Resolution of Chlamydial Genital Infection with Antigen-Specific T-Lymphocyte Lines

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To determine cell-mediated immune mechanisms involved in the resolution of chlamydial genital infection of mice, we utilized an established murine model in which it has been demonstrated that resolution of infection occurs independently of the antibody response. Splenic T lymphocytes were obtained from mice that had previously been immunized with viable elementary bodies of the mouse pneumonitis agent (MoPn), a Chlamydia trachomatis biovar. Antigen-reactive T lymphocytes were maintained and expanded in vitro by frequent restimulation with UV light-inactivated MoPn in the presence of antigen-presenting cells and recombinant interleukin-2 (rIL-2). Flow cytometry indicated that this cell line was at least 92% positive for the panspecific T-cell marker Thy1.2. Stimulation of the cells in the presence of syngeneic antigen-presenting cells plus MoPn antigen and in the absence of exogenous IL-2 induced the cells to produce IL-2 activity in culture supernatants. Following adoptive transfer, this T-lymphocyte line was effective in inducing resolution of an ongoing MoPn genital infection in congenitally athymic nude mice which otherwise maintain chronic unresolved infections. The line was less efficient in resolving the infection after longer periods in culture. An additional T-lymphocyte line was derived from the spleens of athymic mice that had received the first line and had resolved the infection. These T cells were also capable of inducing resolution of the infection. Lastly, this cell line was treated with specific antibody and complement to delete either CD4+ or CD8+ T lymphocytes in an attempt to enrich for T-cell subpopulations prior to transfer into infected athymic mice. The anti-CD4-treated line was essentially depleted of CD4 cells, while the anti-CD8-treated line was only partially enriched for CD4 cells, with a large proportion of CD8 cells still present. Nude mice that received either of the treated T-cell lines or the parental cell line were capable of resolving the infection, although the line with increased numbers of CD4 cells was more efficient than either the parental line or the CD8 line.

The relative roles of the antibody and cell-mediated immune (CMI) responses in chlamydial genital infection of humans remain unclear. Some evidence has been provided that implicates a role for local antibody in reducing the amount of Chlamydia trachomatis isolated from the genital tracts of infected females (3). However, while CMI responses have been measured following infection in humans (20, 21), the exact function of T cells is difficult to discern. Therefore, the immunologic parameters critical to resolution of and resistance to chlamydial genital infection have been studied in animal models. In female guinea pigs infected genitally with the agent of guinea pig inclusion conjunctivitis, a C. psittaci agent, it has been found that both CMI and antibody responses cooperate to resolve the infection and provide resistance to reinfection (24, 25, 27, 28).

Another useful model has been that of female mice infected in the genital tract with mouse pneumonitis agent (MoPn), a C. trachomatis biovar (2). Congenitally athymic nude (nu/nu) mice developed chronic genital infections with MoPn which did not resolve during the period of observation, 265 days, in comparison with their heterozygous (nu/+) littermates which resolved the infection in approximately 20 days (26). However, since the nu/nu mice did not develop significant antibody responses compared with the nu/+ mice, it could not be discerned whether the unresolved infection was due to insufficient CMI responses or a lack of antibody-mediated mechanisms. Nonetheless, in a later study, it was found that B-cell-deficient mice are capable of resolving the infection and are immune to a challenge infection (23). Thus, in this model, T cells eliciting CMI and not antibody responses are essential to resolution of the infection and provision of immunity to reinfection. Additionally, it has been found that despite in vivo depletion of either CD4+ or CD8+ T cells, animals are capable of resolving the infection (22). A common mechanism of these two T-cell subpopulations was implicated when animals treated with monoclonal antibody to murine gamma interferon (IFN-γ) sustained a significantly prolonged infection (22a).

Preliminary to the present study, data indicated that adoptive transfer of either immune or normal nu/+ whole spleen cells, as well as B-cell-enriched and T-cell-enriched populations from immune nu/+ mice, was capable of resolving the infection in nu/nu mice (23a). These results provide an opportunity to utilize adoptive transfer of cultured T-cell lines to further dissect the contribution of CMI mechanisms to resolution of this infection. Here we report the production and partial characterization of MoPn-specific T-cell lines from nu/+ mice that are capable of resolving MoPn genital infections in nu/nu mice.

MATERIALS AND METHODS

Animals. Five- to six-week-old female nu/nu and nu/+ mice on a BALB/c background were obtained from Harlan Sprague-Dawley, Inc., Indianapolis, Ind. All mice were maintained in Plexiglas isolators under pathogen-free conditions and fed sterile food and water ad libitum in an environmentally controlled room with a cycle of 12 h of light and 12 h of darkness.

Immunization of nu/+ mice. Five mice were immunized by intravenous administration of 10⁸ inclusion-forming units of

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viable McCoy cell-grown MoPn in 0.2 ml of 2-sucrose-phosphate buffer transport medium. Two weeks later, mice received a similar intravenous injection with 3 × 10⁶ inclusion-forming units of viable MoPn. After an additional 7 days, mice were sacrificed and used to prepare immune T-lymphocyte lines as described below. This immunization regimen often resulted in marked splenomegaly.

Preparation of splenic T cells for culture. Mice were sacrificed by cervical dislocation while they were under sodium pentobarbital anesthesia. Spleens were aseptically removed and teased into single-cell suspensions in cold RPMI 1640. Both spleens were harvested following centrifugation at 400 × g and suspension in 0.17 M Tris-buffered NH₄Cl (pH 7.3). After centrifugation through fetal bovine serum and extensive washing in cold RPMI 1640, the cell suspension was enriched for T cells by passage and elution over a nylon wool column. The effluent cells were washed and suspended in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 0.05 mM 2-mercaptoethanol, 10 mM HEPES (N-2-hydroxyethylpiperazin-N'2-ethanesulfonic acid) buffer, 0.1 mM nonessential amino acids, 1.0 mM sodium pyruvate, 100 U of penicillin per ml, and 100 µg of streptomycin per ml; henceforth referred to as lymphocyte culture medium (LCM).

Preparation of antigen-presenting cells (APCs). Normal, nonimmune mice (1-2 mice) were sacrificed as described above. Spleens were aseptically removed and teased into single-cell suspensions in cold RPMI 1640. The cells were then washed twice by centrifugation through cold RPMI 1640, gamma irradiated (2,200 rads), washed again, and suspended in LCM. For T-lymphocyte cultures, these cells were incubated for 3 h with 5 µg of Renograin-gradient-purified, UV light-inactivated, HeLa cell-grown MoPn (Ag) per ml prior to addition of T cells.

Initiation and propagation of MoPn-specific T-lymphocyte lines. For the initial in vitro stimulation period, pooled T-enriched spleen cells were suspended to 2 × 10⁶ cells per ml in LCM. Five milliliters (10⁶ cells) was added to each well of six-well culture plates also containing 10⁷ APCs plus Ag in 5 ml of LCM (1:1 ratio of T cells to APCs). Cultures were then incubated at 37°C in a humidified atmosphere containing 5% CO₂. After 18 to 24 h, recombinant murine interleukin-2 (rIL-2; Genzyme Corp., Boston, Mass.) was added at a concentration of 20 U/ml. After an additional 72 to 96 h in culture, the cells were harvested and viable lymphocytes were isolated by recovery at the interface of a Ficoll-Hypaque gradient (density, 1.0875). For secondary stimulation, the cells were extensively washed and suspended to 10⁶ cells per ml in LCM. A 2.5-ml volume of this suspension was added to an equal volume of LCM containing 10⁷ fresh APCs plus Ag in each well of six-well culture plates (1:4 ratio of T cells to APCs), and rIL-2 was added at 20 U/ml. The secondary culture was then incubated as described above for 72 h. At the end of this incubation period, the cultures were fed by gentle addition of 5 ml of fresh LCM containing 40 U of rIL-2 per ml. At the end of an additional 72-h incubation period, cells were harvested and viable lymphocytes were again isolated over a Ficoll-Hypaque gradient. The viable cells were then cultured with antigen, APC, and rIL-2 as described above. The stimulation cycle was then repeated in this manner every 6 days.

Assay for IL-2 activity in culture supernatants of T-lymphocyte lines. Following a secondary stimulation cycle, viable cells were isolated by recovery at the interface of a Ficoll-Hypaque gradient, washed extensively with RPMI 1640, and suspended in LCM to 10⁶ cells per ml. One milliliter of this suspension was added to 4 × 10⁶ APCs plus Ag in 1 ml of LCM in each well of 24-well culture plates. These cultures were then incubated for 24 h in the absence of exogenous IL-2. At the end of each incubation period, cell-free supernatants were collected by centrifugation of harvested cultures. Supernatants were frozen at −70°C until assayed.

IL-2 activity in supernatants was assayed by the method of Larsson and Coutinho (15). Mouse spleen cells were stimulated for 72 h in LCM containing 5 µg of concanavalin A (ConA; Sigma Chemical Co., St. Louis, Mo.) per ml. Cells were recovered and washed twice by centrifugation through Hanks balanced salt solution containing 10 mg of α-methyl mannoside (Sigma). T-cell lymphoblasts were then recovered at the interface of a Ficoll-Hypaque gradient, washed twice more in Hanks balanced salt solution containing α-methyl mannoside and once in Hanks balanced salt solution alone. Lastly, cells were suspended to 2 × 10⁶/ml in LCM, and 0.1-ml volumes (2 × 10⁴ cells per well) were added to 0.1-ml volumes of test supernatants in 96-well plates for 0.2-ml total volumes. Proliferation was measured by addition of 1 µCi of [methyl-3H]thymidine blasts which were refractory to further stimulation by ConA (6). This was confirmed in our study by addition of ConA or rIL-2 to control cultures.

Analysis of T cells by flow cytometry. T-lymphocyte cultures were assessed by flow cytometry for the percentage of cells possessing the surface markers Thyl.1, L3T4, Lyt2.2, and immunoglobulin as previously described (22). Rat anti-Thyl.1 was obtained commercially (Becton Dickinson, Mountain View, Calif.). Concentrated monoclonal antibody preparations were derived by 50% ammonium sulfate fractionation of supernatants of either GK1.5 hybridoma-producing rat anti-L3T4 immunoglobulin G2b (IgG2b) or 2.43 hybridoma-producing rat anti-Lyt2.2 IgG2b (22). Fluorescein isothiocyanate-conjugated goat anti-rat IgG was obtained commercially (Southern Biotechnology Associates, Birmingham, Ala.), as was a fluorescein isothiocyanate goat anti-mouse immunoglobulin (Organon Teknika, Malvern, Pa.). Samples were analyzed on a Cytocfluorograph IFS flow cytometer (Becton-Dickinson, Sunnyvale, Calif.) and gated to exclude nonviable cells. The percentage of positive cells was calculated after subtraction of the percentage of cells that absorbed fluorescence nonspecifically by using fluorescein isothiocyanate-conjugated goat anti-rabbit IgG (Organon Teknika) in a control histogram.

Infection of nu/nu mice. nu/nu mice were infected as previously reported (26). Mice were inoculated intravaginally on three consecutive days while they were under sodium pentobarbital anesthesia with a suspension of McCoy cell-grown MoPn containing 10⁶ inclusion-forming units in 0.03 ml of sucrose phosphate glutamate buffer (pH 7.2). Mice were 6 to 7 weeks old at the time of infection. Those that did not become infected by this protocol were eliminated from the experiment.

Adaptive transfer of T-cell lines. Approximately 48 h into a secondary stimulation cycle, T-cell lines were harvested and viable cells were isolated over a Ficoll-Hypaque gradient. After extensive washing in RPMI 1640, the cells were suspended in RPMI 1640 and 10⁷ cells were injected intravenously into nu/nu mice which had been infected 3 days previously.

Assessment of infection. Infection was assessed by isolation of MoPn from cervical-vaginal swabs in McCoy cells (1). Inclusions were subsequently visualized by indirect immunofluorescence by a standard technique (8, 23).

Quantitation of antibody responses. Sera were obtained
TABLE 1. Experimental design

<table>
<thead>
<tr>
<th>Expt</th>
<th>T-cell line transferred</th>
<th>No. of days in culture</th>
<th>Source</th>
<th>Duration of extp*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>MMT2</td>
<td>13</td>
<td>nul/+ spleen</td>
<td>67</td>
</tr>
<tr>
<td>B</td>
<td>MMT2</td>
<td>56</td>
<td>nul/+ spleen</td>
<td>90</td>
</tr>
<tr>
<td>C</td>
<td>MMT2.1</td>
<td>18</td>
<td>nul/nu spleen</td>
<td>59</td>
</tr>
<tr>
<td>D</td>
<td>MMT2.2</td>
<td>25</td>
<td>nul/nu spleen</td>
<td>80</td>
</tr>
<tr>
<td>E</td>
<td>MMT2.1.4</td>
<td>25</td>
<td>MMT2.1</td>
<td>80</td>
</tr>
<tr>
<td>F</td>
<td>MMT2.1.2</td>
<td>25</td>
<td>MMT2.1.2</td>
<td>80</td>
</tr>
</tbody>
</table>

* Number of days posttransfer when the experiment was terminated.

† This T-cell line was derived from three nul/+ mice that had resolved the infection in experiment A.

‡ The MMT2.1.4 and MMT2.1.2 cell lines were derived from the MMT2.1 T-cell line following treatment with anti-Lyt2.2 or anti-L3T4 monoclonal antibody and complement, respectively.

from anesthetized mice by puncture of the retroorbital venous plexus. Vaginal secretions were obtained by flushing the vaginal vault with phosphate-buffered saline as described elsewhere (23). Serum IgG and secretion IgA responses were measured by an enzyme-linked immunoadsorbant assay (ELISA) as described elsewhere (10).

**Assessment of DTH.** Delayed-type hypersensitivity (DTH) responses were assessed as previously described (1). Briefly, hind footpads were injected with 5 μg of Ag in 0.05 ml of phosphate-buffered saline. Footpad thickness was measured just prior to injection and at 24 h postinjection. As a control for nonspecific inflammation, the contralateral footpads of some mice were injected with phosphate-buffered saline only. The minimum increase in footpad thickness for a positive DTH response was considered to be 0.3 mm by previously described criteria (1). While not performed in the current study, no significant reactions have ever been noted when infected mice were tested with host cell control (HeLa) antigen.

**T-lymphoblast transformation assays.** Both antigen-specific and mitogen-induced lymphoblast transformation assays for nylon wool-enriched splenic T cells were performed as previously described (23). Spleens were aseptically removed, teased into single-cell suspensions, and passed over nylon wool columns. The effluent cells (T enriched) were suspended in LCM and aliquoted into 96-well culture plates at 2 × 10⁵ cells per well with 2 × 10⁵ APCs per well. ConA or Ag was added to wells for a final concentration of 5 μg/ml. The culture plates were incubated at 37°C in a humidified atmosphere containing 5% CO₂. The proliferative response was measured by incorporation of [³H]thyminde over the last 4 h of a 72-h incubation period (ConA) or the last 18 to 24 h of a 5-day incubation period (Ag). Results are reported as mean counts per minute (± the standard deviation) of quadruplicate cultures. Unstimulated T-enriched cells from each animal plus APCs were included as controls, and a positive response is reported for cultures that possessed activity significantly above that of controls. No obvious reaction was seen in the absence of chlamyoidal antigen. In addition, when host cell (HeLa) antigen was tested in culture, no response was noted, indicating that the proliferative response was antigen specific.

**Treatment of T lymphocytes with monoclonal antibody to L3T4 or Lyt2.2 surface markers and complement.** The MMT2.1 T-lymphocyte line (Table 1) was divided into three aliquots. One aliquot was stimulated as described above (MMT2.1). The remaining two aliquots were pelleted by centrifugation (400 × g). The pellets were then suspended in cytotoxicity medium (Accurate Chemicals, Westbury, N.Y.) containing a 1:20 dilution of a concentrated preparation of monoclonal antibody derived from supernatants of either GK1.5 hybridoma-producing rat anti-L3T4 IgG2b, or 2.43 hybridoma-producing rat anti-Lyt2.2 IgG2b (22). The cells were incubated for 45 min at 4°C and washed once in cold cytotoxicity medium, followed by suspension in cytotoxicity medium containing a 1:20 dilution of guinea pig complement (C). The cells were then incubated for 30 min at 37°C, followed by extensive washing in cold RPMI 1640 and restimulation as described above. Hereafter, these cell lines are referred to as MMT2.1.4 (treated with anti-Lyt2.2 plus C) and MMT2.1.2 (treated with anti-L3T4 plus C).

**Experimental design.** The first T-cell line derived (MMT2) was derived from nul/+ mice that had been immunized intravenously with viable MoPn (Table 1). This line was utilized for two adoptive transfer experiments. One transfer was conducted after the line had been in culture for 18 days (experiment A). The second transfer conducted with MMT2 cells was accomplished after 56 days in culture (experiment B).

The second line was derived from three nul/nu mice in experiment A which had resolved the infection. This line, designated MMT2.1, was used in an adoptive transfer after it had been expanded for 18 days in culture (experiment C). It was also the source of two additional lines derived by treatment of MMT2.1 cells with antibody to the L3T4 and Lyt2.2 surface markers and C. These were designated MMT2.1.2 and MMT2.1.4, respectively. Lastly, the MMT2.1, MMT2.1.2, and MMT2.1.4 T-cell lines were expanded in vitro and used in an additional adoptive transfer after a total of 25 days in culture (experiment D).

Blood was collected every 7 to 10 days for the first 30 days posttransfer. Blood and vaginal washings were collected just prior to termination of each experiment. DTH responses were also assessed just prior to the termination of each experiment. Cervical-vaginal swabs were taken just prior to adoptive transfer, every 4 or 5 days to day 30 posttransfer, and every 10 days thereafter. T-lymphoblast transformation responses of individual animals were assessed in each experiment.

**Statistics.** Thymidine incorporation assays were compared by a one-tailed t test. Infection kinetics were compared by Wilcoxon signed-ranks analysis. The kinetics of the antibody response were analyzed by two-factor (treatment group and days) analysis of variance with repeated measures on one variable (days). Differences were considered significant at P < 0.05.

**RESULTS**

Surface phenotype of T-lymphocyte lines. Table 2 shows the percentage of cells in each line that stained positive for the surface markers immunoglobulin (pan-B cell), Thy1.2...
TABLE 3. Proliferation and IL-2 production of T-lymphocyte lines in response to MoPn antigen

<table>
<thead>
<tr>
<th>T-lymphocyte line</th>
<th>Mean ± SD cpm, 10^3 (control)</th>
<th>Proliferative response^a</th>
<th>IL-2 activity^a</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMT2</td>
<td>26.4 ± 3.5 (1.1 ± 0.1)</td>
<td>32.3 ± 4.9 (0.5 ± 0.1)</td>
<td></td>
</tr>
<tr>
<td>MMT2.1</td>
<td>63.9 ± 2.1 (18.3 ± 2.2)</td>
<td>2.1 ± 0.2 (0.3 ± 0.2)</td>
<td></td>
</tr>
<tr>
<td>MMT2.1.4</td>
<td>ND</td>
<td>4.1 ± 0.4 (0.3 ± 0.1)</td>
<td></td>
</tr>
<tr>
<td>MMT2.1.2</td>
<td>ND</td>
<td>2.3 ± 0.3 (0.3 ± 0.1)</td>
<td></td>
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</table>

^a The values are means of quadruplicate cultures of T-enriched spleen cells in response to MoPn during the initial stimulation period in vitro. Controls were unstimulated cultures run in parallel.

The values are means of quadruplicate cultures of ConA blasts in response to culture supernatants of the designated T-cell lines stimulated with MoPn plus APC in the absence of exogenous IL-2. Controls were ConA blasts in medium alone.

ND, Not done.

(pan-T cell), L3T4 (helper-inducer T cell), and Lyt2.2 (suppressor-cytotoxic T cell). The MMT2 cell line most closely resembled the staining pattern of noncultured T-enriched spleen cells from a normal nulnu mouse.

The MMT2.1 cell line was derived from nulnu mice that had received an adoptive transfer from the MMT2 line and subsequently resolved the infection. This T-cell line was predominantly Lyt2.2+ (92%), but histograms revealed a distinct portion of cells that stained positive for the L3T4 surface marker. An attempt was made to enrich this line further for Lyt2.2+ cells by treatment with antibody to the L3T4 surface marker plus C. Simultaneously, another portion of the MMT2.1 cell line was treated with anti-Lyt2.2 plus C. While the treatments with anti-Lyt2.2 plus C did not achieve substantial depletion of the targeted cells (probably because of the predominance of Lyt2.2+ cells), a distinct population of L3T4+ cells was present in the MMT2.1.4 cell line but was not readily apparent in histograms of the MMT2.1.2 line (data not shown). Additionally, these two cell lines showed significantly different characteristics in vitro and in vivo in recipients of adoptive transfers (results discussed below).

Responses of T-lymphocyte lines to antigen stimulation. The responses of T-cell lines to antigen are shown in Table 3. Both the MMT2 and MMT2.1 cell lines showed significant proliferative responses to MoPn above that of APCs in the absence of MoPn (P < 0.0001; two-tailed t test). Also, both cell lines possessed IL-2 activity in culture supernatants in response to MoPn antigen plus APC and in the absence of exogenous IL-2. However, the MMT2 line possessed a significantly higher amount of IL-2 activity in culture supernatants (P < 0.0001; two-tailed t test). The IL-2 activity produced by the MMT2.1.4 cell line, which possessed a distinct population of L3T4+ cells, was significantly higher than that of either the MMT2.1 or MMT2.1.2 line (P < 0.003; two-tailed t test). Preliminary data also indicate that each of these lines is capable of producing IFN-γ in response to MoPn antigen plus APCs.

Several unsuccessful attempts were made to incorporate periods of rest between stimulation cycles in the MMT2 and MMT2.1 T-cell lines. These lines routinely sustained marked loss of viability if placed in culture with irradiated syngeneic filler cells without antigen and IL-2 or with IL-2 alone for longer than 24 h (data not shown). Therefore, the constant presence of MoPn antigen and APCs was required to sustain viability. Although addition of exogenous IL-2 was not required to sustain viability, viable cell yields and proliferative responses were enhanced in the presence of rIL-2 (data not shown).

Adaptive transfer of MoPn-specific T-cell line MMT2. To determine whether the MMT2 cell line derived from immunized +/nu mice could resolve a chlamydial genital infection, two separate experiments were performed in which 10^7 MMT2 cells were adoptively transferred into 5 and 10 (experiments A and B, respectively) previously infected nu/nu mice. Three and nine of these recipients, respectively, resolved their infections. Interestingly, transferred cells which had been in culture for only 13 days resolved the infection more quickly than cells in culture for 56 days (P < 0.02; Wilcoxon signed-ranks test) (data not shown).

All of the mice that resolved the infection developed significant DTH responses, antibody titers in serum, and both antigen-specific and mitogen-induced T-cell blast transformation responses. DTH responses to MoPn were absent in the three mice that did not resolve the infection.

Adaptive transfer of an antigen-specific T-cell line derived from nu/nu mice—MMT2.1. As mentioned previously, the MMT2.1 T cell line was derived from nu/nu mice that resolved the infection in experiment A. This was done to enrich for T-cell populations that were capable of resolving the infection. When infected nu/nu mice received an adoptive transfer with 10^7 MMT2.1 cells, seven of eight animals resolved the infection (experiment C; data not shown). The mean day of resolution posttransfer was day 19.

Of the seven animals that resolved the infection, all developed significant DTH responses. Additionally, seven of seven immune animals showed significant T-lymphoblast transformation reactions to both MoPn and ConA. One of the seven mice never developed an IgG antibody response in serum, while the rest showed high titers. The remaining mouse that did not resolve the infection displayed no DTH reaction. A low antibody titer and an MoPn-specific T-lymphoblast transformation response were also noted for this animal.

Effect of enrichment for T-cell subpopulations on the MMT2.1 T cell line. The MMT2.1 cell line was treated with anti-L3T4 plus C (MMT2.1.2) or anti-Lyt2.2 plus C (MMT2.1.4) and expanded for 2 weeks in culture. Approximately 10^7 cells from each of these lines and the MMT2.1 line were then transferred into infected nu/nu recipients (experiment D). The infection curve obtained with the L3T4-depleted (L3T4+ Lyt2.2+) MMT2.1.2 line was not significantly different from that obtained with the MMT2.1 line (Fig. 1), although 9 of 10 animals resolved the infection with the former, compared with 6 of 10 with the latter, by day 50. However, the infection was significantly shorter in mice that received the MMT2.1.4 (L3T4+ Lyt2.2+) line than in recipients of either the MMT2.1.2 (L3T4+ Lyt2.2+) or MMT2.1 line (P < 0.02; Wilcoxon signed-ranks test). Also, nine of nine animals that received the MMT2.1.4 line resolved the infection.

When immunological parameters were assessed, it was found that, unlike previous experiments, the DTH response was not present in all of the animals that had resolved the infection (Table 4). Also, four of the MMT2.1 recipients that did not resolve the infection had positive DTH responses. A similar pattern was seen for MoPn-specific T-lymphoblast transformation responses. With the exception of two MMT2.1.2 (L3T4+ Lyt 2.2+) recipients, all animals produced IgG antibody to MoPn in serum. However, the development of the antibody response in the recipients of this line was significantly depressed as opposed to that of MMT2.1.4 (L3T4+ Lyt2.2+) or MMT2.1 recipients when...
TABLE 4. Immune parameters of nu/nu mice following adoptive transfer of T-cell lines (experiment D)

| Cell line and outcome (no. of mice) | No. with DTH (response range) | No. antibody positive (titer range) | No. with T-lymphoblast transformation
titer (range [cpm, 10^3]) in response to: |
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<tr>
<td></td>
<td></td>
<td></td>
<td>MoPn</td>
</tr>
<tr>
<td>MMT2.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolved (6)</td>
<td>5 (0.6–0.85)</td>
<td>6 (180–10,240)</td>
<td>6 (6.2–54.1)</td>
</tr>
<tr>
<td>Infected (4)</td>
<td>4 (0.3–1.0)</td>
<td>4 (20–10,240)</td>
<td>3 (6.6–83.3)</td>
</tr>
<tr>
<td>MMT2.1.4, resolved (9)*</td>
<td>9 (0.3–1.25)</td>
<td>9 (5,120–10,240)</td>
<td>8 (2.5–69.8)</td>
</tr>
<tr>
<td>MMT2.1.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resolved (9)</td>
<td>6 (0.35–1.25)</td>
<td>8 (160–2,560)</td>
<td>6 (3.0–79.4)</td>
</tr>
<tr>
<td>Infected (1)</td>
<td>0</td>
<td>1 (80)</td>
<td>1 (2.2)</td>
</tr>
</tbody>
</table>

a The range values represent increases in footpad thickness in millimeters for those showing a positive result.

b The range values represent the IgG titers in serum as measured by ELISA for those showing a positive result.

c Any response significantly above the background response of unstimulated cells in the presence of APCs was considered a positive result.

d None of the mice that received this cell line were infected.

compared by a two-factor (T-cell line and days) analysis of variance with repeated measures on one variable (days) (P < 0.0001; Fig. 2). Responses of infected and resolved animals to ConA were consistently positive in each group.

**DISCUSSION**

In this study, we demonstrated that T-lymphocyte lines specific for the mouse biovar of C. trachomatis, MoPn, could be established and maintained in culture but still retain the ability to transfer immunity to immunologically naive recipients. Two related lines were developed. The original line was derived from spleen cells of nu/nu mice that had been immunized intravenously with viable MoPn elementary bodies. To select for a line which was more enriched for protective T cells, splenic T cells from nu/nu mice that had received the original line and subsequently resolved the infection were also cultured. These T-cell lines were each greater than 90% positive for panspecific T-cell surface marker Thy1.2. The primary line (MMT2) was 64% L3T4+ and 24% Lyt2.2+, whereas the line derived from nu/nu recipients of the primary line was 9% L3T4+ and 92% Lyt2.2+ (MMT2.1). Both lines were protective despite the differences in subpopulation proportions.

IL-2 activity was greatest in the line containing higher percentages of L3T4+ cells, as one might expect, since Lyt2+ cells do not elaborate IL-2. Additionally, preliminary results have indicated that both of these cell lines produce IFN-γ in response to stimulation with antigen plus APCs.

Also of interest is the observation that T-cell lines derived from the spleen were capable of resolving an infection in the genital tract. Normally, one would not anticipate that spleen cells could routinely home to a mucosal tissue, but it is possible that the cells, while circulating, localized in the genital tract because of the presence of chlamydial antigen and did not require specific receptors for mucosal tissue.

It has been previously reported that long-term-cultured T cells display aberrant homing and functional characteristics (7). In our study, an interesting observation was that T cells in culture for shorter periods were more efficient in resolving the infection after adoptive transfer. This could possibly indicate that homing to the site of the infection, while not necessary, expedites resolution of the infection. Alternatively, the transferred cells or their progeny may have been able to regain at least part of their functional characteristics when returned to an in vivo environment.

Following adoptive transfer into nu/nu mice, many of the
recipients developed MoPn-specific DTH reactions. Indeed, the DTH responses observed for some of the transfer recipients were two to three times higher than previously reported (1, 22, 23). While it has been observed elsewhere that nu/nu mice possess some of the early components of the DTH response (9), the response is immature and is not present at 24 h into the assay, the time when our measurements were reported.

MoPn-specific T-lymphoblast responses were also noted in the majority of adoptive-transfer recipients of these two T-cell lines. These responses closely mirrored the DTH responses in that mice that developed significant DTH reactions often possessed significant antigen-specific T-lymphoblast responses. Lastly, in most recipients, transfer of these T-cell lines also induced production of IgG antibody titers in serum that were markedly higher than the low and intermittent titers previously reported for unaltered MoPn-infected nu/nu mice (26). It should also be noted that in the present study, splenic T-cell lines were capable of inducing production of IgA antibody to various degrees in the vaginal secretions of approximately two-thirds of the nu/nu mice tested (data not shown).

Attempts to enrich the MMT2.1 line for the L3T4+ or Lyt2.2+ subpopulation of T cells were made. Although we did not obtain a line that was predominantly L3T4+, we were able to produce a line that was largely Lyt2.2+ but had a distinct population of L3T4+ cells (MMT2.1.4) and another line that was exclusively Lyt2.2+ (MMT2.1.2). Both of these lines were capable of inducing resolution of the infection, with 9 of 9 MMT2.1.4 and 9 of 10 MMT2.1.2 recipients resolving the infection.

The functional difference between these T-cell lines was evident. In vitro, the MMT2.1.4 (L3T4+ Lyt2.2+) line displayed a significantly greater amount of IL-2 activity in culture supernatants in response to MoPn antigen than did the MMT2.1.2 (L3T4+ Lyt2.2+) line. In vivo, adoptive transfer with the MMT2.1.4 (L3T4+ Lyt2.2+) cell line resolved the infection significantly quicker than that with the MMT2.1.2 (L3T4+ Lyt2.2+) or the parent MMT2.1 T-cell line. Also, development of the antibody response was depressed in the absence of L3T4+ cells when MMT2.1.2 line recipients were compared with MMT2.1.4 and MMT2.1 line recipients.

Of additional importance in this study is the derivation of a protective T-cell line from nu/nu mice that had previously received adoptive transfer of another line and subsequently resolved the infection (MMT2.1). It is tempting to speculate that this process selectively enriched for a population of antigen-specific T cells that were important in resolution of the infection. Interestingly, these derived cells were predominantly of the Lyt2.2+ phenotype. It is somewhat paradoxical that animals should select for CD8 cells when, alone, these cells do not appear to be as efficient in resolving the infection. As we have demonstrated, these cells are indeed capable of eliminating the infection, but it is possible that synergism between CD4 and CD8 cells is required for the most efficient protective immune response. Certainly, CD4 T cells are necessary for sensitization of CD8 cells.

The reason for the apparent selection of CD8 cells by nude mice is not clear. Others have reported that nu/nu mice possess a pool of replication-defective Thy1.2+ CD4+ CD8- lineages that, upon certain stimulation, give rise to progeny that are predominantly CD8+ (11, 13). Additionally, aged nu/nu mice have been shown to possess higher ratios of CD8 to CD4 cells than their normal counterparts (16). It is possible that this MMT2.1 cell line represents resident nu/nu T cells that were affected in some way by the transfer of activated T cells. Alternatively, some endogenous factor may have been involved in the selection of Lyt2.2+ cells.

It is interesting that the MMT2.1 cells proliferated in response to MoPn and APCs in culture conditions that normally favor the growth of L3T4+ cells only. Indeed, this cell line routinely had higher viable cell yields in culture than did the original MMT2 T-cell line. Also, a combined total of 16 of 17 recipients of this cell line displayed DTH reactions and 16 of 17 had high antibody titers following transfer. This may be a result of the small population (~10%) of L3T4+ cells present in this line. Alternatively, these reactions could be the result of maturation of resident T cells following transfer as described above.

In the first three experiments, development of DTH reactions seemed to relate to the ability of recipient nu/nu mice to resolve the infection. However, in the latter experiment, one animal that received MMT2.1 cells and three animals that received MMT2.1.2 (L3T4− Lyt2.2+) cells did not display significant DTH reactions and yet resolved the infection. Additionally, four MMT2.1 line recipients developed significant DTH responses and did not resolve the infection. Therefore, it is difficult to relate DTH reactions to resolution of the infection.

We also observed production of antigen-specific T-lymphoblast transformation in many of the nu/nu mice that resolved the infection. As with the DTH reactions, these responses were conspicuously absent in other mice that resolved the infection and were present in several of those that did not. Therefore, it is also difficult to associate this type of T-cell reactivity with resolution of the infection. Interestingly, a vigorous response of T-enriched spleen cells to the T-cell mitogen ConA was observed in all nu/nu recipients in each experiment, indicating universal reconstitution of T cells. Although other reports indicate that some immature T cells are present in nu/nu mice of this age, these mice do not normally respond to mitogen stimulation by proliferation (13). It is certainly possible that different clones from the lines became established in different mice, some having protective capability but not the ability to elicit a detectable DTH response, while others could produce a DTH response but no protection.

As with the DTH reactions and antigen blast transformation, it is difficult to draw conclusions relating antibody production with resolution of the infection in these experiments, since a broad range of antibody titers was found in animals that resolved the infection, as well as in those that did not. However, while antibody may play a participatory role in resolution of this infection, previous reports have indicated that antibody is not essential to resolution of the infection (23). Also, nu/nu mice that received a passive antibody transfer were unable to resolve the infection (23a). Therefore, production of antibody in these experiments indicates that resident B cells receive assistance from T cells that are otherwise not present or functional.

These results support our previous findings that mice depleted of either L3T4+ or Lyt2.2+ T cells are capable of resolving MoPn genital infection (22). Since IFN-γ production is common to both L3T4+ and Lyt2.2+ T cells (11, 12, 17), it is possible that this is a key factor in resolution of the infection. Indeed, others have also shown that IFN-γ can inhibit chlamydial replication in vitro (5, 29). Additionally, IFN-γ has been implicated in protection of mice from lethal MoPn lung infections (4, 30). Lastly, there were several nu/nu recipients of the L3T4− Lyt2.2+ MMT2.1.2 T-cell line that had little or no indication of helper-inducer T-cell
activity, as assessed by low DTH activity, antibody responses, and T-lymphoblast transformation responses. However, these mice still resolved the infection. This observation could implicate cytotoxic T-cell activity against MoPn-infected cells in these animals, although cell-mediated cytotoxicity of Chlamydia-infected cells has not been convincingly established (14, 18, 19).

Nevertheless, the primary importance of successful propagation and adoptive transfer of cultured antigen-specific T-cell lines that induce resolution of MoPn genital infection lies in the possibility of deriving clones from these lines. In future studies, successful propagation and adoptive transfer of cloned MoPn-specific T-cell lines should allow for more clear-cut dissection of the mechanisms by which these cells mediate resolution of this infection, as well as identification of chlamydial antigens capable of eliciting protective cell-mediated immune responses in this model.

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REFERENCES


