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$CD4^+$ T Cells Play a Significant Role in Adoptive Immunity to *Chlamydia trachomatis* Infection of the Mouse Genital Tract

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The ability of $CD4^+$ and $CD8^+$ T cells to adoptively immunize mice against *Chlamydia trachomatis* infection of the mouse genital tract was studied. Adoptive transfer experiments were performed with splenic $CD4^+$ or $CD8^+$ T cells obtained from mice following resolution of a primary genital tract infection and after a secondary chlamydial challenge. The results show that donor $CD4^+$ T cells, but not $CD8^+$ T cells, obtained from mice following resolution of a primary infection or after secondary challenge were effective in transferring significant antichlamydial immunity to the genital tracts of naive animals. The lymphokine profiles in the culture supernatants of proliferating *Chlamydia*-specific $CD4^+$ T cells obtained from mice following resolution of a primary infection and after secondary challenge were assayed by an enzyme-linked immunoadsorbent assay. Protective $CD4^+$ T cells restimulated in vitro secreted interleukin 2, gamma interferon, and interleukin 6, lymphokine profiles characteristic of both Th1- and Th2-like responses. Resting $CD4^+$ T cells obtained from mice 4 months following resolution of a primary infection were also capable of conferring significant levels of adoptive protective immunity to naive mice. These findings support an important role for $CD4^+$ T cells in acquired immunity to chlamydial infection of the genital tract and indicate that protective $CD4^+$ immune responses in this model are relatively long lived.

Chlamydia trachomatis is an obligate intracellular bacterial pathogen that infects epithelial cells of the conjunctivae and genital tract mucosae. Oculogenital infections of humans caused by *C. trachomatis* are major causes of preventable blindness and sexually transmitted diseases worldwide (11, 17, 32). Immune intervention is a possible approach for the prevention and control of chlamydial disease, but despite considerable efforts to develop an effective chlamydial vaccine, there have not been significant advances toward this end. The paucity of progress in vaccine development can be attributed in part to an inadequate knowledge of immune mechanisms that are important in the development of acquired immunity to mucosal chlamydial infections.

It is clear that T-cell responses are an important component in protective immunity to chlamydial infection of the mouse. Rank et al. (30) have shown that athymic nude (nu^+/nu^+) mice fail to clear chlamydial infection of the genital tract. There is evidence supporting a role for both $CD4^+$ and $CD8^+$ T cells in protective immunity to chlamydial infection in the mouse. *Chlamydia*-specific $CD4^+$ and $CD8^+$ T-cell lines afford partial protection to infection following adoptive immunization (29). More recently, a class II-restricted $CD4^+$ T-cell clone (15) and a class I-restricted cytotoxic $CD8^+$ T-cell line (34) have been shown to confer partial protection against chlamydial infection following adoptive transfer to nude and naive mice, respectively. Although these findings indicate a potential role for both $CD4^+$ and $CD8^+$ T-cell subsets in protective immunity to chlamydial infection, they do not address a critical issue which is the actual contribution of these T-cell subsets in naturally acquired immune responses to chlamydial infection.

Adoptive immunity conferred by polyclonal T cells from immune animals is a powerful tool for defining the importance of T-cell subsets in acquired immunity to infection. This approach has been used successfully to study immunity to a number of intracellular parasites, the most notable being *Listeria monocytogenes* (3, 4, 18, 21, 25, 28). Surprisingly, there is a paucity of published reports on the use of adoptive transfer of polyclonal immune T cells in protection against chlamydial infection. Buzoni-Gatel et al. (5) showed that immune $CD8^+$ T cells but not $CD4^+$ T cells partially protected mice against *Chlamydia psittaci* infection of the spleen. However, there are no published reports describing protective immunity following adoptive immunization with polyclonal immune T cells or their subsets in animal models of chlamydial mucosal infection. Such models are likely more relevant for understanding acquired immunity to *C. trachomatis* infections of humans which are primarily associated with infection of mucosal surfaces.

The *C. trachomatis* murine biovar, mouse pneumonitis (MoPn), produces localized infection and disease of the mouse genital tract that closely parallels human infections caused by human *C. trachomatis* biovars (1, 26, 36). Mice infected vaginally with MoPn develop an acute self-limiting infection. Subsequent rechallenge is characterized by a significant decrease in chlamydial cervicovaginal shedding and a rapid resolution of infection. Thus, this animal model provides an excellent experimental system for the study of naturally acquired immunity to chlamydial genital tract infection.

In the present study, splenic $CD4^+$ and $CD8^+$ T cells were recovered from mice following resolution of primary chlamydial genital tract infection or following a secondary challenge, a time when mice exhibit significant protective immunity to chlamydial infection. These T-cell subsets were used in adoptive transfer studies to investigate the ability of either subset to protect naive mice against chlamydial genital tract infection. The results show that $CD4^+$, but not $CD8^+$, T cells were capable of conferring significant levels of protective immunity against *C. trachomatis* infection of the genital tract.

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MATERIALS AND METHODS

Chlamydiae. The MoPn strain was grown in HeLa 229 cells, and elementary bodies (EBs) were purified on discontinuous gradients of diatrizoate meglumine (Renographin 76; Squibb Diagnostics, New Brunswick, N.J.) as described previously (8). Infectivity of purified EBs was assayed by enumeration of chlamydial inclusion-forming units (IFUs) on monolayers of HeLa 229 cells grown in 24-well tissue culture plates. The same seed stock of MoPn was used throughout the study.

Mice. Female C57BL/10 mice were purchased from Jackson Laboratory (Bar Harbor, Maine). Mice were used for experimentation between 6 to 10 weeks of age.

Infection of mice. Mice received 2.5 mg of Depo-Provera (medroxy-progesterone acetate) (The Upjohn Co., Kalamazoo, Mich.) subcutaneously at 10 and 3 days prior to vaginal infection. Mice were infected by inoculating 5 μl of 250 mM sucrose–10 mM sodium phosphate–5 mM L-glutamic acid (pH 7.2) containing 1500 IFUs (100 50% infection doses [ID_{50}]) of MoPn into the vaginal vault. The course of infection was monitored by swabbing the vaginal vault with a Calgiswab (Spectrum Medical Industries, Los Angeles, Calif.) at various times postinfection and enumerating IFUs by culturing onto HeLa 229 cell monolayers. Inclusions were visualized by indirect immunofluorescence using the genus-specific monoclonal antibody EVI-H1 and fluorescence-labeled goat anti-mouse immunoglobulin G (IgG).

Isolation of T cells. T cells and their subsets were isolated from the spleens of mice following resolution of a primary infection and 10 days following a secondary challenge. Mice receiving secondary challenges were rechallenged 40 days after resolution of their primary infection. Resting T cells were isolated from the spleens of mice 4 months after resolution of their primary infection. Thus, donor T cells obtained at these time periods were considered to be representative of immune T cells that function in (i) resolution of a primary infection, (ii) resistance to reinfection, and (iii) memory T-cell responses. Mice were sacrificed, and their spleens were harvested. Single-cell suspensions were prepared from the spleens, and erythrocytes were lysed with 0.17 M Tris-buffered ammonium chloride. Splenocytes were resuspended in phosphate-buffered balanced salt solution (pH 7.2) containing 5% fetal calf serum. T cells, $CD4^+$ T cells, and $CD8^+$ T cells were isolated from splenocyte suspensions by negative selection using immunoaffinity chromatography. T cells and $CD4^+$ cells were isolated by using the IsoCell mouse T-cell and IsoCell mouse CD4 isolation kits (Pierce, Rockford, Ill.), respectively. $CD8^+$ T cells were isolated from splenocyte suspensions by immunoaffinity chromatography using Cellect plus mouse CD8 isolation kits (Biotex Laboratories, Inc., Edmonton, Alberta, Canada). Immunoaffinity isolation of T cells was performed as directed by the manufacturer.

Flow cytometric analysis. T cells, $CD4^+$ cells, and $CD8^+$ cells isolated by immunoaffinity chromatography were stained with fluorescein isothiocyanate-conjugated monoclonal antibodies specific to B cells (anti-B220, clone RA36B2), T cells (anti-Thy-1.2, clone 53-2.1), or CD8 (anti-Lyt-2, clone 169.4) or a phycoerythrin (PE)-conjugated anti-mouse CD4 antibody (anti-L3T4, clone GK1.5) and analyzed with a FACStar instrument (Becton Dickinson Immunocytometry Systems, San Jose, Calif.). A fluorescein isothiocyanate-conjugated mouse anti-rat kappa light-chain antibody (clone MAR 18.5) was used as a negative control. Each sample was collected in a list mode file of 10,000 events, and the results were plotted as histograms or contour plots showing fluorescence-staining intensity.

Adoptive transfer. Adoptive immunization against chlamydial challenge infection was done by inoculating naive recipient C57BL/10 mice intravenously (i.v.) with 10^7 T cells (one spleen equivalent), $CD4^+$ cells, or $CD8^+$ cells (two to three spleen equivalents for each T-cell subset). In pilot experiments, recipient C57BL/10 mice were infused with T cells or enriched $CD4^+$ T cells (10^7 cells per mouse) isolated from the spleens of nonimmune mice and then challenged vaginally with MoPn. Mice that received nonimmune T cells or $CD4^+$ T cells shed similar numbers of recoverable IFUs as infected naive animals. Therefore, in subsequent experiments, naive mice were used as negative controls. Groups of six to eight mice were used for each adoptive transfer experiment. Mice were challenged vaginally with 100 ID_{50} of MoPn 2 to 4 h prior to adoptive transfer. Protection was assessed by culturing chlamydiae from cervicovaginal swabs of adoptively immunized mice throughout the infection period.

Lymphokine assays. Chlamydial antigen-presenting cells were prepared from irradiated (3,000 rad) syngeneic splenocytes and heat-killed MoPn EBs as previously described (35). Immune $CD4^+$ T cells were isolated from the spleens of mice following resolution of a primary infection, following a secondary challenge, or 4 months following resolution of a primary infection. For these experiments, $CD4^+$ T cells were isolated from the spleens of six mice. Pooled $CD4^+$ T cells suspended in Dulbecco's modified Eagle medium (Life Technologies/BRL, Gaithersburg, Md.) supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), gentamicin (10 μg/ml), and 50 μM 2-mercaptoethanol were added to TC24 well plates (5×10^6 cells per well) containing 5×10^6 antigen-presenting cells. The plates were incubated at 37°C in 5% CO_2. Supernatant fractions were harvested at 24 and 72 h postincubation and stored in aliquots at −70°C until used. Interleukin 2 (IL-2), IL-4, IL-5, IL-6, and gamma interferon (IFN-γ) were measured by using an enzyme-linked immunoadsorbent assay (ELISA). Capture antibodies, detecting antibodies, and recombinant lymphokine standards were purchased from PharMingen (San Diego, Calif.). ELISA standard curves and assays were performed as described by the manufacturer. We observed that IL-2 levels declined over the 72-h culture period, whereas the levels of IFN-γ and IL-6 increased. Consequently, the results presented show the concentrations of IL-2 in 24-h supernatants and the concentrations of the remaining lymphokines in 72-h culture supernatants.

Statistical analysis. Analyses of the differences between mean IFU counts of control and experimental groups at various time intervals after adoptive transfer were performed by using Student's *t* test. Mean IFU values were log transformed prior to analysis to eliminate positive skewing of the variances.

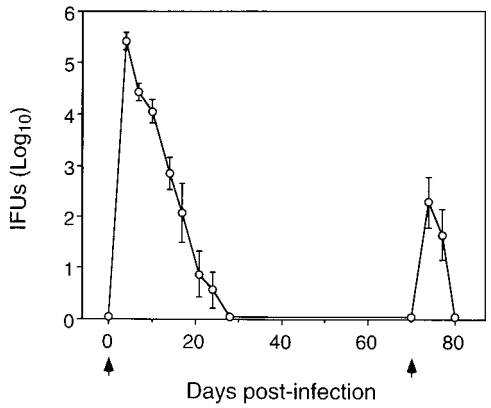

FIG. 1. Acquired immunity to chlamydial genital tract infection in the mouse. Ten mice were infected vaginally with 100 ID_{50} of MoPn and allowed to resolve their primary infection. The same animals were rechallenged vaginally with 100 ID_{50} of MoPn 40 days after resolution of the primary infection. These time points are designated by arrows. Mice were cultured throughout the infection period until all animals within the group became culture negative. The mean number of recoverable IFUs ± standard error of the mean is shown for each of the culture times postinfection. Secondary challenged mice shed significantly fewer chlamydiae from their genital tracts, and the infection was of much shorter duration than in animals experiencing a primary infection.

RESULTS

Chlamydial infection and immunity in the mouse genital tract. Since a primary objective of this work was to define the protective roles of $CD4^+$ and $CD8^+$ T cells in adaptive immunity to chlamydial genital tract infection, it was necessary to describe both the qualitative and quantitative aspects of infection and resistance to reinfection of mice from which donor T cells were to be used for subsequent study. Figure 1 shows the recovery of MoPn IFUs obtained from cervicovaginal cultures following a primary infection and following a secondary chlamydial challenge administered 40 days after the mice had resolved their primary infection. Mice infected vaginally with 100 ID_{50} of MoPn developed a self-limiting infection of approximately 4 weeks' duration, with peak chlamydial shedding (10^5 to 10^6 IFUs per mouse) occurring 3 to 7 days postinfection. In contrast, mice that had resolved a primary infection and were then rechallenged 40 days later exhibited infections of much shorter duration (7 to 10 days). Moreover, at the time of peak chlamydial shedding (day 3 postchallenge), rechallenged mice exhibited a marked reduction in recoverable infectious organisms (≤ 3 log_{10}). It is important to emphasize, however, that all mice given a secondary challenge were infected. Therefore, protective immunity in this model is not absolute. Nevertheless, these results demonstrate that mice develop a significant level of acquired immunity to chlamydial infection of the genital tract and support the use of this model for the study of protective cellular immunity to chlamydial infection.

Flow cytometry of T cells. Immunoaffinity-purified splenic T cells and their subsets were phenotyped immediately following their isolation and subsequently used for adoptive immunization of naive mice. A representative cytometric analysis of the

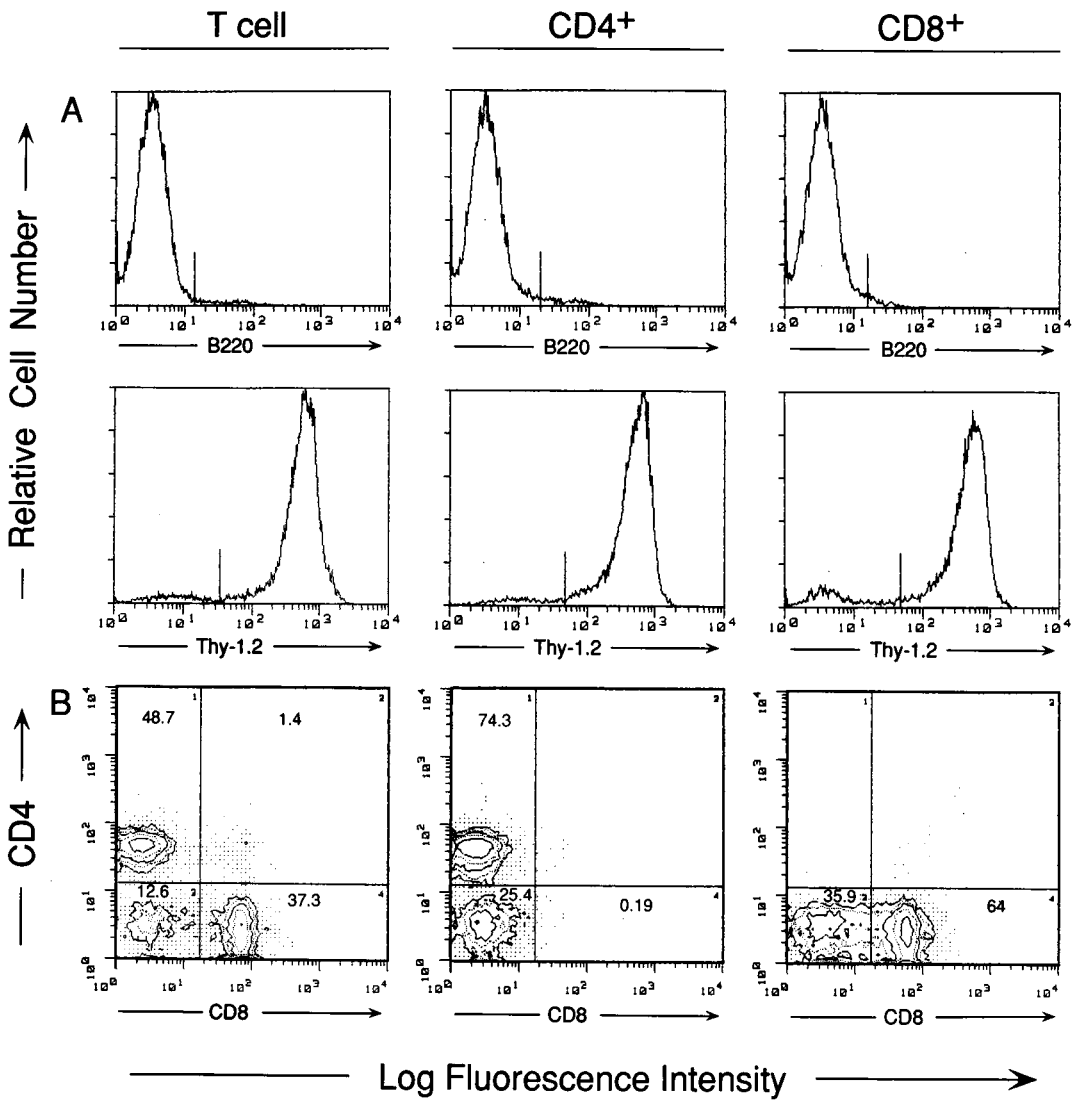

FIG. 2. Flow cytometry of T cells and their subsets used for adoptive immunization. (A) Single-color histograms of cells stained with anti-B-cell monoclonal antibody B220 (top) and with anti-T-cell antibody Thy-1.2 (bottom). (B) Contour plots of T cells analyzed for both $CD4^+$ and $CD8^+$ expression. Numbers refer to the percentage of total cells found in each gate. The cytometric profiles shown for $CD4^+$ and $CD8^+$ T-cell populations are representative of populations used in adoptive immunization throughout this study.

cell populations used in adoptive immunization experiments is shown in Fig. 2. Total splenic immune T-cell suspensions were 94% or greater T cells and contained less than 3% B cells. The enriched T cells were 49% $CD4^+$ and 37% $CD8^+$, with approximately 13% of the cells being both $CD4^-$ and $CD8^-$. Negatively selected immune $CD4^+$ and $CD8^+$ cells were 74 and 64% of the total T-cell population, respectively. Enriched $CD4^+$ and $CD8^+$ cells contained 0.2% or less contaminating $CD8^+$ and $CD4^+$ cells, respectively. Twenty to thirty percent of both the $CD4^+$ and $CD8^+$ populations did not stain with anti-CD4 or anti-CD8 antibodies. The phenotype of this double-negative population is unknown. Since distinct differences were observed in the protective capabilities of $CD4^+$ and $CD8^+$ populations in the adoptive immunization experiments described below, it is reasonable to conclude that this $CD4^-$ $CD8^-$ population had no significance in the outcome or interpretation of the experimental results described.

Adoptive transfer with donor T cells, $CD4^+$ cells, and $CD8^+$ cells obtained from mice following resolution of a primary chlamydial infection. Figure 3 shows the results of adoptive immunization of naive mice, using immune splenic T cells, $CD4^+$ cells, and $CD8^+$ cells obtained from donor mice following resolution of a primary genital tract infection. Mice were challenged 2 to 4 h prior to i.v. infusion of 10^7 T cells. The effect of adoptive immunization was evaluated by quantitating the number of MoPn IFUs recovered from cervicovaginal swabs taken from mice at various time periods after chlamydial challenge. At 4 days postchallenge, there was no significant difference in recoverable IFUs between control mice and mice that received immune T cells, $CD4^+$ cells, or $CD8^+$ cells. In contrast, mice that received donor $CD4^+$ T cells shed significantly less chlamydiae at 7 days postchallenge (≥ 1 $\log_{10}$) and at each of the subsequent culture periods thereafter. Donor T cells were less effective in adoptive immunization than enriched $CD4^+$ T cells. This result is consistent with the fact that the total T-cell population contained fewer *Chlamydia*-specific $CD4^+$ T cells. Immune $CD8^+$ T cells were not protective. Mice that received immune $CD8^+$ T cells shed numbers of infectious organisms similar to those of control mice throughout the infection period. These findings support a protective role for

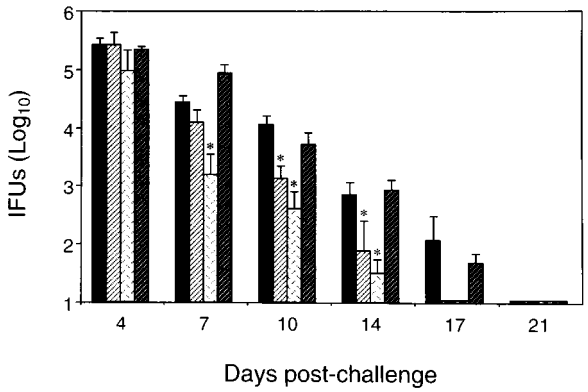

FIG. 3. Adoptive immunization using total T cells, $CD4^+$ cells, or $CD8^+$ cells isolated from mice following resolution of a primary infection. Donor T cells, $CD4^+$ cells, and $CD8^+$ cells were obtained from mice following resolution of a primary infection and were injected i.v. into syngeneic recipient mice. The mice were challenged vaginally with 100 ID_{50} of MoPn EBs 2 to 4 h prior to injection of T-cell populations. Mice were cultured at various time periods postchallenge, and the number of IFUs recovered from cervicovaginal swabs was determined at each culture period. ■, control; ▨, immune T cells; ▩, immune $CD4^+$ T cells; ▨, immune $CD8^+$ T cells. The data show the mean recoverable IFUs for each experimental group ± standard errors of the means. Student's *t* test was used to compare differences between control and experimental groups. Asterisks above bars indicate a significant difference in IFU recovery between naive mice and mice given immune T cells. *, $P < 0.05$.

$CD4^+$ T cells in the resolution of a primary chlamydial genital tract infection.

Adoptive transfer with donor T cells, $CD4^+$ cells, and $CD8^+$ cells obtained from mice following resolution of a secondary chlamydial infection. We next performed adoptive immunization studies using T cells, $CD4^+$ T cells, and $CD8^+$ T cells obtained from mice that were rechallenged with chlamydiae 40 days following resolution of a primary infection to determine the types of cells involved in acquired resistance to chlamydial infection. Adoptively transferred T cells and $CD4^+$ cells isolated from secondary challenged mice were capable of conferring significant levels of protective immunity to naive mice (Fig. 4). Mice adoptively immunized with $CD4^+$ T cells shed significantly less (1.5 to 2 $\log_{10}$) infectious chlamydiae at each

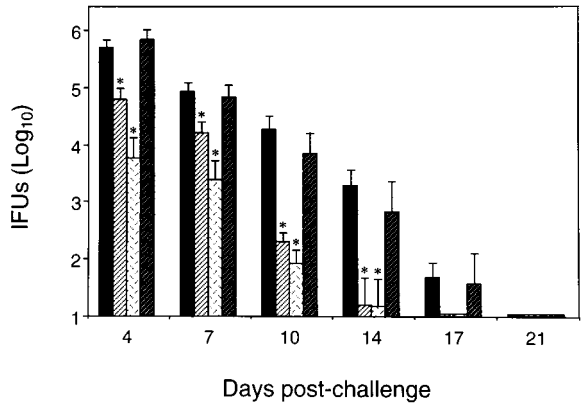

FIG. 4. Adoptive immunization using total T cells, $CD4^+$ cells, or $CD8^+$ cells isolated from mice following resolution of a secondary infection. Donor T cells, $CD4^+$ cells, and $CD8^+$ cells were isolated from the spleens of mice following resolution of a secondary chlamydial infection. T cells were then injected i.v. into syngeneic recipient mice that had been infected with MoPn. Mice were cultured for recoverable IFUs at various time periods following adoptive transfer. ■, control; ▨, immune T cells; ▨, immune $CD4^+$ T cells; ▩, immune $CD8^+$ T cells. *, $P < 0.05$.

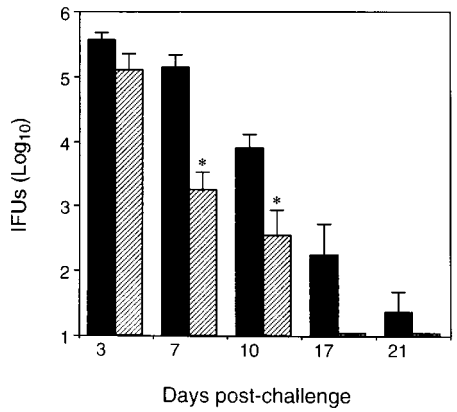

FIG. 5. Adoptive immunization using $CD4^+$ cells isolated from mice 4 months following resolution of a primary chlamydial infection. Donor $CD4^+$ T cells were injected i.v. into six syngeneic recipient mice. Mice were challenged with MoPn EBs and cultured for recoverable IFUs as described for Fig. 1. ■, control; ▨, immune $CD4^+$ T cells. *, $P < 0.05$.

of the culture periods postchallenge. Protection was observed in mice adoptively immunized with $CD4^+$ cells as early as day 4 postchallenge. Adoptively transferred $CD8^+$ T cells obtained from mice following secondary chlamydial challenge were not protective. These results indicate that $CD4^+$ T cells are the principal protective T-cell subset that functions in acquired resistance to chlamydial reinfection of the genital tract.

Adoptive transfer with primed resting $CD4^+$ T cells. It was important to determine the duration of $CD4^+$ T-cell-mediated immunity in this model. For these studies, mice that had resolved a primary genital tract infection were rested for 4 months and were then rechallenged vaginally or not challenged. Immunity was assessed in challenged mice by cervicovaginal culture, and it was found that these animals were still highly resistant to chlamydial infection (data not shown). Splenic $CD4^+$ T cells were isolated from the unchallenged groups of mice and used to adoptively immunize naive mice. Only $CD4^+$ T cells were used in these studies since they were the primary protective subset in the experiments described above. Rested immune donor $CD4^+$ T cells conferred significant protective immunity to naive mice following adoptive transfer (Fig. 5). Mice that received rested $CD4^+$ T cells shed 1.5 to 2 $\log_{10}$ fewer IFUs from day 7 and thereafter postchallenge. These findings demonstrate that *Chlamydia*-specific $CD4^+$ T-cell immunity in the mouse is long lived.

Antigen-specific lymphokine profiles of protective $CD4^+$ T cells. Murine $CD4^+$ T cells can be differentiated by the lymphokines that they secret following their activation (23). The production of IFN-γ and IL-2 is indicative of a Th1-like response, whereas the production of IL-4, IL-5, IL-6, or IL-10 reflects a Th2-like response (13, 31). It was of interest to analyze the antigen-specific lymphokine profiles of the immune $CD4^+$ T cells obtained from infected mice following resolution of a primary genital tract infection and after rechallenge in attempts to correlate specific lymphokine profiles or changes in profiles that might reflect functional differences in $CD4^+$ T-cell responses in immunity to chlamydial infection at these time periods. The antigen-specific induction of Th1- and Th2-like responses was investigated by quantifying the levels of lymphokines in the culture supernatants of proliferating immune and control $CD4^+$ T cells by ELISA (Fig. 6). Results are presented for IL-2, IFN-γ, and IL-6 only. IL-4 and IL-5 were also assayed in these experiments, but these lymphokines were undetectable (IL-5) or present at very low levels (IL-4) in 24-

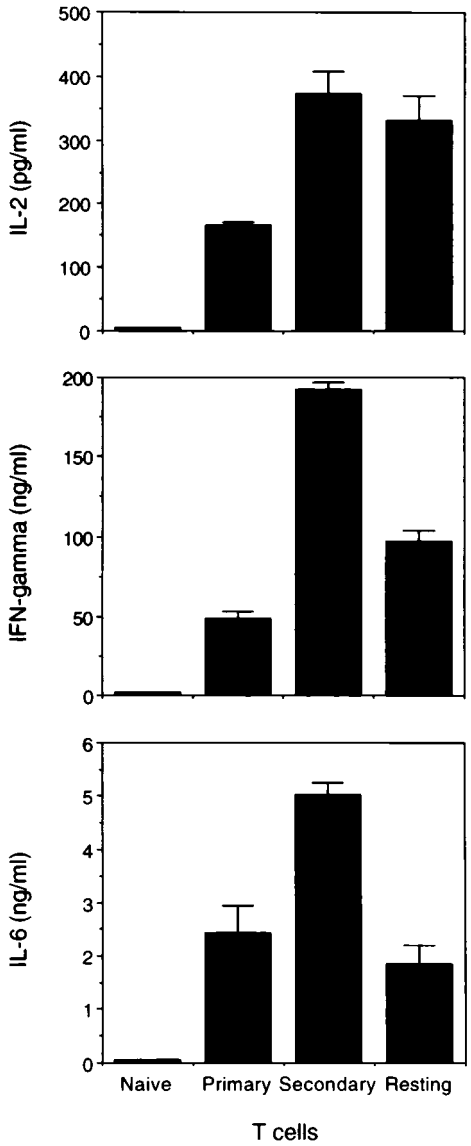

FIG. 6. Pattern of lymphokine secretion by *Chlamydia*-specific $CD4^+$ T cells isolated from mice following resolution of a primary infection, following a secondary challenge, or 5 months after a primary infection. The levels of IL-2, IFN-γ, and IL-6 in culture supernatants of proliferating $CD4^+$ T cells were assayed by ELISA as described in Materials and Methods. IL-2 levels shown were determined from 24-h culture supernatants. IFN-γ and IL-6 levels were assayed from 72-h culture supernatants. Primary, $CD4^+$ T cells obtained from mice after resolution of a primary infection; secondary, $CD4^+$ T cells obtained from mice 10 days following a secondary challenge; resting, $CD4^+$ T cells obtained from mice 4 months after a resolution of a primary infection. The culture supernatants were stored as aliquots, and ELISA was performed on individual replicate samples. The data shown are the mean values of triplicate replicate tests ± standard errors of the means.

and 72-h culture supernatants. Following resolution of a primary infection, $CD4^+$ cells restimulated in vitro secreted IL-2, IFN-γ, and IL-6, profiles indicative of both Th1- and Th2-like responses. Following secondary challenge, the pattern of lymphokine secretion by $CD4^+$ cells did not change; however, moderate increases in the levels all three lymphokines were evident compared with the levels in primary restimulated $CD4^+$ T cells. Lastly, the lymphokine profiles of resting restimulated $CD4^+$ T cells were similar to those of $CD4^+$ T cells obtained from mice following either a primary infection or a secondary chlamydial challenge. These lymphokine profiles are indicative of both $CD4^+$ Th1 and Th2 responses and implicate a functional role of both $CD4^+$ subsets in acquired protective immunity against chlamydial infection of genital tract mucosae.

DISCUSSION

In this study, we have used adoptive immunization to define the protective roles of $CD4^+$ and $CD8^+$ T cells in the murine model of *C. trachomatis* genital tract infection. We believe these findings to be important because they are the first to describe protective adoptive immunization studies using polyclonal T-cell populations obtained from mice following chlamydial genital tract infection and are therefore arguably more representative of in vivo responses than experiments with T-cell lines or clones. Moreover, we have performed adoptive immunizations using polyclonal T cells at times postinfection that correspond to immune responses that are operative in primary and secondary chlamydial infections. Cellular immune responses elicited following a secondary infection are important to vaccine development because they reflect adaptive antigen-specific responses that provide enhanced levels of protective immunity to chlamydial infection of the genital tract mucosae.

Our results show that splenic $CD4^+$ T cells obtained from mice following resolution of a primary chlamydial genital tract infection or after a secondary challenge were capable of conferring significant levels of protection against chlamydial genital tract infection to naive animals. In contrast, adoptively transferred $CD8^+$ T cells were not capable of conferring immunity in this model. We also showed that $CD4^+$ T cells isolated from mice 4 months after resolution of primary genital tract infection were capable of adoptively immunizing naive animals. Collectively, our findings support an important role for $CD4^+$ T cells in both the primary and secondary immune responses to chlamydial genital tract infection and indicate that protective $CD4^+$-mediated T-cell immunity in the mouse is relatively long lived.

Lymphokine profiles of protective activated *Chlamydia*-specific $CD4^+$ T cells taken from mice following a primary infection or secondary challenge or 4 months following resolution of a primary infection were indicative of both Th1- and Th2-like responses. It is not clear what the relative protective roles of these $CD4^+$ subsets are or how they might function in protection against chlamydial infection of the genital tract. The presence of locally activated $CD4^+$ Th1 cells secreting IFN-γ could function in protective immunity against chlamydial infection by a variety of mechanisms. It is well documented that IFN-γ inhibits chlamydial growth in vitro (6, 9, 20, 33). This inhibitory effect is mediated by the depletion of intracellular tryptophan, an amino acid essential for chlamydial growth (7), or by the induction of nitric oxide synthesis and other reactive nitrogen intermediates that possess antichlamydial activity in vitro (22). IFN-γ is a potent activator of macrophages (10, 19, 27), which are important effectors in cell-mediated immunity and could aid in the control of infection by phagocytosis and subsequent killing of ingested chlamydiae. Class II-restricted $CD4^+$ Th1-like cells that lyse *Mycobacterium* antigen-pulsed target cells have been recently described (24). It is therefore possible that *Chlamydia*-specific $CD4^+$ T cells possess cytolytic activity, a possibility that is supported by a recent report by Igietseme et al. (16) demonstrating that the antichlamydial activity of a MoPn-specific $CD4^+$ T-cell clone requires lymphoepithelial interactions. The most likely mechanism by which $CD4^+$ Th2 cells function in protective immunity against

chlamydial genital tract infection would be by providing cognate help to B cells for the production of *Chlamydia*-specific antibodies. Our findings show that *Chlamydia*-specific activated $CD4^+$ T cells secret IL-6. Murine responsive IL-6 B cells are present in Peyer's patches (2), and IL-6 selectively stimulates the production of IgA by a non-isotype-switching mechanism that is thought to involve terminal differentiation of IgA^+ B cells into plasma cells (37). Thus, the elevated levels of IL-6 produced by activated splenic $CD4^+$ T cells might reflect their helper function in the genital tract mucosae by providing cognate help to mucosal B cells for the production of *Chlamydia*-specific IgA.

It is apparent from the results presented here that splenic T cells are capable of conferring adoptive immunity to chlamydial infection in the genital tract. Since chlamydial infection in this model is restricted to the genital mucosal epithelium, these findings indicate that mucosally stimulated $CD4^+$ T cells migrated to and resided in the spleen. Moreover, our findings suggest that resident splenic $CD4^+$ T cells that originated from genital mucosal infection retain surface receptors important for their homing to and retention within the genital tract epithelium. Thus, splenic $CD4^+$ T cells appear to be representative of the T-cell immune response to chlamydial genital tract infection and therefore constitute an appropriate source of *Chlamydia*-specific T cells for future studies of cell-mediated immunity to chlamydial infection in this model.

The findings reported here corroborate and extend those of others (15, 29) showing that $CD4^+$ MoPn-specific T-cell lines or clones are capable of adoptively immunizing mice against chlamydial genital tract infection. However, the results of this study appear to be in contrast to reports demonstrating evidence for a protective role of $CD8^+$ T cells in immunity against chlamydial infection. Igietseme et al. (14) reported a *Chlamydia*-specific $CD8^+$ T-cell clone that was protective in the mouse genital tract infection model; however, it is evident from those studies that protection conferred by the $CD8^+$ clone was less efficient than that conferred by the $CD4^+$ clone (15). Other studies describing a protective role for $CD8^+$ T cells have assessed the protective capabilities of a class I-restricted $CD8^+$ T-cell line (34) or enriched $CD8^+$ polyclonal T cells (5) by adoptive transfer followed by i.v. challenge with chlamydiae and then monitoring infection in the spleen. It is not possible to directly compare their studies with those reported here since different infection routes and target organs were used; however, it is possible that $CD8^+$ T cells are important for protection against chlamydial infection of the spleen, where macrophages are the likely target cells of infection, while they are not as critical for protection in the genital tract, where epithelial cells are the primary host cells. A study by Rakhmilevich (28) provides evidence for a differential protective role of $CD4^+$ and $CD8^+$ *Listeria*-specific T cells for the liver and spleen, respectively. In that work, enriched $CD4^+$ and $CD8^+$ T cells were equally protective against *Listeria* infection of the liver, whereas $CD8^+$ T cells were significantly more efficient than $CD4^+$ T cells in transferring protection in the spleen. Moreover, a recent report by Harding and Pfeifer (12) provides evidence that macrophages and epithelial cells infected with *Salmonella typhimurium*, a bacterium like chlamydiae that does not escape from vacuolar compartments following phagocytosis, have different major histocompatibility complex (MHC) class I antigen-presenting capabilities. Infected macrophages were shown to mediate MHC class I recognition of processed peptide antigen to cytotoxic $CD8^+$ T cells whereas infected epithelial cells did not, leading these investigators to conclude that cytotoxic $CD8^+$ T cells may have a limited role in epithelial immunity in salmonellosis. Thus, it is possible that discordant results in ascertaining the protective roles of $CD4^+$ and $CD8^+$ T cells may reflect differences in models and target cells used to detect effector T-cell function. This may be particularly important in regard to the work reported here, in which protection was assessed in the genital tract mucosae, arguably a more relevant model with respect to medically important chlamydial infections of humans. It is formally possible that $CD8^+$ T cells provide an in vivo role in antichlamydial protection but cannot function independently of $CD4^+$ T-cell help. However, we believe this unlikely since no enhanced protection was noted in experiments in which unseparated immune T cells were used for adoptive immunization. It is more likely that the seclusion of chlamydial organisms in vesicles of epithelial cells eludes foreign proteins from entering the MHC class I antigen processing pathway, thus severely limiting the role of cytotoxic $CD8^+$ T cells in immunity to chlamydial infection. It is also possible that the inability of $CD8^+$ T cells to adoptively transfer protection against mucosal infection might be due to differences in the capacity of $CD4^+$ and $CD8^+$ splenic T cells to recirculate to the genital tract mucosa.

Defining the mechanisms by which $CD4^+$ T-cell subsets mediate protection against chlamydial infection of the genital mucosae and identification of chlamydial peptides complexed to MHC class II molecules recognized by protective $CD4^+$ T cells are logical future goals in studying cell-mediated immunity in the murine model. We have recently reported (35) on the use of murine bone marrow-derived macrophages as chlamydial antigen-presenting cells and defined experimental conditions that provide optimum presentation of processed chlamydial antigen to protective *Chlamydia*-specific $CD4^+$ T cells. It is possible that bone marrow-derived macrophages will be a useful source for the isolation of naturally processed chlamydial antigen from MHC class II molecules. Identification of naturally processed antigen bound to MHC molecules that are recognized by protective $CD4^+$ T cells would provide an important first step in the rational design of synthetic or recombinant-based chlamydial vaccine formulations.

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