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Role of L3T4-Bearing T-Cell Populations in Experimental Murine Chlamydial Salpingitis

DANIEL V. LANDERS,[1]* KIM ERLICH,[2]† MINNIE SUNG,[3]‡ AND JULIUS SCHACHTER[3]

Department of Obstetrics, Gynecology and Reproductive Sciences,[1] Department of Medicine,[2] and Department of Laboratory Medicine,[3] San Francisco General Hospital, University of California, San Francisco, 1001 Potrero Avenue, San Francisco, California 94110

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A role for both the cellular and humoral components of the immune response has been established for chlamydial infection. The significance of helper (L3T4) T cells was evaluated by using a *Chlamydia trachomatis* murine salpingitis model for upper genital tract chlamydial infection. Mouse oviducts were inoculated with *C. trachomatis* by using the mouse pneumonitis agent (MoPn) or control medium. Mice depleted of L3T4-bearing lymphocytes had significantly higher ($P < 0.05$) numbers of organisms recovered at day 7 postinoculation. The rate of hydrosalpinx formation was significantly higher in the mice depleted of L3T4-bearing lymphocytes (27 of 31 [87%]) than in the infected undepleted group (8 of 16 [50%]) ($P < 0.01$). The geometric mean antichlamydial immunoglobulin G titers at day 54 postinoculation were significantly higher in the L3T4-depleted mice (mean titer, 2,030) than in the undepleted group (mean titer, 776; $P < 0.05$). The rate of fertility was lower in the L3T4-depleted group (2 of 31 [6%]) than in the infected, undepleted mice (2 of 16 [13%]), but this difference did not reach statistical significance. In conclusion, the greater persistence of organisms in the oviduct and higher rates of hydrosalpinx formation in mice depleted of L3T4-bearing cells suggests that these cells play a role in the clearing of organisms following infection and thus in reducing the degree of oviduct obstruction and damage.

The importance of *Chlamydia trachomatis* in genital tract infections has been well established (3, 11). This organism has been implicated as a major etiologic agent in pelvic inflammatory disease, involuntary infertility, and ectopic pregnancy (4, 5). However, the immune response to chlamydial infection continues to be poorly understood. It is clear, however, that the amount of oviduct damage cannot be explained simply by infection of cells leading to cell death and lysis. The pathogenesis and subsequent genital tract damage must involve components of the immune response. In a murine respiratory disease model, it has been established that both specific antibody production and cell-mediated immunity to the mouse pneumonitis agent (MoPn) of *C. trachomatis* are T-cell dependent (12). While even less is known about the immune response to genital tract chlamydial infections, investigations of the immune response and its contribution to the pathogenesis are essential if rational approaches to these infections are to be developed.

The mouse model for chlamydial salpingitis and subsequent infertility developed in our laboratory has been used successfully to study various antimicrobial and anti-inflammatory treatments. Because we are able to correlate antibody production with antigen load, inflammation, pathologic changes, and infertility, it is a useful system with which to study the immune responses.

It is known that administration of a rat immunoglobulin G2b (IgG2b) monoclonal antibody against L3T4 rapidly depletes the T-helper/inducer cell populations in mice (13). This is associated with suppression of humoral immunity to T-dependent antigens and also some suppression of cellular immunity. These findings suggest that monoclonal antibodies directed against specific lymphocyte antigens may serve as probes to delineate specific lymphocyte populations contributing to the immune response. The antibodies may also have potential in the development of treatment strategies directed towards reducing inflammatory damage and subsequent infertility.

Helper T cells are known to play a major role in orchestrating the immune response to viral and other foreign antigens. This study was undertaken to evaluate the hypothesis that the helper/inducer T-cell population identified by the L3T4 monoclonal antibody plays a significant role in the immune response to murine chlamydial salpingitis caused by the MoPn biovar of *C. trachomatis*.

MATERIALS AND METHODS

Mice. Random-bred, female Swiss Webster mice were obtained from Charles River Breeding Laboratories, Inc. (Wilmington, Mass.). Females were 4 to 6 weeks of age; males were at least 8 weeks of age and proven breeders.

Organisms. The mouse pneumonitis (MoPn) strain of *C. trachomatis*, grown in McCoy cell monolayers and frozen in aliquots at −70°C, as previously described by Swenson et al. (8a), was used for inoculations at a concentration of approximately 2×10^6 inclusion-forming units (IFU) per ml.

Inoculation procedure. Tribromoethanol was administered intraperitoneally to anesthetize mice for inoculations. The left uterine horn, oviduct, and ovary were delivered through a lateral flank incision. Approximately 0.03 ml of *C. trachomatis* suspension was injected into the left ovarian bursa through the ovarian fat pad, and another 0.03 ml was injected into the left uterine horn. Control mice were inoculated with sterile tissue culture medium in the same manner. Flank incisions were closed with 4-0 silk suture (peritoneum) and surgical skin clips.

* Corresponding author.
† Present address: Suite 302, 901 Campus Drive, Daly City, CA 94015.
‡ Present address: 348 Hazelwood Avenue, San Francisco, CA 94127.

Fertility. One or more mice from each group were killed 6 to 7 days postinoculation to confirm acute inflammation. The remaining mice were caged with males (five females to one male) during days 42 to 50 postinoculation for mating. Males were rotated between cages after 5 days. Animals were killed on day 54 postinoculation, blood was collected by cardiac puncture for serology, and postmortem examination was performed to assess fertility and gross morphology. Reisolation and histologic examination were performed on representative animals from each group.

Serology. Serum samples were tested for IgG and IgM by the microimmunofluorescence test of Wang and Grayston (9), with yolk sac-grown MoPn as the antigen and fluorescein-conjugated anti-mouse immunoglobulins (Cappel Laboratories Inc., Cochranville, Pa.).

Histology and microbiology. Microscopic examination of the oviduct and uterine horn of the inoculated side was performed on 10% Formalin-fixed sections stained with hematoxylin and eosin. Tissue samples of the entire oviduct on the inoculated side were disrupted, inoculated onto McCoy cell monolayers, and cultured by previously described methods (7) with vials, double-blind passage, and iodine staining.

Monoclonal antibodies. Mice in the L3T4 group received three weekly injections of anti-L3T4 monoclonal antibody. Mice were given 0.4 mg on the day prior to infection and then 0.2 mg on day 7 postinoculation and again on day 14 postinoculation. Control mice were given equivalent volumes of normal saline in the same manner.

Lymphocyte analysis. Peripheral blood lymphocytes from three mice in each group postinoculation were analyzed on days 2 and 20 by fluorescence-activated cell sorting (FACS). Cells were stained by the double-antibody technique with L3T4 (mouse equivalent to human CD4), Lyt2 (mouse equivalent to human CD8), or Thy1.2 (a pan-T-cell antigen) as the first antibody and fluorescein isothiocyanate-labeled goat anti-rat Igs as the second antibody.

Statistical analysis. Pregnancy and hydrosalpinx rates among treatment groups were compared by chi-square analyses or Fischer's exact test. Geometric mean serologic titers were compared by the *t* test performed on log-transformed data.

RESULTS

Mice treated with L3T4 were noted to have near total depletion of the L3T4-bearing lymphocyte population by day 2 postinoculation. This depletion persisted through at least day 20 (Fig. 1). The L3T4 depletion did not significantly alter the percentages of cells expressing Thy1.2 (a pan-T-cell antigen) or Lyt2 (suppressor/cytotoxic T-cell antigen equivalent to CD8 in humans) (Fig. 2).

Mice treated with L3T4 monoclonal antibody prior to infection were compared with infected untreated mice. Control groups included fertility controls (no surgery), infection controls (inoculated with tissue culture medium), and L3T4 controls (treated with L3T4 but inoculated with medium only). The results are summarized in Table 1. The acute inflammation 7 days after infection was indistinguishable in the infected groups regardless of L3T4 treatment. Significantly more organisms were recovered from the oviduct on day 7 ($P < 0.05$) in the L3T4-treated group (mean of 13,848 IFU versus 8,078 IFU). Following mating (day 50), when fertility outcomes were assessed, no organisms could be reisolated from oviducts. Histologic examination of the oviduct revealed normal morphology, with complete resolution of the acute inflammation, as determined by light microscopy, except for distension of the tubal lumen and flattening of the tubal epithelium in those with hydrosalpinges.

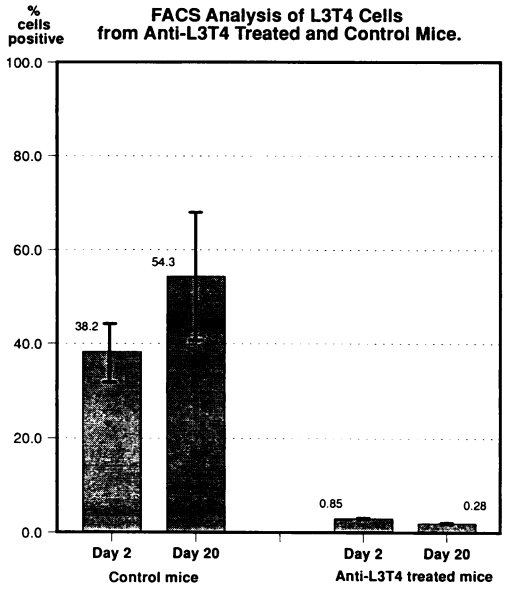

FIG. 1. Early and persistent loss of cells binding fluorescent-labeled anti-L3T4 antibody.

There were no significant differences in the normal bilateral pregnancy rates between any of the uninfected control groups following mating 41 to 50 days after inoculation. The normal bilateral pregnancy rate in the infected untreated group was 2 of 16 (13%), which was significantly lower than in the infection control group ($P < 0.0001$). The rate of hydrosalpinx formation by day 54 was 8 of 16 (50%), and the geometric mean IgG titer was 776. The normal bilateral pregnancy rate in infected mice pretreated with L3T4 was only 2 of 31 (6%). This was markedly different from the rate

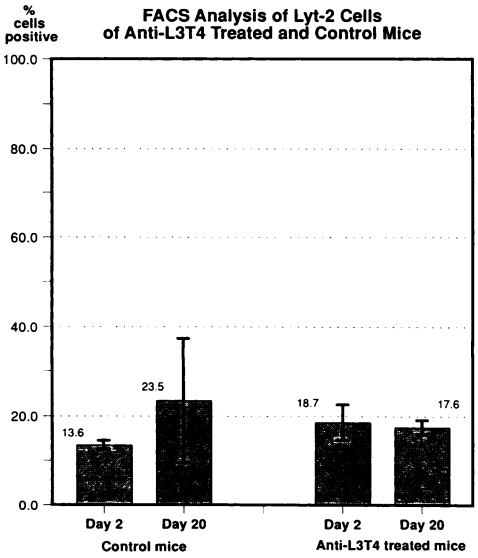

FIG. 2. Persistence of anti-Lyt2-binding cells in mice treated with anti-L3T4 antibody.

TABLE 1. Effect of L3T4 treatment

Group		Acute inflammation on day 7	Chlamydia isolation (IFU/oviduct day 7)	No. of mice with hydrosalpinx on day 54/no. in group (%)	No. of mice with normal bilateral pregnancy/no. in group (%)	IgG GMT,[a] day 54
Inoculation	Treatment					
None	None	No	None	0/15	11/15 (73)	0
Medium	None	No	None	0/10	9/10 (90)	0
Medium	L3T4	No	None	0/6	4/6 (67)	0
MoPn	None	Yes	8,078	8/16 (50)	2/16 (13)	776
MoPn	L3T4	Yes	13,848	27/31 (87)	2/31 (6)	2,030

[a] GMT, geometric mean titer.

in uninfected mice treated with L3T4 ($P < 0.005$). While the L3T4-treated infected mice had a somewhat lower rate of normal pregnancies than infected untreated controls (6 versus 13%), with these small numbers, the difference did not reach statistical significance. However, the rate of hydrosalpinx formation (87%) in the L3T4-treated infected group was significantly higher than in the infected untreated controls (50%) ($P < 0.01$). Furthermore, the geometric mean antichlamydial IgG titer at day 54 postinoculation was significantly higher in the L3T4-pretreated infected group than in the untreated infected group (2,030 versus 776).

DISCUSSION

Our goal with this mouse model is to successfully recapitulate the inflammatory response and subsequent infertility found in human chlamydial salpingitis. We used this system to further define the lymphocyte subsets involved in the immune response to murine chlamydial salpingitis. While it is generally accepted that the cell-mediated immune response plays a role in chlamydial infection, the exact nature of the role remains unclear (1, 2, 12). Among the newer immunologic reagents available, antilymphocyte monoclonal antibodies have become a valuable research tool. In this study, we used one such antibody to selectively deplete the L3T4-bearing (helper cell) lymphocyte subset prior to infection with *C. trachomatis*. This resulted in several alterations in the course of this experimentally induced salpingitis. While the acute inflammatory response appeared to be similar, mice depleted of $L3T4^+$ cells had higher long-term antichlamydial IgG titers and significantly more hydrosalpinx formation. The elevated IgG titer implies a greater exposure to chlamydial antigen, perhaps due to slower clearing and/or increased proliferation of the organism. In fact, the mean number of organisms reisolated was significantly higher in the L3T4-treated group. The greater number of organisms may be related to the absence of the L3T4-bearing lymphocyte contribution to the immune response.

It has been shown that deleterious immune responses elucidated by a 57-kDa chlamydial stress response protein are cell mediated and associated with a delayed hypersensitivity response (6, 10). Recently, it has further been demonstrated that T-cell epitopes of the major outer membrane protein (Momp) of *C. trachomatis* are important in the B-cell production of Momp-specific IgG (8). These findings support the importance of T cells, particularly T helper cells, in the immune response to chlamydial infection. They do not, however, address the mechanisms by which chlamydial infection is controlled and eradicated in the absence of T helper cells. The data presented in this article suggest that B-cell production of antichlamydial IgG may occur by mechanisms other than by T-helper-cell stimulation. However, nonspecific B-cell stimulation has been shown to occur in response to chlamydial elementary bodies (2). Alternatively, it may be possible that the small number of T helper cells remaining following anti-L3T4 treatment are activated to release excessive amounts of B-cell differentiation factor, leading to greater antibody production. However, the very small numbers of remaining L3T4-bearing cells would make this possibility seem unlikely. While the degree of infiltration of the oviduct with polymorphonuclear lymphocytes and plasma cells was not appreciably different in the anti-L3T4-treated group, this method of determining the degree of inflammatory response may not be sufficiently sensitive. The production and secretion of various antibodies and cytokines at the site of infection may, however, be substantially different.

The increased rate of hydrosalpinx formation indicates greater damage to the oviduct in mice treated with anti-L3T4 antibodies. Two possible explanations for this phenomenon are that greater numbers of organisms persist due to the lack of a helper cell population, in turn leading to more oviduct damage, and that additional, more destructive components of the immune response are necessary because of the lack of helper T lymphocytes. Infection that is less well controlled because of the absence of the helper T-cell population may lead to more antigen, with more subsequent inflammatory damage to the oviduct. Helper T-cell populations are known to secrete cytokines that play key roles in B- and T-cell proliferation, T-cell cytolysis, and monocyte-macrophage chemotaxis. Without this system intact, a more destructive inflammatory process may be necessary to eradicate the infection, leading to increased oviduct obstruction and hydrosalpinx formation. However, the exact mechanism of oviduct damage in this T-helper-cell-deficient model remains unclear.

Additional studies will be required to further delineate the role of L3T4-bearing lymphocytes in the immune response to chlamydial salpingitis. Those studies should involve not only depletion of specific cell populations but also replacement of the major cytokines produced by those cells, particularly T helper cells, in response to infection.

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