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Local Th1-Like Responses Are Induced by Intravaginal Infection of Mice with the Mouse Pneumonitis Biovar of *Chlamydia trachomatis*

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A critical role for cell-mediated immunity (CMI) has been demonstrated for effecting the resolution of genital infections of mice infected intravaginally with the mouse pneumonitis biovar of *Chlamydia trachomatis* (MoPn). However, little is known about expression of CMI in the murine genital tract. The mouse MoPn model was used to examine CMI responses in the genital tract and associated lymph nodes during the course of infection. MoPn-specific lymphocytes were present in the genital mucosa, with the maximum level of proliferation in response to MoPn at 3 weeks postinfection. MoPn-stimulated cells secreting gamma interferon were also detected in the cells from the genital mucosa, but few interleukin-4-secreting cells were seen at any time postinfection, indicating the induction of a Th1-like response in the cells of the genital mucosa. The iliac node draining the genital tract was the major node stimulated as a result of a genital infection and exhibited a predominant Th1-like pattern of cytokine secretion as well. Mesenteric lymph node cells demonstrated poor proliferative responses to MoPn and few antigen-stimulated cytokine-secreting cells after the primary infection. However, 7 days after a second infection administered 50 days following the primary infection, there was a marked increase in both proliferative responses and the frequencies of MoPn-stimulated gamma interferon- and interleukin-4-secreting cells. These studies provided information regarding the local CMI response to MoPn in mice which may prove valuable in the development of vaccination strategies for the prevention of chlamydial genital infections.

Insights into the immune mechanisms leading to the clearance of chlamydial disease have been gained by the study of a murine model of chlamydial genital disease caused by the mouse pneumonitis biovar of *Chlamydia trachomatis* (MoPn). Mice infected intravaginally with MoPn develop a self-limiting infection which generally resolves in 18 to 21 days (27). Previous studies from this laboratory have indicated that T cells and cell-mediated immunity (CMI) are essential for effecting the resolution of MoPn genital infections. Originally, it was found that athymic nude mice developed chronic genital infections which can persist over 200 days (27), while their immunologically intact littermates cleared the infection in 2 to 3 weeks. Therefore, mature T cells are essential for the resolution of disease in this model. Moreover, mice rendered B-cell deficient by treatment with anti-immunoglobulin M serum were unimpaired in their ability to resolve either genital disease (25) or pneumonia (37). Further evidence for the critical role of CMI in the resolution of infection was obtained with the demonstration that immune spleen cells (24), CD4- and CD8-enriched cell lines (24), or an MoPn-specific Th1 clone (9) transferred intravenously into infected nude mice led to the clearance of the disease.

Nevertheless, little is known about the expression of CMI in the murine genital tract. Studies examining the immune response of the genital tract have relied on identification of isotypes and levels of antibodies (6, 13, 14, 33) or descriptions of cell types present based on histochemical staining and electron microscopy (17, 18, 21). In the mouse MoPn model the local production of MoPn-specific antibodies from vaginal washings following genital infections has been demonstrated (23), but specific CMI responses to these infections in the local site have not been described. Delayed-type hypersensitivity reactions, as indicated by footpad or ear swelling (24, 25, 37, 38), and proliferation of splenic T cells in response to MoPn antigen (24, 37, 38) have been shown to be present; however, these tests assessed the systemic immune response to a local infection. Since MoPn genital infections are restricted to the superficial epithelial layer of the genital tract (1), a mucosal tissue, a study of the immune response elicited in the local area is potentially important in understanding the mode of disease resolution and immunity to reinfection.

Two subsets of murine $CD4^+$ T cells have been defined on the basis of their differing cytokine profiles (16). Th1 cells produce interleukin-2 (IL-2) and gamma interferon (IFN-γ) and mediate several functions associated with cytotoxicity and local inflammatory reactions, while Th2 cells produce IL-4, IL-5, IL-6, and IL-10 and are more effective at stimulating B cells to produce antibody. A role for these subsets has been demonstrated in several models of disease (29), but the selective activation of either subset has not been defined with regard to chlamydial infections.

Therefore, in this study, the mouse model of MoPn genital infections was used to examine the development of CMI in the genital tract and associated lymph nodes during the course of infection. The kinetics of the response were defined with regard to the MoPn-specific in vitro proliferative response and the induction of Th1- or Th2-like subsets in the local tissues of mice infected intravaginally with MoPn.

MATERIALS AND METHODS

Animals. Female BALB/c mice (5 to 6 weeks old) were purchased from Harlan Sprague-Dawley, Indianapolis, Ind. Mice were given food and water ad libitum in an environmentally controlled room with a cycle of 12 h of light and 12 h of darkness.

Infection. Mice were inoculated intravaginally while under sodium pentobarbital anesthesia with McCoy cell-grown MoPn containing 10^7 inclusion-forming units in 0.03 ml of sucrose phosphate-glutamate buffer (pH 7.2). Other experi-

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ments have shown that all mice may not be infected after a single inoculation and that this resistance to infection may be related to the estrus cycle (19, 25). To overcome this resistance, mice were inoculated on three consecutive days. The third day of inoculation is routinely considered day 0 of infection. Infection was confirmed by isolation of MoPn from cervicovaginal swabs in McCoy cells (25).

Preparation of genital-tract MNCs. Mice were sacrificed by cervical dislocation, and genital tracts (cervix, uterine horns, and oviducts) were removed, weighed, and placed in RPMI 1640 containing 0.6 mg of collagenase type I (Sigma, St. Louis, Mo.) per ml. Genital tracts were extensively minced with a scalpel, and the suspension was incubated for 1 h at 37°C in a 5% CO_2 atmosphere. Mononuclear cells (MNCs) were obtained by centrifugation of the cell suspension over a layer of Lympholyte M (Cedarlane Laboratories, Hornby, Ontario, Canada). These cells were washed twice and resuspended in RPMI 1640 containing 10% fetal bovine serum, 100 U of penicillin per ml, 100 μg of streptomycin per ml, 2 mM L-glutamine, and 50 μM 2-mercaptoethanol (complete medium).

The yield of MNCs from murine genital tracts varied depending on the time after genital infection and the hormonal status of the animal when it was killed. To compensate for the potential variation due to these parameters and to obtain a satisfactory number of cells for the assays, tissues from at least 10 mice were pooled. To normalize the cell quantitation data for groups of animals sacrificed at different times after infection and for the differences in weights of genital tracts obtained from various groups, the genital-tract cell counts were expressed as the number of MNCs per gram of genital tract tissue. The cell yield per gram was calculated as the total number of MNCs from pooled tissues divided by the weight of the pooled tissues (10).

Preparation of MNCs from lymphoid tissue. Spleens and lymph nodes (iliac, cervical, and mesenteric), which were pooled from 10 mice, were placed separately in RPMI 1640, and single-cell suspensions were prepared by mincing with forceps. For iliac and cervical nodes, the tissues were also teased on a 40-mesh stainless steel wire screen. These suspensions were treated with 0.17 M Tris-buffered NH_4Cl (pH 7.3) to lyse erythrocytes, were washed twice, and were then resuspended in complete RPMI 1640. MNCs were then used in either in vitro proliferation assays or enzyme-linked immunospot (ELISPOT) assays.

Proliferation assays. Cells were seeded into 96-well tissue culture plates (Falcon; Becton-Dickinson, Lincoln Park, N.J.) at 2×10^5 cells per well. MoPn antigen, which was prepared by UV inactivation of MoPn elementary bodies derived from HeLa cells and purified on a Renografin gradient (3), was added to wells at 5 μg/ml. In some cases the T-cell mitogen concanavalin A (Sigma) was added to cultures at 2.5 μg/ml. Control wells received medium alone to determine background response in the absence of antigen or mitogen. The proliferative response was measured by the incorporation of 1 μCi of [*methyl*-^{3}H]thymidine per well over the last 18 to 24 h of a 5-day culture period. Cells were harvested onto Self-Aligning RG glass fiber filters (Packard, Meridien, Conn.) with a Packard FilterMate cell harvester. Uptake of [*methyl*-^{3}H]thymidine was measured by a determination of total counts over a 6-min period with a Packard Matrix 96 Direct Beta Counter. The mean response was obtained from an average of data from triplicate cultures. Data were expressed as total counts (for cells from lymphoid tissue) or the response per gram of tissue (for cells from genital tract tissue).

ELISPOT assay. The ELISPOT assay for detection of IFN-γ- and IL-4-secreting cells was modified from a technique described by Taguchi et al. (32). Coating and detecting pairs of antibodies were purchased from PharMingen (San Diego, Calif.). Nitrocellulose-based 96-well plates (Millititer HA; Millipore Corporation, Marlborough, Mass.) were coated with a primary antibody (2 μg/ml) directed against either murine IFN-γ (R46A2) or murine IL-4 (1D11). After being coated overnight at 4°C, plates were emptied and blocked with RPMI 1640 containing 5% fetal bovine serum for 1 h at 37°C in a 5% CO_2 atmosphere.

We have found that for satisfactory spot development, cells must receive stimulation with either antigen or mitogen prior to the addition of cells to the ELISPOT assay plate. Therefore, cells (10^7/ml) were routinely stimulated with 5 μg of MoPn antigen per ml or 2.5 μg of concanavalin A per ml overnight before addition to the plates. After blocking of the plates, stimulated MNCs were added in triplicate at various dilutions, ranging from 10^5 to 10^3 cells per well, to the plates. Cells were incubated at 37°C in a 5% CO_2 atmosphere for 20 h. Plates were washed extensively with phosphate-buffered saline (PBS) containing 0.05% Tween to remove cells and then were incubated overnight at 4°C with a secondary biotinylated antibody (4 μg/ml) directed against either IFN-γ (XMG1.2) or IL-4 (24G2). Plates were then washed with PBS-Tween, incubated with 2.5 μg of avidin-peroxidase (Vector, Burlingame, Calif.) per ml for 1 h, and subjected to color development with 3-amino-9-ethylcarbazole (Vector). Spots indicative of locations of cells secreting cytokines were enumerated with the aid of a dissecting microscope. The mean number of cytokine-secreting cells counted in triplicate samples at a single dilution was used to calculate the number of spot-forming cells (SFCs) per million cells.

RESULTS

Kinetics of the MoPn-specific proliferative response of genital mucosal lymphocytes. The role of local genital mucosal lymphocytes in mice infected intravaginally with MoPn has not been defined. In order to quantitate the MoPn-reactive lymphocytes in the genital mucosae of mice infected with MoPn, groups of mice were infected intravaginally with MoPn and the proliferative response of genital MNCs to MoPn antigen was assessed at weekly intervals postinfection. The numbers of MNCs in the genital tract were also determined at weekly intervals after infection. An influx of MNCs was noted at 2 weeks postinfection, but the maximum cell yield was seen at 3 weeks postinfection, concomitant with the resolution of infection (27) (Fig. 1). Cell yields decreased thereafter but still remained elevated in comparison with those for uninfected animals at 7 weeks postinfection.

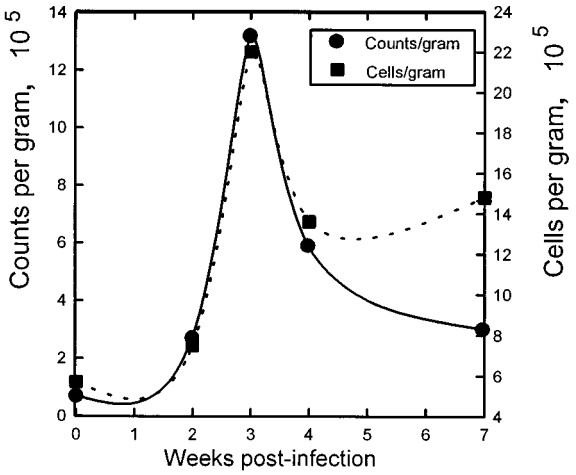

FIG. 1. Proliferation of genital tract MNCs in response to MoPn antigen. The response per gram of tissue was calculated as follows: [(experimental count − background count)/number of cells in culture] × number of cells per gram of tissue (10). Experimental and background counts represent the means of counts from triplicate cultures of cells pooled from 10 mice. Similar results were seen in two separate experiments.

When the MoPn-stimulated proliferation of these cells was assessed, it was found that the patterns of proliferation after infection paralleled the cell yield data in that a peak level of proliferation occurred at 3 weeks postinfection, after which the level declined (Fig. 1). Therefore, in response to an intravaginal infection with MoPn, there is an influx into the genital mucosa of immune cells which proliferate in response to MoPn antigen. Following the clearance of infection, the level of proliferation in response to MoPn antigen decreases sharply, although an increased number of MNCs remains in the genital tract. Upon repetition of the experiment, similar results were found.

Cytokine secretion by genital-tract MNCs in response to MoPn infection. The mechanism by which local immune cells can participate in effecting the resolution of infection is not clear. Production of IFN-γ is thought to be one of the major effector mechanisms for the clearance of chlamydial infections (26, 36). To investigate the presence of locally produced IFN-γ, the frequencies of IFN-γ-secreting cells were determined by ELISPOT assay. Moreover, since the production of IFN-γ and IL-2 is indicative of a Th1-like response, while the production of IL-4, IL-5, IL-6, and IL-10 reflects a Th2-like response (16), the selective induction of a Th1- or Th2-like response was investigated by comparing the frequencies of IFN-γ-secreting (Th1-like) and IL-4-secreting (Th2-like) cells. Antigen-stimulated MNCs from the genital tracts of mice infected with MoPn were examined for cytokine secretion at various intervals following infection. High levels of IFN-γ-

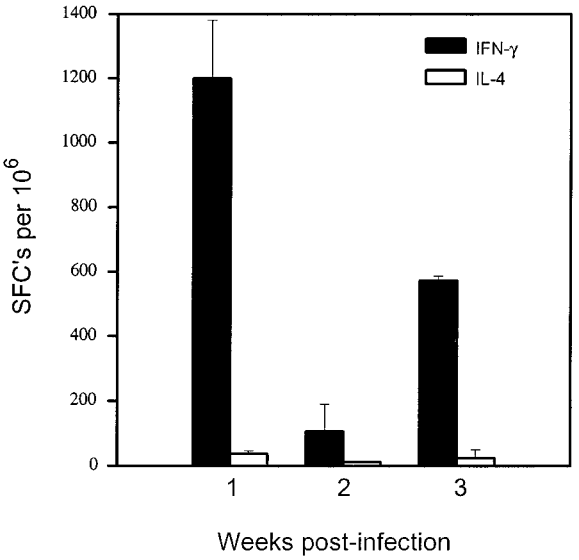

FIG. 2. Cytokine secretion by MoPn-stimulated genital tract MNCs. The mean number of cytokine-secreting cells counted in triplicate samples at a single dilution was used to calculate the number of SFCs per million cells. MNCs were stimulated with MoPn antigen (5 μg/ml) overnight before use in the ELISPOT assay. Values are mean numbers of cytokine-secreting cells counted in triplicate cultures at a single dilution (error bars show standard deviations). Similar results were seen in two separate experiments.

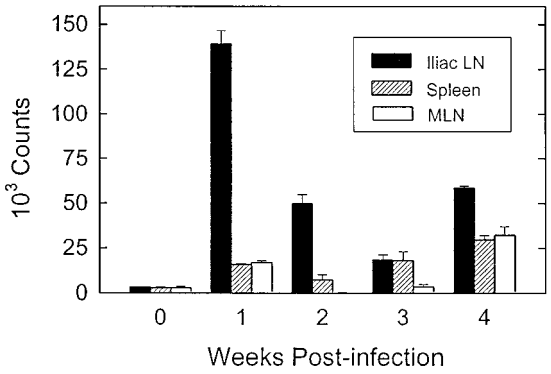

FIG. 3. Proliferation of MNCs from the iliac lymph node (LN), spleen, and MLN in response to MoPn antigen. Values are mean counts of triplicate cultures after subtraction of background counts (error bars show standard deviations). Cells (2×10^5 per well) were stimulated with MoPn antigen (5 μg/ml) in culture for 5 days. Similar results were seen in three separate experiments.

secreting cells were detected among these cells at 1, 2, and 3 weeks postinfection, with the highest level at 1 week postinfection. While there was a decrease in the frequency of IFN-γ-producing cells at 2 weeks, an increase was once again noted at 3 weeks postinfection, coinciding with the resolution of infection (Fig. 2). In contrast, very few IL-4-secreting cells were seen at any of the time points after infection. Genital-tract cells from uninfected mice did not secrete either cytokine at a detectable level when stimulated with MoPn (data not shown). Thus, it appears that a dominant Th1-like response is elicited in the genital mucosa by chlamydial genital infection. Similar results were found when the experiment was repeated.

Proliferation of genital-tract-associated lymph node cells. CMI responses against MoPn genital infections have been described mostly for cells from the spleen and peripheral blood. Because these infections do not disseminate but are restricted to the epithelium of the genital mucosa (1), the immune response in locally stimulated lymphoid tissues is potentially important in the resolution of infection. Therefore, in addition to assessing the proliferative response of cells obtained directly from the genital mucosa, we also determined the response of MNCs from lymphoid tissue associated with the genital tract. Parr and Parr (20) demonstrated that the iliac lymph node was a major node directly draining the genital tract. Cells from the mesenteric lymph node (MLN) may also participate in the immune response to genital infections because of the major role of this tissue in the mucosa-associated lymphoid system. The MLN is a key source of both immunoglobulin A and immunoglobulin G plasmocytes in the mouse genital tract (14). Splenic lymphocytes and cervical lymph node MNCs were also examined to assess the systemic immune response to genital infections.

MNCs from the iliac node, MLN, and spleen were assessed for proliferative responses to MoPn antigen at various intervals postinfection. The iliac node was found to be a major site stimulated as a result of an MoPn genital infection. The proliferative response of cells from the iliac node increased dramatically at 1 week postinfection, after which the response declined (Fig. 3). MLN cells and spleen cells from infected animals also exhibited higher levels of proliferation than those from uninfected animals; however, this proliferative response was never as vigorous as that seen in iliac node cells at 1 week postinfection. Nevertheless, there did appear to be a gradual increase in responsiveness of cells from the spleen and MLN by 4 weeks after infection. Responses of cervical lymph node MNCs were consistently low at all time points (data not shown). Two subsequent experiments revealed similar patterns of response.

Cytokine secretion by cells from genital-tract-associated lymphoid tissues. To investigate a possible effector mechanism for the cells of the genital-tract-associated lymph nodes, the frequencies of MoPn-stimulated IFN-γ and IL-4-producing cells in these tissues were determined. The maximum frequency of IFN-γ-secreting iliac node cells was seen 1 week following infection (Fig. 4). Very few, if any, IL-4-secreting cells were detected in this tissue, suggesting that, as in the genital mucosa, a preferential activation of the Th1-like subset occurs. Splenic cells stimulated with MoPn exhibited a predominance of IFN-γ-secreting cells as well (Fig. 4). Analysis of MoPn-stimulated MLN cultures revealed no IFN-γ- or IL-4-producing cells (data not shown).

Effect of reinfection on antigen-stimulated patterns of proliferation and cytokine production. The patterns of response in the genital-tract-associated tissues were examined for mice which had received a second infection 50 days following the primary infection. The experiment was designed such that tissues were collected from the primary infection group and the reinfection group at the same time so that direct comparisons could be made between the groups under exactly the same experimental conditions. When the proliferative responses of spleen, MLN, and iliac node cells were determined at 7 days after both a primary and secondary infection, a higher level of response to MoPn antigen was seen in cells from all of these tissues after a reinfection (Fig. 5). Cells from the MLN, which had consistently demonstrated modest proliferative responses to MoPn 7 days after a primary infection, had a dramatic increase in proliferation at 7 days after a second infection (Fig. 5). Three separate experiments yielded similar results.

Frequencies of cytokine-secreting cells in tissues of mice which were reinfected 50 days following the primary infection were also determined. The frequencies of MoPn-stimulated

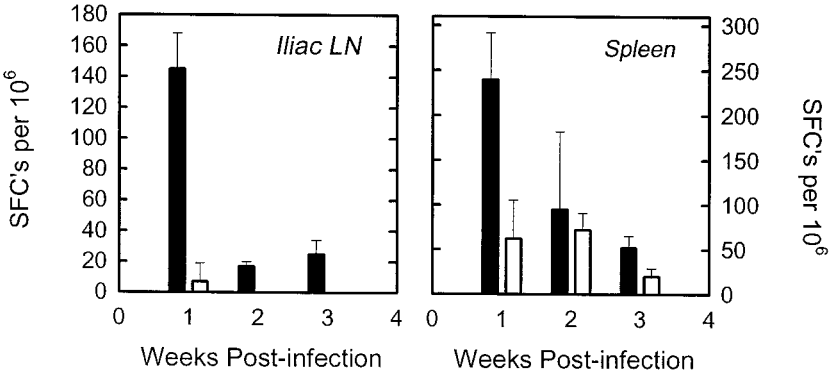

FIG. 4. Cytokine secretion by MoPn-stimulated iliac lymph node (LN) and spleen MNCs. The mean number of cytokine-secreting cells counted in triplicate samples at a single dilution was used to calculate SFCs per million. MNCs were stimulated with MoPn antigen (5 μg/ml) overnight before use in the ELISPOT assay. Values are mean numbers of cytokine-secreting cells counted in triplicate cultures at a single dilution (error bars show standard deviations). Similar results were seen in two separate experiments. ■, IFN-γ; □, IL-4.

spleen cells secreting both IFN-γ and IL-4 were increased slightly at 7 days after a reinfection (Fig. 6). In antigen-stimulated iliac node cultures from mice 7 days after a second infection, as seen following a primary infection, a predominantly Th1-like phenotype was detected (Fig. 6).

Very few IFN-γ- or IL-4-secreting cells could be detected in MoPn-stimulated MLN cultures after a primary infection, presumably because of the paucity of antigen-specific cells in this tissue at the given times after infection, although cells secreting both cytokines could be detected in concanavalin A-stimulated MLN cultures (data not shown). Seven days following a second infection, both IFN-γ- and IL-4-secreting cells could be detected in MoPn-stimulated MLN cultures (Fig. 6); however, the predominance of IFN-γ-producing cells relative to IL-4-producing cells was not as marked as that seen with iliac node cells.

DISCUSSION

It has been well documented that CMI mechanisms are essential for the resolution of murine chlamydial genital infections by the MoPn biovar of *C. trachomatis* (9, 24, 25, 27). This study supports and extends these findings by demonstrating that cellular immune responses in the local tissues associated with MoPn genital infections are dominated by Th1-like responses.

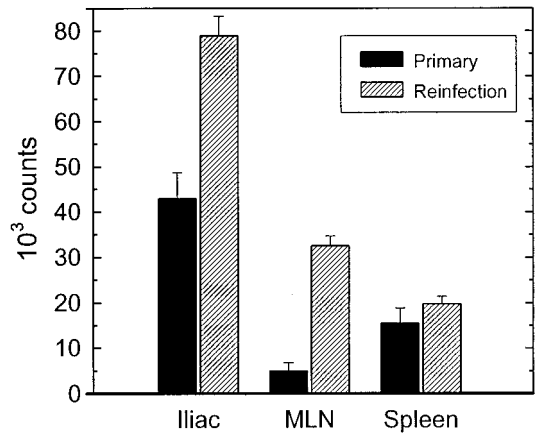

FIG. 5. Comparison of proliferative responses to MoPn antigen after a primary and secondary infection. One group of mice received a second genital inoculation of MoPn 50 days after the primary infection, while the other group received only a primary infection. Infections were timed such that the tissues from both groups were processed on the same day. Values are mean counts of triplicate cultures after subtraction of background counts (error bars show standard deviations). Similar results were seen in three separate experiments.

When the kinetics of the CMI response in the local mucosal sites were examined, cells capable of proliferating in response to MoPn antigen were demonstrated in the genital mucosa, with the maximum level of proliferation and peak MNC yields occurring at 3 weeks postinfection, a time at which the infection is normally cleared (27). It can be hypothesized that as local antigen dissipates in the genital tract, antigen-specific cells leave the local area because of the lack of antigenic stimulus. The kinetics of the proliferative response in the genital tract suggest that cells in this tissue are contributing to the immune response that effects the clearance of the infection. These findings are similar to those from studies of genital infections of guinea pigs by the guinea pig inclusion conjunctivitis biovar of *Chlamydia psittaci*. In those studies, T cells from the genital mucosae of infected animals were shown to proliferate in response to the antigen of this *C. psittaci* biovar, and the disappearance of antigen-specific cells in the genital tract was correlated with susceptibility to reinfection (10).

The exact mechanism by which CMI brings about the resolution of disease is not clear. A potential effector mechanism is the inhibition of chlamydial growth by cytokines such as IFN-γ. IFN-γ certainly exhibits antichlamydial activity (2, 28) and indeed may be a primary effector mechanism in the clearance of infection. With regard to MoPn infections in particular, it was demonstrated that in vivo depletion of IFN-γ with monoclonal antibody to IFN-γ made mice more susceptible to murine pneumonia (36) and genital infections (26). Therefore, a noteworthy finding in the present studies was the predominance of IFN-γ-producing cells in the genital mucosae of infected mice after chlamydial infection. The local production of IFN-γ may be an important mechanism whereby MoPn genital infections are controlled.

Production of IFN-γ is also the hallmark of a Th1 response. Mosmann and Coffman (16) classified two subsets of murine T-cell clones, Th1 and Th2, on the basis of their differing cytokine profiles. A role for these subsets in infectious disease has been demonstrated in several models (5, 7, 8, 11, 39). The secretion of IFN-γ, but not IL-4, by antigen-stimulated cells of the genital tracts of mice infected with MoPn suggests a selective induction of a Th1-like population directly at the site of infection, i.e., the genital mucosa.

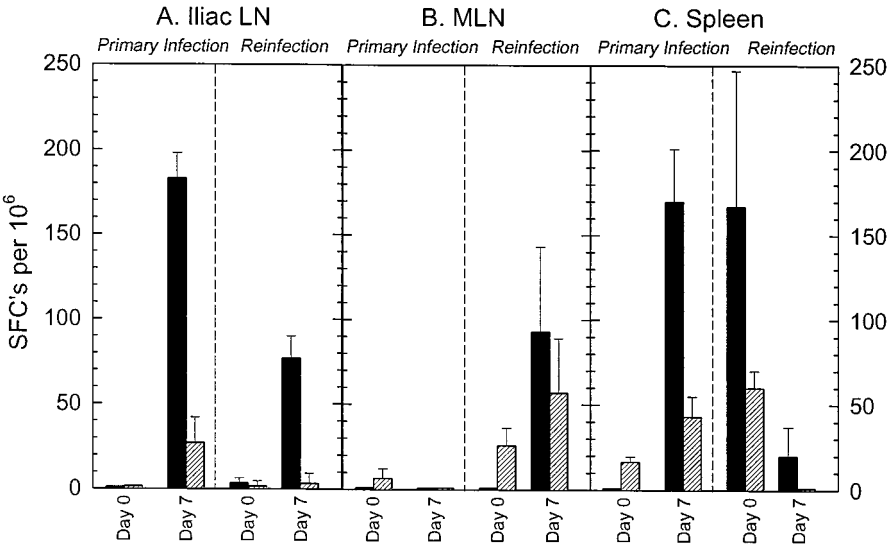

FIG. 6. Comparison of frequencies of cytokine-secreting cells after a primary and second infection. One group of mice received a second genital inoculation of MoPn 50 days after the primary infection, while a second group received only a primary infection. Infections were timed such that tissues from both groups were processed on the same day. The time point Day 0 indicates uninfected animals (primary infection) or animals infected 50 days previously (reinfection), while the time point Day 7 indicates animals sacrificed 7 days following either a primary or second infection. Values are mean numbers of cytokine-secreting cells counted in triplicate cultures at a single dilution (error bars show standard deviations). MNCs were stimulated with MoPn antigen (5 μg/ml) overnight before use in the ELISPOT assay. Similar results were seen in two separate experiments. ■, IFN-γ; ▨, IL-4.

Surprisingly, while the maximum proliferation of genital MNCs was not seen until 3 weeks postinfection, the maximum frequency of IFN-γ-secreting cells was seen at 1 week postinfection, with a second peak, although somewhat reduced, at 3 weeks. Since the cells examined were whole MNC populations and not purified $CD4^+$ T cells, it is possible that some of the IFN-γ-producing cells seen at 1 week postinfection could be attributed to non-$CD4^+$ cell types, such as $CD8^+$ T cells, $\gamma\delta^+$ T cells, or natural killer cells. It is generally believed that natural killer cells play a role in controlling the early phase of infection before the appearance and expansion of pathogen-specific T cells (4, 12, 22). In addition, $CD8^+$ T cells and $\gamma\delta^+$ T cells secrete IFN-γ in response to antigenic stimuli (29). The IFN-γ-secreting cells detected at 3 weeks postinfection could represent the induction of an antigen-specific T-cell-mediated immune response.

Therefore, it is apparent that CMI responses can be initiated directly in the murine reproductive tract. However, since there is little resident lymphoid tissue in the genital tract, it is likely that other lymphoid tissues act as a source of effector cells. The MLN, which is a major tissue of the mucosa-associated lymphoid system, may also participate in the immune responses to infections of the genital tract, which is part of the mucosal immune system as well. McDermott and Bienenstock demonstrated a preference of MLN cells for populating mucosal sites (13). After a primary MoPn genital infection, low-level proliferative responses and low levels of MoPn-stimulated cytokine production were seen in MLN cultures; however, responses were markedly elevated at 7 days following a second infection. Therefore, it appears that while the MLN is not a source of MoPn-specific cells early after a primary infection, this tissue may be playing an important role in the generation of effector cells in the immune response to repeated infections.

To investigate the role of the regional lymph nodes, cells from the iliac nodes were examined for their proliferative response to MoPn and for the presence of IFN-γ- and IL-4-secreting cells. The iliac nodes were found to be the major tissue stimulated as a result of a genital infection. Studies by Parr and Parr demonstrated that tracers administered into the vaginal walls of mice appeared in the iliac, caudal, and/or renal nodes, with the iliac nodes being the major nodes draining the vagina (20). In a model of murine genital herpes simplex virus-2 infections, protective immunity involves antigen stimulation of migratory T cells in the lymph nodes draining the genital tract (15). In this system, mice receiving a transfer of cells from genital lymph nodes of mice infected with attenuated herpes simplex virus 2 are protected from subsequent challenge with wild-type virus. However, mice receiving a transfer of spleen cells are not protected from challenge.

As seen with MNCs from the genital tract, antigen-stimulated cells from the iliac node also exhibited primarily a Th1-like phenotype following both a primary and secondary infection. These studies suggest that the cells from the local tissues (the genital tract and regional nodes) stimulated as a result of an MoPn genital infection are predominantly Th1-like cells. The activation of a predominantly Th1-like subset is consistent with the protective immune response to other intracellular pathogens such as *Leishmania* spp. (7) and *Mycobacterium leprae* (40). Moreover, the selective activation of Th1-like responses in the local tissues seems to be an appropriate immune response in this system in which a crucial role for CMI has been demonstrated. Transfer of an MoPn-specific Th1 clone allows nude mice genitally infected with MoPn to resolve the infection (9). Interestingly, a panel of *Chlamydia*-specific T-cell clones derived from the synovial fluid of a patient with sexually acquired reactive arthritis exhibited a predominantly Th1 phenotype as well (30). Th1 cells produce cytokines such as IFN-γ and tumor necrosis factor alpha which may help to effect the clearance of disease. However, Th1 cells also participate in delayed-type hypersensitivity reactions which may contribute to the pathological changes in the upper tract seen when mice are infected with chlamydiae by an intrauterine route (31, 34, 35). Therefore, activation of Th1 subsets could bring about both protective and deleterious effects.

Antigen-stimulated proliferative responses, as well as IFN-γ- and IL-4-secreting cells, were seen in cells from the spleen.

However, the proliferative responses of cervical lymph node cells, which are not associated with lymphatic draining of the genital tract, were similar to background levels.

These studies defined aspects of the local CMI response following a natural genital infection. On the basis of these findings, it appears that the stimulation of local immunity in the genital tract and associated lymph nodes may be a requirement for the clearance of chlamydial infections of the reproductive tract.

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