In Situ Analysis of the Evolution of the Primary Immune Response in Murine Chlamydia trachomatis Genital Tract Infection

SANDRA G. MORRISON AND RICHARD P. MORRISON*

Department of Microbiology, Montana State University, Bozeman, Montana 59717

Received 12 October 1999/Returned for modification 13 December 1999/Accepted 18 January 2000

Adaptive immune responses contribute to the resolution of Chlamydia trachomatis genital tract infection and protect against reinfection, but our understanding of the mechanisms of those protective responses is incomplete. In this study, we analyzed by in situ immunohistochemistry the progression of the inflammatory and cytokine responses in the genital tracts of mice vaginally infected with C. trachomatis strain mouse pneumonitis. The cellular inflammatory response was characterized by an initial elevation in myeloid cells in the vagina (day 3) and uterine horns (day 7), followed by a marked rise in the number of T cells, predominantly CD4+ cells. CD8+ T cells and CD45R+B cells were also detected but were much less numerous. Perivascular clusters of CD4+ T cells, which resembled clusters of T cells seen in delayed-type hypersensitivity responses, were evident by 2 weeks postinfection. Following the resolution of infection, few CD8+ T cells and CD45R+B cells remained, whereas numerous CD4+ T cells and perivascular clusters of CD4+ T cells persisted in genital tract tissues. Interleukin-12 (IL-12)- and tumor necrosis factor alpha (TNF-α)-producing cells were observed in vaginal tissue by day 3 of infection and in uterine tissues by day 7. Cells producing IL-4 or IL-10 were absent from vaginal tissues at day 3 of infection but were present in uterine tissues by day 7 and were consistently more numerous than IL-12- and TNF-α-producing cells. Thus, the evolution of the local inflammatory response was characterized by the accumulation of CD4+ T cells into perivascular clusters and the presence of cells secreting both Th1- and Th2-type cytokines. The persistence of CD4+-T-cell clusters long after infection had resolved (day 70) may provide for a readily mobilizable T-cell response by which previously infected animals can quickly respond to and control a secondary infectious challenge.

In the murine model of Chlamydia trachomatis genital tract infection, intravaginal inoculation of C. trachomatis strain mouse pneumonitis (MoPn) produces an initial infection of vaginal and cervical epithelial cells, which progresses to involve the uterine horns and oviducts (1, 22, 24, 40). Animals generally resolve the infection and are culture negative by 4 weeks (22). The resolution of primary C. trachomatis genital tract infection in mice is dependent on T-cell-mediated immune responses. Genital infection of major histocompatibility complex class II-deficient gene knockout mice results in a chronic course of infection compared to that in normal immunocompetent mice (22). Furthermore, depletion of CD4+ T cells prior to infection (20) delays the resolution of infection, and the transfer of immune T cells or T-cell clones or lines confers a moderate level of protective immunity to naive recipients (3, 12, 31, 36). Conversely, chlamydia-specific antibodies are not needed to bring about the resolution of primary infection (14, 37) but may contribute to the protection of mice from reinfection (37).

Th1-type cytokines, such as interleukin-12 (IL-12) and gamma interferon (IFN-γ), are needed for the resolution of chlamydial infection. Mice treated with anti-IL-12 resolve primary infection more slowly than nontreated mice (25). Mice deficient in IFN-γ are unable to completely resolve genital tract infection, and chlamydial infection in those mice disseminates to systemic sites (5, 25). The importance of other cytokines and immunological mediators in protective immunity to primary chlamydial genital tract infection has been studied, but none are known to be as critical as IL-12 and IFN-γ in controlling and resolving primary chlamydial infection (25–27).

Our understanding of the systemic immune responses that contribute to the resolution of primary chlamydial genital tract infection has been broadened through the use of specific gene knockout mice. However, our knowledge of the evolution of the local immune response during the course of infection has not been thoroughly documented. Previous studies have examined to some extent the cellular and cytokine compositions of the local inflammatory response to chlamydial genital tract infection (13, 15, 24, 25, 27, 28, 41), and others have analyzed the systemic immune responses (16, 26, 27, 37). Various methodologies have been used in those studies to detect the presence or absence of cytokines, including detection of cytokine mRNA by reverse transcription-PCR in homogenates of genital tract tissue from infected mice or detection of cytokines in culture supernatants from antigen-stimulated splenic lymphocytes by enzyme-linked immunosorbent assay. However, to understand the antimicrobial properties of the adaptive immune response to chlamydial infection, it is important to gain an understanding of the local inflammatory responses elicited during the course of an infection that resolves without therapeutic intervention.

The purpose of the present study was to characterize the evolution of the local inflammatory response to chlamydial genital tract infection. In situ immunohistochemistry was used to depict changes in lineage-specific cell populations and the pattern of cytokine production in the murine genital tract during the course of chlamydial infection. Our results provide a foundation from which we can study the effects of experimen-
tally induced perturbations in the systemic immune response on the development of local (genital tract) immunity.

MATERIALS AND METHODS

Mice. Female C57BL/6J mice were purchased from the National Cancer Institute (Bethesda, Md.) and maintained in the animal facilities at Montana State University. Mice 8 to 12 weeks of age were used throughout the study.

Germ-free purification and enumeration of C. trachomatis. The MoPn strain of C. trachomatis was grown in HeLa 229 cells. Elementary bodies were purified and inclusion-forming units (IFU) were determined as described previously (4).

Antibodies. The following reagents were purchased from Pharmingen (San Diego, Calif.): (i) Staining of cell surface antigens. The MoPn strain of C. trachomatis MoPn as previously described (22). The course of infection in a group of nine mice was monitored by enumerating the number of IFU recovered from cervicovaginal swabs (Cultigam; Spectra Medical Industries, Los Angeles, Calif.) taken at various times following infection (22). Inclusions were visualized by indirect immunofluorescence using the MoPn-specific anti-major outer membrane protein monoclonal antibody Mo-33b and fluorescein isothiocyanate-labeled goat anti-mouse IgG (1:200). Because swabbing disrupted the vaginal and cervical mucosal epithelium, tissues from these mice were not used for immunohistochemical analysis. A separate group of 45 mice was infected concurrently, and their genital tracts were harvested and used for immunohistochemistry. At days 3, 7, 10, 14, 21, 28, 35, 42, and 70 following infection, five mice at each time point were sacrificed; the entire genital tract was removed, placed in embedding medium (OCT; Tissue-Tek; Sakura Finetek, Torrance, Calif.), snap frozen in dry ice-cooled 2-methylbutane, and stored at −85°C until sectioned and processed for immunohistochemistry. Because chlamydial inclusions were not performed on mice used for histological analysis, infection was confirmed either by staining genital tract tissues for chlamydial inclusions (days 3, 7, and 10 postinfection) (22).

RESULTS

In situ analysis of lineage-specific cells in genital tract tissue during the course of C. trachomatis infection. At various times following infection, animals were sacrificed and the entire genital tract (vagina, uterine horns, and oviducts) was removed and analyzed by immunohistochemistry for cell surface markers that define specific cell lineages. Cells of the myeloid lineage express cell surface molecules recognized by anti-CD11b, a phenotype shared by macrophages/monocytes and polymorphonuclear neutrophils (PMN) (21). Anti-Ly-6G reacts with a surface molecule on mature granulocytes (8). Anti-CD3e and anti-CD45R (B220) were used to identify T cells and B cells, respectively.

The course of chlamydial genital tract infection is shown in Fig. 1. Tissues were harvested from infected mice at various times following infection and correspond to different stages of infection. For example at the time of peak infection (day 7) lymphocytes accumulate in the uterine horns (Fig. 1C and F) and localized to the uterine lumen, mucosal epithelium, and lamina propria (Fig. 2E and F). Cells of the B-cell lineage (CD45R) were rare (Fig. 2H), and a few T cells (CD3e+ cells) (Fig. 2G) were found in the mucosal epithelium and submucosa. As infection progressed, cells began to infiltrate and accumulate in the uterine horns (Fig. 3). Ly-6G+ and CD11b+ positive cells were numerous by day 7 postinfection (Fig. 3E and F) and localized to the uterine lumen, mucosal epithelium, and lamina propria. As the infection resolved (3 to 4 weeks postinfection), the PMN population decreased (Fig. 3Q and R). T cells (CD3e+ cells) and B cells (CD45R+ cells) were rarely observed in uterine tissues until about day 7 postinfection, at which time they contributed significantly to the inflammatory infiltrate (Fig. 3G and H). T cells and B cells localized primarily to the lamina propria but were also occasionally

normal serum-0.1% saponin. Tissues were incubated with the Vastactin ABC reagent and developed as described above, except that all washes and incubation mixtures contained 0.1% saponin and the incubation with the ABC reagent was for 1 h.

Biotinylated, monoclonal polyclonal antibodies (anti-IL-12 and anti-TNF-α) were used for the detection of some cytokines. In those assays tissues were treated as described for the detection of cytokines with monoclonal antibodies, except that tissues were incubated for 2 h at room temperature in a humidified chamber with biotinylated primary antibody and no secondary antibody was used.

To ensure that all anticytokine antibodies would stain their respective cytokines under the conditions described above, positive control cells (MiCK-1, -2, and -3) (Pharmingen) were spread onto Super Frost slides, fixed, and stained by the procedures described above for cytokine staining. Positive control cell populations for TNF-α, and IFN-γ were MiCK-1 cells, those for IL-4 and IL-10 were MiCK-2 cells, and those for IL-12 were MiCK-3 cells.

Qualitative evaluation of genital tract inflammation. Tissue sections from four or five mice at each indicated time point postinfection were stained for cell surface antigens and cytokines as described above. Cell populations and subpopulations were enumerated by counting positive-staining cells in 10 high-power (40×) microscopic fields, an area of approximately 2.3 mm². Tissues were then assigned an inflammatory score for each cell surface phenotype, as follows: 0, <1 positive cells/mm²; 1, 1 to 50 positive cells/mm²; 2, 51 to 250 positive cells/mm²; 3, 251 to 500 positive cells/mm²; 4, >500 positive cells/mm² without evidence of cell clusters; and 5, >500 positive cells/mm² with cell clusters present. Cytokine-producing cells were enumerated by counting the number of positive-staining cells in 10 20× fields, an area of approximately 9.5 mm².

Downloaded from http://iai.asm.org/ on November 7, 2019 by guest
observed within the mucosal epithelium. CD3\(^+\) T cells were the predominant cell type during and following the resolution of infection (days 14 to 70).

An interesting feature of the T-cell inflammatory response in the uterus, and to some extent in the vagina, was the development of perivascular T-cell clusters. Clusters became apparent by day 21 postinfection (Fig. 3O) and were observed as late as 70 days postinfection (data not shown). B-cell clusters were less evident, but anti-CD11b also stained clusters of cells. Low-power magnification of immunostained tissues (Fig. 3U to X) clearly demonstrated the clustering of T cells throughout the uterine horns.

In the murine model of chlamydial genital tract infection, the oviducts are the primary site for inflammatory damage, which subsequently results in infertility. The cellular infiltrate of the oviducts following chlamydial infection was evaluated and found to be similar to that of uterine tissues (data not shown). Briefly, as infection ascended into the oviducts (approximately day 7 postinfection), PMN predominated in the luminal exudate and in mucosal and submucosal tissues. T cells and B cells accumulated as the course of infection progressed. Unlike that of uterine tissues, however, the cellular infiltrate of oviducts diminished greatly, and only a few scattered T cells were observed following the resolution of infection. Thus, fewer inflammatory cells remained in oviductal tissue, but as reported previously (22), tubal ectasia, loss of ciliated columnar epithelial cells, and hydrosalpinx were frequently observed.

In situ analysis of CD4 and CD8 T-cell subsets. Both CD4\(^+\) and CD8\(^+\) T cells have been implicated in the protective immune response to C. trachomatis genital tract infection (11, 22, 34–36). At 3 days following infection, CD4\(^+\) and CD8\(^+\) T cells were observed in the vaginal mucosal epithelium and lamina propria (Fig. 4C and D). By 7 days postinfection, the uterine mucosal epithelium and lamina propria contained CD4\(^+\) and CD8\(^+\) T cells (Fig. 5). CD4\(^+\)-T-cell clusters were evident by 14 to 21 days postinfection (Fig. 5C and D). Clusters were observed throughout the uterine horns, and, in general, clusters of CD4\(^+\) T cells were more numerous and were comprised of more cells than CD8\(^+\)-T-cell clusters (Fig. 5F and L).

The frequencies of cell populations in genital tract tissue during the course of infection are depicted in Fig. 6. Myeloid-lineage cells (CD11b) and CD3\(^+\) T cells were the predominant cell types in early infection (day 7), but T cells, and particularly CD4\(^+\) T cells, were the predominant cell type during the resolution (days 14 to 21) and following the resolution (days 28 to 70) of infection. The kinetics of B-cell infiltration into infected genital tract tissue was similar to that of T cells, but the number

![Image](http://iai.asm.org/Downloaded from http://iai.asm.org)
FIG. 3. Immunohistochemical staining of myeloid and lymphoid cell populations in uterine tissue. At weekly intervals uterine horns were harvested from chlamydia-infected mice and stained for CD11b, Ly6G, CD3e, or CD45R cell surface antigens. (A to D) Noninfected mice; (E to H) 7 days postinfection; (I to L) 14 days postinfection; (M to P) 21 days postinfection; (Q to T and U to X) 28 days postinfection. Anti-CD11b (A, E, I, M, Q, and U), anti-Ly6G (B, F, J, N, R, and V), anti-CD3e (C, G, K, O, S, and W), and anti-CD45R (D, H, L, P, T, and X) were used. Magnifications, ×300 (A to T) and ×60 (U to X). Representative tissues from four or five mice at each time point are shown.
of B cells never approached the level of T cells. The inflammatory infiltrate of the genital tract tissues diminished as infection resolved, but appreciable numbers of T cells remained localized to uterine tissue for at least 70 days postinfection.

**Cellular characteristics of the vagina and uterus following the resolution of genital tract infection.** Previous studies have shown that the adaptive immune responses which develop during the course of primary chlamydial infection confer a considerable degree of protection from a secondary infectious challenge (22). The protective response is characterized by the shedding of fewer chlamydiae and a much shortened course of infection. To determine the characteristics of the inflammatory cell response in genital tract tissue at a time when infection had resolved and animals demonstrated a level of immunity to reinfection, we examined vaginal and uterine tissues for various cell populations at 42 days following primary infection (2 weeks past the first culture-negative time point).

Considerable numbers of inflammatory cells remain localized to the vaginal and uterine tissues following the resolution of primary infection (Fig. 7). At 42 days following infection, moderate numbers of CD11b+ cells and CD3+ T cells were detected in the vagina. CD45R+ B cells were also detected but were less numerous than T cells. B cells and T cells were found to localize to both the lamina propria and mucosal epithelium. CD4+-T-cell clusters were evident, and CD8+ T cells were often observed to be localized to the mucosal epithelium. The uterus displayed a similar inflammatory picture; T cells pre-
Although single CD11b+ theilium and sparsely scattered throughout the lamina propria. IL-12- and TNF-α-producing cells throughout the course of infection and was not significantly different at any time point analyzed. Similar variability in the TNF-α response has been reported previously (6). In contrast, the number of IL-4- and IL-10-producing cells was greatest at 3 weeks postinfection, a time when infection is nearing resolution, and declined thereafter. However, even at 70 days postinfection the number of cytokine-producing cells in genital tract tissues was greater than that in naive mice.

Tissues were also probed for IL-1β, IL-2, IL-5, IL-6, and IFN-γ, but we were unable to convincingly demonstrate the production of those cytokines using immunohistochemical staining. Those data do not necessarily imply that the cytokines were not produced, but may instead simply reflect the limitations of the detection methodology. Our enumeration of IFN-γ-producing cells in genital tract tissues of infected mice was also confounded by the observation that immunoaffinity-purified anti-IFN-γ stained chlamydial inclusions quite intensely. The reason for the cross-reactivity of anti-IFN-γ with chlamydial inclusions is not understood, but because of that reactivity we were unable to confidently enumerate IFN-γ-producing cells.

FIG. 6. Characterization of the local cellular inflammatory response following genital tract infection with C. trachomatis. Uterine horns were harvested at various times postinfection, processed, stained, and enumerated as described in Materials and Methods. Bars represent the mean inflammatory score ± standard deviation in uterine tissues from groups of four or five mice at each time point.

dominated, and only scattered CD45R0 B cells and Ly6G+ PMN were observed. CD4+ T cells were scattered throughout the lamina propria and within clusters, whereas the less numerous CD8+ T cells were observed within the mucosal epithelium and sparsely scattered throughout the lamina propria. Although single CD11b+ cells were present in the uterine tissue at this time, a more dominant feature was the diffuse CD11b staining of regions that appeared to be near perivascular clusters of lymphocytes.

Detection of cytokine-producing cells in the genital tracts of infected mice. To determine the kinetics of cytokine production and the location of cytokine-producing cells during genital tract infection, vaginal and uterine tissues were analyzed for cells producing specific cytokines at various times throughout the course of infection (Fig. 8; Table 1). Cells producing IL-12 or TNF-α were detected in vaginal tissues by day 3 postinfection (Fig. 8); however, neither IL-4- nor IL-10-producing cells were detected at that time. At 7 days postinfection, cytokine-producing cells were evident in uterine tissue (Table 1). The individual variation in the number of IL-12- and TNF-α-producing cells also occurred during the course of infection and was not significantly different at any time point analyzed. Similar variability in the TNF-α response has been reported previously (6). In contrast, the number of IL-4- and IL-10-producing cells was greatest at 3 weeks postinfection, a time when infection is nearing resolution, and declined thereafter. However, even at 70 days postinfection the number of cytokine-producing cells in genital tract tissues was greater than that in naive mice.

Tissues were also probed for IL-1β, IL-2, IL-5, IL-6, and IFN-γ, but we were unable to convincingly demonstrate the production of those cytokines using immunohistochemical staining. Those data do not necessarily imply that the cytokines were not produced, but may instead simply reflect the limitations of the detection methodology. Our enumeration of IFN-γ-producing cells in genital tract tissues of infected mice was also confounded by the observation that immunoaffinity-purified anti-IFN-γ stained chlamydial inclusions quite intensely. The reason for the cross-reactivity of anti-IFN-γ with chlamydial inclusions is not understood, but because of that reactivity we were unable to confidently enumerate IFN-γ-producing cells.

DISCUSSION

Previous studies of the primary immune response to C. trachomatis genital tract infection have utilized methods that disrupt the genital tract tissue to enumerate infiltrating cell populations or to determine the pattern of cytokine production by infiltrating cells (25, 27, 41). The in vitro production of cytokines by antigen-stimulated splenic lymphocytes has also been used to define the systemic cellular immune response following primary chlamydial genital tract infection (27, 37–39). In recent studies, the molecules that traffic lymphocytes to genital tract tissue following chlamydial infection have been defined using in situ immunohistochemical staining for cell surface antigens and for the production of intracellular cytokines. Our intent was to identify and characterize changes in the local inflammatory and cytokine responses induced during the course of chlamydial genital tract infection.

The influx of hematopoietic cells into the vagina at day 3 of infection, and into the uterus at day 7 of infection, correlated with a large increase in cells of the myeloid lineage (CD11b+ cells). Although we did not distinguish unequivocally between PMN and macrophages, Ly6G is a cell surface antigen found predominantly on PMN (8), and previous studies using hematoxylin and eosin staining of infected genital tract tissue have indicated that the early inflammatory cellular infiltrate is comprised primarily of PMN (22). The mere presence of PMN at the site of infection is not sufficient to resolve infection, however. For example, mice deficient in T-cell responses do not resolve infection even though a marked PMN inflammatory response is evident (22), and in vivo depletion of PMN with anti-Ly6G only slightly prolongs the course of chlamydial infection (2). It is not known if that modest effect on chlamydial shedding induced by PMN depletion results from decreased killing of chlamydiae by PMN or from the loss of mediators produced by PMN that direct lymphoid cells to the site of infection. PMN also comprised the predominant cell type in the vaginal and uterine luminal exudates, and cells of the lymphoid lineage were rarely observed in the lumens. Thus, the analysis of vaginal washes for specific cytokines might not necessarily reflect the predominant cytokines produced during infection but instead may provide information only on those
cytokines released by cells of the exudate (PMN) or on mediators released by epithelial cells.

Changes in the cellular composition of infected genital tract tissues were determined throughout the course of infection. In addition to measuring the myeloid cells, the influx of T and B cells was monitored. By 7 days postinfection, cells of the lymphoid lineage had increased significantly in vaginal and uterine tissues. The delay in lymphoid cell expansion is not surprising, since signals from myeloid cells are necessary for their activation. The accumulation of CD3ε⁺ cells (T cells) and CD45R/B220⁺ cells (B cells) into genital tract tissue was very rapid and thus was unlikely to result from the in situ expansion of antigen-specific cells. Instead, the increased number of lymphocytes probably resulted from the influx of non-antigen-specific cells. T cells outnumbered B cells throughout the course of infection, and CD4⁺ T cells were more numerous than CD8⁺ cells at all times evaluated. Consistent with the findings of others (28), T cells were predominantly T-cell receptor αβ positive, and T-cell receptor γδ-positive cells were only rarely observed (data not shown).

CD45R/B220 and CD19 are B-cell lineage differentiation antigens expressed on the surface of B lymphocytes from the pro-B-cell through the mature B-cell stage (10, 19). Both CD45R/B220 and CD19 have been used as pan-B-cell markers, although CD19 expression is reported to be more restricted to the B-cell lineage (18). Neither CD45R nor CD19 is found on plasma cells. In our initial studies we attempted to use anti-CD19 antibody to identify cells of the B-lymphocyte lineage. Although we found that splenic B cells were visualized using anti-CD19 antibody, only a few weakly staining cells were observed in genital tract tissues from infected mice (data not shown). However, numerous cells were easily visualized when anti-CD45R/B220 was used as a marker for B cells. We have interpreted the CD45R/B220⁺ cells as representing cells of the B-lymphocyte lineage, even though we were unable to confirm those results using anti-CD19. Those disparate results may simply reflect differences in the utility of the antibodies in immunohistochemistry and may not have occurred if a different detection methodology, such as fluorescence-activated cell sorting was used for these studies. However, because splenic B cells stained with anti-CD19 antibody under identical experimental conditions, our results may indicate the presence of a CD45R⁺ CD19⁻ cell population in chlamydia-infected genital tract tissues. Natural killer (NK) cells do not express CD19 or other B-cell markers but have been reported to express CD45R (33). The CD45R-positive cells in genital tract tissue may therefore represent a population of NK cells. Our attempts to stain genital tract tissues for NK cells using other NK cell markers (anti-NK1.1 or anti-asialo-GM1) were unsuccessful. Alternatively, the CD45R/B220-staining cells may represent a population of activated T cells (42). At this time we know only that a population of CD45R/B220⁺ cells infiltrate genital tract tissue following chlamydial infection and that few cells expressing the B-cell lineage CD19 cell surface antigen are present. If this CD45R⁺ population of cells does represent an NK cell or activated T-cell population, then quite possibly those cells might contribute to the resolution of intracellular chlamydial infection. Additional studies using other methods...
to separate cell populations (e.g., fluorescence-activated cell sorting) will be needed to address that question.

A hallmark of both ocular (trachoma) and urogenital chlamydial infections is the development of lymphoid follicles (7, 9, 17, 23, 32, 43). The follicles present in children with active trachoma appear to have germinal centers composed of B cells (7), but in adults with conjunctival scarring, the follicles lack germinal centers and T cells (CD4^+^) are much more numerous than B cells (32). The composition of follicles that develop during chlamydial genital tract infection is less well characterized (9, 13, 17, 23, 43). The mechanism(s) of follicular hyperplasia has not been elucidated, but it may hold important clues to the adaptive immune responses that confer immune protection and/or the pathogenetic immune responses that are thought to contribute to the severe sequelae of chlamydial infection. Although typical lymphoid follicles (i.e., with the architecture of follicles found in primary or secondary lymphoid tissues) were not observed in the genital tract tissues of infected mice, perivascular lymphocyte clusters were easily discernable (Fig. 3, 5, and 7). Clusters were composed predominantly of CD3e^+^ cells and CD4^+^ cells, but much smaller clusters of CD45R^B^- B cells and CD8^+^ T cells were also present. The genitourinary tract, as well as the skin and the pulmonary and gastrointestinal tracts, are the most immunologically active tertiary lymphoid sites. The clusters of T cells that develop during chlamydial genital tract infection are reminiscent of clusters of T cells that accumulate in the dermis following a delayed-type hypersensitivity reaction (30). T-cell clusters were not apparent until about 10 to 14 days following genital tract infection (Fig. 3 and 5), and some clusters remained for as long as 70 days postinfection (data not shown). In a recent study we demonstrated that the level of protective immunity that deve-
producing cells in genital tract tissue of infected mice, compared to results of other investigators who have failed to detect IL-4 transcripts from infected genital tract tissue or IL-4 protein by enzyme-linked immunosorbent assay of supernatants of chlamydia-stimulated T cells (25, 29, 38, 41). Our staining appears to be specific, because we used monoclonal antibodies and we did not experience false-positive reactions due to incompletely blocked endogenous peroxidase activity. Thus, the discrepancy may be due to differences in detection methodologies or in the tissue preparation. Nevertheless, by immunohistochemistry, considerable numbers of IL-4-producing cells contribute to the cellular infiltrate in genital tract tissue of chlamydia-infected mice.

Clearly, the Th1-type T-cell response contributes importantly to the resolution of intracellular infection, and studies generally do not support an essential role for a Th2-type T-cell response. However, we previously reported that antibody-deficient gene knockout mice were more susceptible to reinfection, and we attributed that susceptibility to the lack of antichlamydial antibody (37). Thus, it may be of interest to further investigate the role of IL-4 and IL-10 in the development of Th2-type antichlamydial immune responses and of specific antibody in acquired resistance to chlamydial genital tract infection.

ACKNOWLEDGMENT

This work was supported by grant AI-38991 from the National Institutes of Health.

REFERENCES

1. Barron, A. L., H. J. White, R. G. Rank, B. L. Soloff, and E. B. Moses. 1981. A new animal model for the study of Chlamydia trachomatis genital infections: infection of mice with the agent of mouse pneumonitis. J. Infect. Dis. 143:63–66.
2. Barteneva, N., I. Theodore, E. M. Peterson, and L. M. de la Maza. 1996. Role of neutrophils in controlling early stages of a Chlamydia trachomatis infection. Infect. Immun. 64:4830–4835.
3. Cain, T. K., and R. G. Rank. 1995. Local Th1-like responses are induced by intravaginal infection of mice with the mouse pneumonitis biovar of Chlamydia trachomatis. Infect. Immun. 63:1784–1789.
4. Caldwell, H. D., J. Kromhout, and J. Schachter. 1981. Purification and partial characterization of the major outer membrane protein of Chlamydia trachomatis. Infect. Immun. 31:1161–1176.
5. Cotter, T. W., K. H. Ramsey, G. S. Miranpur, C. E. Poulsen, and G. I. Byrne. 1997. Dissemination of Chlamydia trachomatis chronic genital tract infection in gamma interferon gene knockout mice. Infect. Immun. 65:2145–2152.
6. Darville, T., C. W. Andrews, Jr., K. K. LaFon, W. Shymasani, L. R. Kishen, and R. G. Rank. 1997. Mouse strain-dependent variation in the course and outcome of chlamydial genital tract infection is associated with differences in host response. Infect. Immun. 65:3065–3073.
7. el-Asrar, A. M., J. J. van den Oord, K. Geboes, L. Missotten, M. H. Emarah, and V. Desmet. 1989. Immunopathology of trachomatous conjunctivitis. Br. J. Ophthalomol. 73:276–282.
8. Fleming, J. T., M. L. Fleming, and T. R. Malek. 1993. Selective expression of Ly6G on myeloid lineage cells in mouse bone marrow. J. Immunol. 151:2399–2408.
9. Hare, M. J., E. Toone, D. Taylor-Robinson, R. T. Evans, P. M. Furr, P. Cooper, and J. K. Oates. 1981. Follicular cervices: colposcopic appearances and associations with Chlamydia trachomatis. Br. J. Obstet. Gynaecol. 88:174–180.
10. Hatfield, K. S., H. Hirano, S. Murakami, and R. J. Hodes. 1992. CD45 expression by B cells. Expression of different CD45 isoforms by subpopulations of activated B cells. J. Immunol. 149:2286–2294.
11. Ijigianometric, 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.
12. Ijigianometric, J. U., K. H. Ramsey, D. M. Magee, D. M. Williams, T. J. Kinca, and R. G. Rank. 1993. Resolution of murine chlamydial genital infection by the adoptive transfer of a biovar-specific, Th1 lymphocyte clone. Regional Immunol. 5:317–324.
13. Johannson, M., K. Schon, M. Ward, and N. Lycke. 1997. Genital tract infection with Chlamydia trachomatis fails to induce protective immunity in gamma interferon receptor-deficient mice despite a strong local immunoglobulin A response. Infect. Immun. 65:1032–1044.
14. Johannson, M., M. Ward, and N. Lycke. 1997. B-cell-deficient mice develop complete immune protection against genital tract infection with Chlamydia
15. Kelly, L. A., and R. G. Rank. 1997. Identification of homing receptors that mediate the recruitment of CD4 T cells to the genital tract following intravaginal infection with Chlamydia trachomatis. Infect. Immun. 65:5198–5208.

16. Kelly, L. A., E. A. Robinson, and R. G. Rank. 1996. Initial route of antigen administration alters the T-cell cytokine profile produced in response to the mouse pneumonitis biovar of Chlamydia trachomatis following genital infection. Infect. Immun. 64:4976–4983.

17. Kiviat, N. B., J. A. Pavaonen, P. Wolner-Hanssen, C. W. Critchlow, W. E. Stamm, J. Douglas, D. A. Eschenbach, L. A. Corey, and K. K. Holmes. 1990. Histopathology of endocervical infection caused by Chlamydia trachomatis, herpes simplex virus, trichomonas vaginalis, and Neisseria gonorrhoeae. Hum. Pathol. 21:831–837.

18. Krop, I. A. R. de Fougerolles, R. R. Hardy, M. Allison, M. S. Schlissel, and D. T. Fearon. 1996. Stimulation of monocyte Mac-1 and p150,95 adhesion proteins from an intracellular vesicular compartment to the cell surface. J. Clin. Invest. 100:535–544.

19. Krop, I. A. R. de Fougerolles, D. T. Fearon, and M. S. Schlissel. 1996. The signaling activity of CD19 is regulated during B cell development. J. Immunol. 157:48–56.

20. Landers, D. V., K. Erlich, M. Sung, and J. Schachter. 1991. Role of L3T4-bearing T-cell populations in experimental murine chlamydial salpingitis. Infect. Immun. 59:3774–3777.

21. Miller, L. J., D. F. Bainton, N. Borregaard, and T. A. Springer. 1987. Stimulation of adhesion and T-cell proliferation by monocyte Mac-1 and p150,95 adhesion proteins from an intracellular vesicular compartment to the cell surface. J. Clin. Invest. 80:535–544.

22. Morrison, R. P., K. Feilzer, and D. B. Tumas. 1995. Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in Chlamydia trachomatis genital tract infection. Infect. Immun. 63:1390–1400.

23. Pavaonen, J., E. Vesterinen, B. Meyer, and E. Saksela. 1982. Colposcopic and histologic findings of cervical chlamydial infection. Obstet. Gynecol. 59:712–715.

24. Patton, D. L., D. V. Landers, and J. Schachter. 1989. Experimental Chlamydia trachomatis salpingitis in mice: initial studies on the characterization of the leukocyte response to chlamydial infection. J. Infect. Dis. 159:1105–1110.

25. Perry, L. L., K. Feilzer, and H. D. Caldwell. 1997. Immunity to Chlamydia trachomatis is mediated by T helper 1 cells through IFN-γ-dependent and -independent pathways. J. Immunol. 158:3344–3352.

26. Perry, L. L., K. Feilzer, and H. D. Caldwell. 1998. Neither interleukin-6 nor inducible nitric oxide synthase is required for clearance of Chlamydia trachomatis from the murine genital tract epithelium. Infect. Immun. 66:1265–1269.

27. Perry, L. L., K. Feilzer, S. Hughes, and H. D. Caldwell. 1999. Clearance of Chlamydia trachomatis from the murine genital mucosa does not require perforin-mediated cytolysis or Fas-mediated apoptosis. Infect. Immun. 67:1379–1385.

28. Perry, L. L., K. Feilzer, J. L. Portis, and H. D. Caldwell. 1998. Distinct homing pathways direct T lymphocytes to the genital and intestinal mucosae in Chlamydia-infected mice. J. Immunol. 160:2905–2914.

29. Perry, L. L., H. Su, K. Feilzer, R. Messer, S. Hughes, W. Whitmore, and H. D. Caldwell. 1999. Differential sensitivity of distinct Chlamydia trachomatis isolates to IFN-γ-mediated inhibition. J. Immunol. 162:3541–3548.

30. Picker, L. J., and M. H. Siegelman. 1999. Lymphoid tissues and organs. p. 479–531. In W. E. Paul (ed.), Fundamental immunology, 4th ed. Lippincott-Raven Publishers, Philadelphia, Pa.

31. Ramsey, K. H., and R. G. Rank. 1991. Resolution of chlamydial genital infection with antigen-specific T-lymphocyte lines. Infect. Immun. 59:925–931.

32. Reacher, M. H., J. Pe’er, P. A. Rapoza, J. A. Whittem-Hudson, and H. R. Taylor. 1991. T cells and trachoma: their role in cicatricial disease. Ophthalmology 98:334–341.

33. Rolink, A., E. ten Broeke, F. Melchers, D. T. Fearon, I. Krop, and J. Andersson. 1996. A subpopulation of B220+ cells in murine bone marrow does not express CD19 and contains natural killer cell progenitors. J. Exp. Med. 183:187–194.

34. Starnbach, M. N., M. J. Bevan, and M. F. Lampe. 1995. Murine cytotoxic T lymphocytes induced following Chlamydia trachomatis intraperitoneal or genital tract infection respond to cells infected with multiple serovars. Infect. Immun. 63:3277–3280.

35. Starnbach, M. N., M. J. Bevan, and M. F. Lampe. 1994. Protective cytotoxic T-lymphocytes are induced during murine infection with Chlamydia trachomatis. J. Immunol. 153:5183–5189.

36. Su, H., and H. D. Caldwell. 1995. CD4+ T cells play a significant role in adoptive immunity to Chlamydia trachomatis infection of the mouse genital tract. Infect. Immun. 63:3302–3308.

37. Su, H., K. Feilzer, H. D. Caldwell, and R. P. Morrison. 1997. Chlamydia trachomatis genital tract infection of antibody-deficient gene knockout mice. Infect. Immun. 65:1993–1999.

38. Su, H., R. Messer, W. Whitmore, E. Fischer, J. C. Portis, and H. D. Caldwell. 1998. Vaccination against chlamydial genital tract infection after immunization with dendritic cells pulsed ex vivo with nonviable Chlamydiae. J. Exp. Med. 188:809–818.

39. Watanabe, Y., and T. Akaike. 1994. The signaling pathways of B1 lymphocytes is dependent on CD19. J. Exp. Med. 183:422–428.

40. Andersson. 1991. T cells and trachoma: their role in cicatricial disease. Ophthalmology 98:334–341.

41. Tseng, C.-T. K., and R. G. Rank. 1994. Activation signal induces the expression of B cell-specific CD45R epitope (6B2) on murine T cells. Scand. J. Immunol. 39:535–544.

42. Swenson, C. E., E. Donegan, and J. Schachter. 1983. Chlamydia trachomatis-induced salpingitis in mice. J. Infect. Dis. 148:1101–1107.

43. Watanabe, Y., and T. Akaike. 1994. Activation signal induces the expression of B cell-specific CD45R epitope (6B2) on murine T cells. Scand. J. Immunol. 39:419–425.

44. Winkler, B., and C. Crum. 1984. Chlamydia trachomatis infection of the female genital tract. Pathogenic and clinicopathologic correlations. Pathol. Ann. 5:193–221.