. Doherty. 1993. Divergence between cytotoxic effector function and tumor necrosis factor α production for inflammatory CD4⁺ T cells from mice with Sendai virus pneumonia. *J. Virol.* **67**:6299–6302.
12. Howard, L., N. S. Orenstein, and N. W. King. 1974. Purification on Renografin density gradients of *Chlamydia trachomatis* grown in the yolk sac of eggs. *Appl. Microbiol.* **27**:102–106.
13. Huffnagle, G. B., J. L. Yates, and M. F. Lipscomb. 1991. Immunity to a pulmonary *Cryptococcus neoformans* infection requires both CD4⁺ and CD8⁺ T cells. *J. Exp. Med.* **173**:793–800.
14. Igietseme, J. U., D. M. Magee, D. M. Williams, and R. G. Rank. 1994. Role for CD8⁺ T cells in antichlamydial immunity defined by chlamydia-specific T-lymphocyte clones. *Infect. Immun.* **62**:5195–5197.
15. Igietseme, J. U., K. H. Ramsey, D. M. Magee, D. M. Williams, S. A. Theus, T. J. Kincy, and R. G. Rank. 1993. Resolution of murine chlamydial genital infection by the adoptive transfer of a CD4⁺, TH1 lymphocyte clone. *Reg. Immunol.* **5**:311–317.
16. Kemeny, D. M., A. Nobel, B. J. Holmes, and D. Diaz-Sanchez. 1994. Immune regulation: a new role of the CD8⁺ T cell. *Immunol. Today* **15**:107–110.
17. Lamouse-Smith, E., V. K. Clements, and S. Ostrand-Rosenberg. 1993. $\beta_2M^{-/-}$ knockout mice contain low levels of CD8⁺ cytotoxic T lymphocytes that mediate specific tumor rejection. *J. Immunol.* **151**:6283–6290.
18. Landers, D. V., K. Erlich, M. Sung, and J. Schachter. 1991. Role of L3T4-bearing T-cell populations in experimental chlamydial salpingitis. *Infect. Immun.* **59**:3774–3777.
19. Locksley, R. M., S. L. Reiner, F. Hatam, D. R. Littman, and N. Kilean. 1993. Helper T cells without CD4: control of leishmaniasis in CD4-deficient mice. *Science* **261**:1448–1451.
20. Magee, D. M., J. U. Igietseme, J. G. Smith, C. A. Bleicker, J. Schachter, R. G. Rank, and D. M. Williams. 1993. *Chlamydia trachomatis* pneumonia in the severe combined immunodeficiency (SCID) mouse. *Reg. Immunol.* **5**:305–311.
21. Magee, D. M., D. M. Williams, E. J. Wing, C. A. Bleicker, and J. Schachter. 1991. Production of colony-stimulating factors during pneumonia caused by *Chlamydia trachomatis*. *Infect. Immun.* **59**:2370–2375.
22. Magee, D. M., and E. J. Wing. 1988. Cloned L3T4⁺ T lymphocytes protect mice against *Listeria monocytogenes* by secreting interferon- γ . *J. Immunol.* **141**:3203–3207.
23. Muller, D., B. H. Koller, J. L. Whitton, K. E. LaPan, K. K. Brigman, and J. A. Frelinger. 1992. LCMV-specific, class II-restricted cytotoxic T cells in beta 2-microglobulin-deficient mice. *Science* **255**:1576–1577.
24. Overath, P., and D. Harbecke. 1993. Course of leishmania infection in beta 2-microglobulin-deficient mice. *Immunol. Lett.* **37**:13–17.
25. Patton, D. L., S. A. Halbert, C.-C. Kuo, S.-P. Wang, and K. K. Holmes. 1983. Host response to primary *Chlamydia trachomatis* infection of the fallopian tube in pig-tailed monkeys. *Fertil. Steril.* **40**:829–833.
26. Pedrazzini, T., K. Hug, and J. A. Louis. 1987. Importance of L3T4⁺ and Lyt2⁺ cells in the immunologic control of infection with *Mycobacterium bovis* strain bacillus Calmette-Guerin in mice. *J. Immunol.* **139**:2032–2037.
27. Podak, E. R., and A. Kupfer. 1991. T cell effector functions: mechanisms for delivery of cytotoxicity and help. *Annu. Rev. Cell Biol.* **7**:479–504.
28. Poston, R. M., and R. J. Kurlander. 1991. Analysis of the time course of IFN- γ mRNA and protein production during primary murine listeriosis. *J. Immunol.* **146**:4333–4337.
29. Ramsey, K. H., and R. G. Rank. 1990. Role of T cell subpopulations in resolution of chlamydial genital infection, p. 241. *In* W. R. Bowie, H. D. Caldwell, R. P. Jones, P. A. Mardh, G. L. Ridgway, J. Schachter, W. E. Stamm, and M. E. Ward (ed.), *Chlamydia infections*. Cambridge University Press, Cambridge.
30. Ramsey, K. H., and R. G. Rank. 1991. Resolution of chlamydial genital infection with antigen-specific T-lymphocyte lines. *Infect. Immun.* **59**:925–931.
31. Roberts, A. D., D. J. Ordway, and I. M. Orme. 1993. *Listeria monocytogenes* infection in β_2 microglobulin-deficient mice. *Infect. Immun.* **61**:1113–1116.
32. Sasaki, T., M. Mieno, H. Udono, K. Yamaguchi, T. Usui, K. Hara, H. Shiku, and E. Nakayama. 1990. Roles of CD4⁺ and CD8⁺ cells, and the effect of administration of recombinant interferon gamma in listerial infection. *J. Exp. Med.* **171**:1141–1154.
33. Schachter, J., J. Moncada, C. R. Dawson, J. Sheppard, P. Courtright, M. E. Said, S. Zaki, S. F. Hafez, and A. Loinez. 1988. Nonculture methods for diagnosing chlamydial infection in patients with trachoma: a clue to the pathogenesis of the disease? *J. Infect. Dis.* **158**:1347–1352.
34. Tarleton, R. L., B. H. Koller, A. Latour, and M. Postan. 1992. Susceptibility of beta 2-microglobulin-deficient mice to *Trypanosoma cruzi* infection. *Nature (London)* **356**:338–340.
35. Thejls, H., J. Gnarp, O. Lundkvist, G. Heimer, G. Larsson, and A. Victor. 1991. Diagnosis and prevalence of persistent *Chlamydia* infection in infertile women: tissue culture, direct antigen detection, and serology. *Fertil. Steril.* **55**:304–310.
36. van der Heyde, H. C., D. D. Manning, D. C. Roopenian, and W. P. Weidanz. 1993. Resolution of bloodstage malarial infections in CD8⁺ cell-deficient beta 2-m^{0/0} mice. *J. Immunol.* **151**:3187–3191.
- 36a. Williams, D. Unpublished data.
37. Williams, D. M., G. I. Byrne, B. Grubbs, T. J. Marshall, and J. Schachter. 1988. Role in vivo for gamma interferon in control of pneumonia caused by *Chlamydia trachomatis* in mice. *Infect. Immun.* **56**:3004–3006.
38. Williams, D. M., J. Kung, and J. Schachter. 1986. Immunity to the mouse pneumonitis agent (murine *Chlamydia trachomatis*).p.465. *In* D. Oriel, G. Ridgway, J. Schachter, D. Taylor-Robinson, and M. Ward (ed.), *Chlamydia infections*. Cambridge University Press, Cambridge.
39. Williams, D. M., D. M. Magee, L. F. Bonewald, J. G. Smith, C. A. Bleicker, G. I. Byrne, and J. Schachter. 1990. A role in vivo for tumor necrosis factor α in host defenses against *Chlamydia trachomatis*. *Infect. Immun.* **58**:1572–1576.
40. Williams, D. M., and J. Schachter. 1985. Role of cell-mediated immunity in *Chlamydia* infection: implications for ocular immunity. *Rev. Infect. Dis.* **7**:754–759.
41. Williams, D. M., J. Schachter, J. J. Coalson, and B. Grubbs. 1984. Cellular immunity to the mouse pneumonitis agent. *J. Infect. Dis.* **149**:630–639.
42. Williams, D. M., J. Schachter, D. J. Drutz, and C. V. Sumaya. 1981. Pneumonia due to *Chlamydia trachomatis* in the immunocompromised (nude) mouse. *J. Infect. Dis.* **143**:238–241.
43. Williams, D. M., J. Schachter, and B. Grubbs. 1987. Role of natural killer cells in infection with the mouse pneumonitis agent (murine *Chlamydia trachomatis*). *Infect. Immun.* **55**:223–226.
44. Williams, D. M., J. Schachter, M. H. Weiner, and B. Grubbs. 1984. Antibody in host defense against mouse pneumonitis agent (murine *Chlamydia trachomatis*). *Infect. Immun.* **45**:674–678.
45. Zhong, G., E. M. Peterson, C. W. Czarniecki, R. D. Schreiber, and L. M. de la Maza. 1989. Role of endogenous gamma interferon in host defense against *Chlamydia trachomatis* infections. *Infect. Immun.* **57**:152–157.