

Antigen-Specific CD8⁺ T Cells Respond to *Chlamydia trachomatis* in the Genital Mucosa¹

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Following sexual transmission, *Chlamydia trachomatis* specifically targets genital tract epithelial cells. Because epithelial cells are readily recognized by CD8⁺ T cells, the response of CD8⁺ T cells to *Chlamydia* infection has been explored in a number of studies. It has been shown that CD8⁺ T cells are present in the genital tracts of mice following *C. trachomatis* infection, but the specificity of these T cells has remained undefined. To determine whether *Chlamydia*-specific CD8⁺ T cells migrate to the genital tract in response to *Chlamydia* infection, we generated retrogenic mice that express a TCR specific for a *Chlamydia*-specific T cell Ag CrpA. T cells from the retrogenic mice were transferred into naive recipient animals to increase the frequency of *Chlamydia*-specific T cells to a level at which they could be tracked during primary infection. We observed that the *Chlamydia*-specific retrogenic T cells proliferated in lymph nodes draining the genital tract in response to genital infection with *C. trachomatis*. Furthermore, we found that these cells acquired the ability to produce IFN- γ and migrated into the genital mucosa of the infected mice. *The Journal of Immunology*, 2006, 177: 7974–7979.

The bacterium *Chlamydia trachomatis* is an obligate intracellular pathogen that is the most common cause of bacterial sexually transmitted disease in the United States and a major public health concern. In mouse models of *Chlamydia* infection, CD8⁺ T cells can enhance the clearance of the bacteria. CD8⁺ T cells can be detected at the site of infection (1, 2) and adoptive transfer of immune CD8⁺ T cells can protect against *Chlamydia* challenge (3–5).

Interestingly, Ags recognized by CD8⁺ T cells are typically derived from cytosolic proteins, yet *C. trachomatis* is strictly confined to a vacuole during the intracellular stages of development. Because the majority of *C. trachomatis* proteins also are confined to the vacuole, they are not typically processed for recognition by CD8⁺ T cells. However, during replication there appears to be a limited subset of *Chlamydia* proteins that do have access to the cytoplasm, subjecting them to possible recognition by CD8⁺ T cells. These include membrane proteins that lie at the interface between the *Chlamydia* vacuole and the host cytosol (6). Two of these proteins, Cap1 and CrpA, are recognized by CD8⁺ T cells following systemic *Chlamydia* infection (7, 8). Of the two Ags, CrpA elicits the more dominant CD8⁺ T cell response following *Chlamydia* infection. Immunization of mice with recombinant vaccinia expressing CrpA provides immunity against systemic *Chlamydia* challenge, suggesting that CrpA-specific CD8⁺ T cells can play a role in limiting infection with *C. trachomatis* (8).

Infection with *C. trachomatis* occurs naturally in the genital tract, where the organisms have a strict tropism for epithelial cells (9). Although CD4⁺ T cells contribute significantly in controlling

Chlamydia infection (10, 11), they may not recognize *Chlamydia*-infected epithelial cells in the genital mucosa because epithelial cells typically express low levels of MHC class II. Because epithelial cells express abundant levels of MHC class I, we sought to determine whether *Chlamydia*-specific CD8⁺ T cells are recruited to the genital epithelium in response to *Chlamydia* infection of the genital tract. Specifically, we wanted to track the localization of CrpA-specific T cells, their proliferation, and their effector functions during the early stages of primary *Chlamydia* infection. However, one difficulty in studying Ag-specific T cell responses in vivo is the low frequency of these cells in a naive mouse. To increase the number of Ag-specific T cells so that the initial events following infection can be monitored, investigators have transferred T cells from TCR transgenic mice into naive mice (12–14). Alternatively, Ag-specific T cells can be obtained from TCR retrogenic mice generated using a newly developed technique that combines retroviral transduction and bone marrow reconstitution (15). Retrogenic mice offer a number of advantages over traditional TCR transgenic mice, including a faster production time. We have used this new approach to generate retrogenic mice expressing a TCR specific for CrpA. T cells from these mice have allowed us to examine the priming and trafficking of *Chlamydia*-specific CD8⁺ T cells following genital infection with *C. trachomatis*. We discovered that CrpA-specific retrogenic T cells proliferate in the draining lymph nodes (DLN)³ following genital infection with *C. trachomatis*. These T cells then develop effector function and migrate into the infected genital mucosa. We describe in this study a new tool for studying Ag-specific CD8⁺ T cells in the genital mucosa following infection.

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Materials and Methods

Mice

Female C57BL/6 mice (H-2^b) and RAG1^{-/-} mice (H-2^b) were obtained from The Jackson Laboratory and used at 7–9 wk of age. All experiments were approved by the Institutional Animal Care and Use Committee.

³ Abbreviations used in this paper: DLN, draining lymph node; BMM, bone marrow-derived macrophage; IFU, inclusion forming unit.

Tissue culture and cell lines

Cell line 1308.1 was maintained in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FBS, L-glutamine, 50 μ M 2-ME, 50 U/ml penicillin, and 50 μ g/ml streptomycin. Cell lines 293T and GP plus E86 (16) were maintained in complete DMEM (Invitrogen Life Technologies) supplemented with 10% FBS, L-glutamine, 50 U/ml penicillin, and 50 μ g/ml streptomycin. Bone marrow derived macrophages (BMM) were cultured from C57BL/6 mice by culturing bone marrow cells in RPMI 1640 (Invitrogen Life Technologies) supplemented with 20% FBS, L-glutamine, 50 U/ml penicillin, 50 μ g/ml streptomycin, and 20% supernatant from L929 cells (American Type Culture Collection).

Growth, isolation, and detection of *C. trachomatis*

Elementary bodies of *C. trachomatis* serovar L2 434/Bu were propagated within McCoy cell monolayers grown in MEM (Invitrogen Life Technologies) supplemented with 10% FCS, 1.5 g/L sodium bicarbonate, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate and purified by density gradient centrifugation as described (17). Aliquots were stored at -70°C in a medium containing 250 mM sucrose, 10 mM sodium phosphate, and 5 mM L-glutamic acid (SPG medium) (18) and thawed immediately before use. UV-inactivated *C. trachomatis* organisms were generated by subjecting purified elementary bodies to 10 min of UV irradiation.

Stimulation and maintenance of CD8^{+} T cells

C57BL/6 females were immunized with a recombinant vaccinia construct expressing CrpA (8). Splenocytes from mice harvested 21 day after immunization were cultured with irradiated (2000 rad) *Chlamydia*-infected BMM (multiplicity of infection 5:1) and naive, syngeneic splenocytes in RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% FBS, L-glutamine, 50 μ M 2-ME, 50 U/ml penicillin, 50 μ g/ml streptomycin, α -methyl mannoside, and 5% supernatant from Con A-stimulated rat spleen cells. CD4^{+} T cells were depleted using Dynabeads Mouse CD4 according to the manufacturer's protocol (Invitrogen Life Technologies). The CD8^{+} T cells were restimulated with fresh *Chlamydia*-infected BMM every 7 days. The resulting T cell line was cloned by limiting dilution to generate an individual T cell clone we designated NR23.4. Chromium release assays were conducted as previously described (3).

Protection assay

Ten days following restimulation, 5×10^6 NR23.4 T cells were transferred into naive C57BL/6 recipient mice. Control immune mice were infected 3 wk earlier with 10^7 inclusion forming units (IFU) of *C. trachomatis* L2. All mice were then challenged i.v. with 10^7 IFU of *C. trachomatis* L2 and spleens were harvested 3 days later. To quantitate *C. trachomatis*, spleens from infected mice were homogenized, sonicated, diluted in SPG medium, and applied to McCoy cell monolayers. Inclusions were enumerated by immunofluorescent microscopy 36 h postinfection and reported as IFU per spleen.

Flow cytometry and Abs

FITC, PE, PerCP, or allophycocyanin conjugated Abs specific for CD4, CD8, CD90.2, and IFN- γ were purchased from BD Biosciences. The D^{b} /CrpA₆₃₋₇₁ tetramer was obtained from the National Institute of Allergy and Infectious Diseases tetramer facility, whereas the D^{b} /GP₃₃₋₄₁ tetramer was purchased from Immunomics. Data were collected on a BD Biosciences FACSCalibur flow cytometer and analyzed using CellQuest software. Intracellular cytokine staining of NR23.4 was performed by stimulating cells for 5 h in the presence of GolgiPlug (BD Biosciences) and untreated, peptide-pulsed (100 nM of either CrpA₆₃₋₇₁ or YopH₂₄₉₋₂₅₇) or *Chlamydia*-infected (multiplicity of infection 5:1) BMM. Intracellular cytokine staining of retrogenic cells was performed by stimulating cells for 4 h in the presence of PMA (50 ng/ml; MP Biomedicals), ionomycin (1 μ g/ml; Sigma-Aldrich), and GolgiPlug (BD Biosciences). Cells were permeabilized with the Cytofix/Cytoperm Plus kit (BD Biosciences). PE-conjugated rat IgG1 (BD Biosciences) was used as an isotype control Ab. In experiments with SNARF, the SNARF-labeled retrogenic cells injected into the flow cytometer were live and identified by gating on GFP⁺ cells. In experiments with CFSE, retrogenic cells needed to be fixed and permeabilized following cell surface staining to eliminate GFP fluorescence, which masks CFSE fluorescence when analyzed by flow cytometry. The CFSE-labeled retrogenic T cells were identified by staining for CD90.2 instead of gating on GFP⁺ cells.

Retroviral constructs and producer cell lines

The rearranged TCR from NR23.4 used the V α 4JTA13 and V β 8.2J β 2.5 receptor chains. Total RNA was isolated from NR23.4 using Ultraspec

RNA (Biotech), treated with RNase-free DNase (Promega), and converted into cDNA using MMLV Reverse Transcriptase (Invitrogen Life Technologies). A TCR construct was produced by recombinant PCR and cloned into a 2A peptide-linked, GFP-labeled retroviral vector previously described (15, 19, 20). Expression of the construct was verified using TransIT-293 (Mirus) to transiently transfect 293T cells with each construct along with a multicistronic CD3 vector provided by D. Vignali (St. Jude Children's Research Hospital, Memphis, TN). A retroviral producer cell line was generated as described (19, 20). Briefly, 293T cells were transiently transfected using TransIT-293 (Mirus) and a retroviral producer cell line was generated by repeated transduction of GP plus E86 cells with retroviral supernatant. The OTI retroviral producer cell line was also provided by D. Vignali, St. Jude Children's Research Hospital.

Retroviral-mediated stem cell gene transfer

Retroviral transduction of murine bone marrow cells was performed as described (15, 19, 20). Briefly, 8- to 10-wk-old RAG1^{-/-} donor mice were injected with 5 mg of 5-fluoracil (InvivoGen). Two days later, bone marrow cells were harvested and cultured in complete DMEM (Invitrogen Life Technologies) with 20% FBS, 20 ng/ml IL-4, 50 ng/ml IL-6, and 50 ng/ml stem cell factor (PeproTech). One day later, bone marrow cells were added to the retroviral producer cell lines described earlier and transduced for another 2 days. Approximately 3×10^6 transduced bone marrow cells were injected i.v. with 2 U/ml heparin (Sigma-Aldrich) into irradiated (450 rad) RAG1^{-/-} recipient mice. Mice were analyzed for TCR expression by orbital bleeding 6–10 wk after transplantation.

Adoptive transfer and tissue preparation

A single cell suspension of spleens and peripheral lymph nodes was harvested from retrogenic mice and stained with the fluorescent dyes SNARF (3 μ g/ml; Molecular Probes) or CFSE (5 μ M; Molecular Probes). Recipient mice were injected i.v. with $1-2 \times 10^6$ labeled retrogenic cells. Where indicated, mice were infected i.v. with 10^7 IFU of *C. trachomatis* L2 1 day after the transfer of cells. For genital tract infections, mice were treated with 2.5 mg of medroxyprogesterone acetate s.c. 1 wk before infection to synchronize the mice into a diestrus state (21, 22). Intrauterine infection was conducted by inoculating the uterus with 10^6 IFU of *C. trachomatis* serovar L2 in 5 μ l of SPG medium. At various times postinfection, single cell suspensions of splenocytes or DLN taken from the iliac lymph nodes were prepared and analyzed by flow cytometry as described earlier. To isolate cells from the genital mucosa, genital tracts (oviduct, uterus, and cervix) were removed from mice and digested with collagenase (type XI; Sigma-Aldrich) for 1 h before staining and flow cytometry.

Results

Generation and characterization of a CrpA₆₃₋₇₁-specific CD8^{+} T cell clone

To generate the retrogenic mice used in this study, we first identified and cloned a CrpA-specific TCR that the mice would be engineered to express. This TCR was obtained from a T cell clone designated NR23.4 that was generated by stimulating splenocytes from a CrpA immune mouse on *Chlamydia*-infected BMM. NR23.4 bound a tetramer consisting of H2-D^b complexed to the CrpA epitope CrpA₆₃₋₇₁ but not a control tetramer consisting of H2-D^b complexed to GP₃₃₋₄₁ from lymphocytic choriomeningitis virus (Fig. 1A), suggesting that the TCR expressed by NR23.4 was CrpA₆₃₋₇₁-specific.

To further verify that NR23.4 was specific for CrpA₆₃₋₇₁, we tested whether the clone produced IFN- γ in response to CrpA₆₃₋₇₁. Previous work from our laboratory has shown that the protective ability of *Chlamydia*-specific CD8^{+} T cells depends on the ability of these cells to secrete IFN- γ (23). We incubated NR23.4 with CrpA₆₃₋₇₁-pulsed BMM and measured IFN- γ production by intracellular cytokine staining. As shown in Fig. 1B, NR23.4 produced significant levels of IFN- γ in response to CrpA₆₃₋₇₁-pulsed BMM. The response was specific for CrpA₆₃₋₇₁ because BMM pulsed with the H2-D^b-restricted peptide epitope YopH₂₄₉₋₂₅₇ from *Yersinia enterocolitica* did not stimulate NR23.4 to secrete IFN- γ . Furthermore, NR23.4 produced IFN- γ in response to BMM infected with *C. trachomatis*, demonstrating that NR23.4 recognized CrpA₆₃₋₇₁ during *Chlamydia* infection of cells in vitro. Lastly,

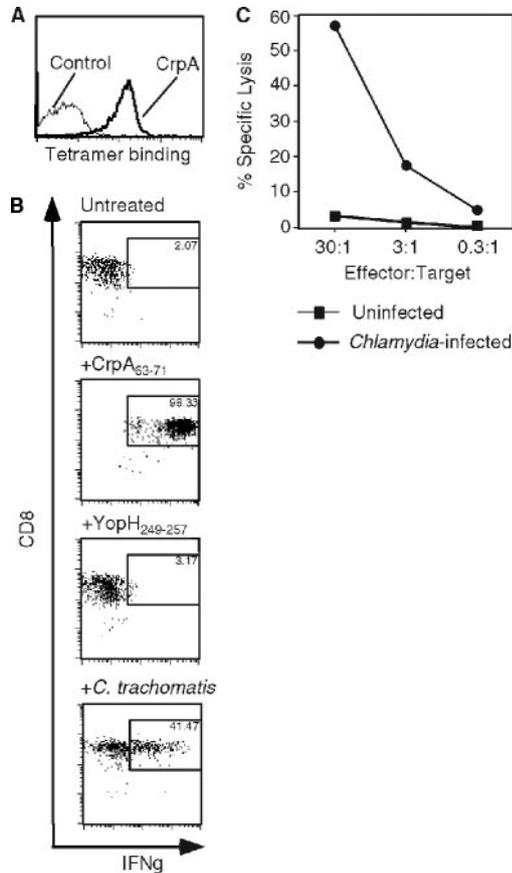


FIGURE 1. CD8⁺ T cell clone NR23.4 is CrpA₆₃₋₇₂-specific and recognizes *Chlamydia*-infected cells in vitro. **A**, NR23.4 was stained with a control GP₃₃₋₄₁-specific (thin line histogram) or a CrpA₆₃₋₇₁-specific (thick line histogram) tetramer. Results were gated on live, CD8⁺ T cells. **B**, NR23.4 was incubated with BMMs that were untreated, CrpA₆₃₋₇₁-pulsed, YopH₂₄₉₋₂₅₇-pulsed, or *Chlamydia*-infected as indicated. Intracellular IFN- γ levels were determined after 5 h in the presence of brefeldin A. The values shown in the gated region correspond to the percentage of cells that are CD8⁺IFN γ ⁺. **C**, NR23.4 was tested on uninfected or *Chlamydia*-infected 1308.1 epithelial cells in a ⁵¹Cr release assay.

NR23.4 also recognized and lysed *Chlamydia*-infected 1308.1 epithelial cells, demonstrating that this T cell clone recognized *Chlamydia*-infected cells in the context of an epithelial cell infection (Fig. 1C).

Adoptive transfer of NR23.4 protects against *C. trachomatis* challenge

To determine whether T cell clone NR23.4 conferred protection against *C. trachomatis* infection, NR23.4 T cells were transferred into naive C57BL/6 mice. The mice were then challenged i.v. with 10⁷ IFU of *C. trachomatis* L2, and spleens were titrated 3 days later. As shown in Fig. 2, mice that had received NR23.4 had ~10-fold fewer *C. trachomatis* IFU in their spleen relative to mice that had not received NR23.4. The level of protection observed in naive mice that had received NR23.4 appeared to be comparable to the level of protection observed in immune mice that had recovered from *C. trachomatis* infection.

Generation of CrpA₆₃₋₇₁-specific retrogenic mice

Although we were able to use NR23.4 to demonstrate the specificity and protective ability of CrpA₆₃₋₇₁-specific T cells, the NR23.4 clone described has been restimulated extensively in vitro. In vitro restimulation renders the T cells less useful in trafficking

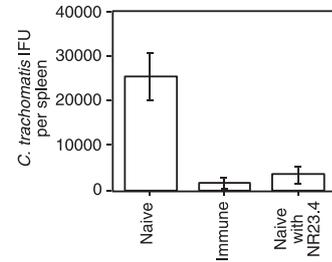


FIGURE 2. Adoptive transfer of NR23.4 protects against *C. trachomatis* infection. Naive mice, immune mice that had recovered from a prior infection, or naive mice transferred with 5 × 10⁶ NR23.4 T cells were infected with 10⁷ IFU of *C. trachomatis* L2. The number of infectious organisms present in the spleens of these mice was determined 3 days later.

studies in vivo because cells that have been cultured extensively do not retain properties of cells that have never encountered Ag (24). To generate an abundant source of CrpA₆₃₋₇₁-specific T cells that have not previously encountered Ag, we used a recently described technique to generate retrogenic mice (15, 20). We isolated cDNA from the NR23.4 T cell clone and cloned the CrpA₆₃₋₇₁-specific TCR into a retroviral vector marked by an internal ribosomal entry site-encoded GFP (19). Retroviral particles encoding the NR23.4 TCR were packaged and used to transduce RAG1^{-/-} bone marrow cells. We then reconstituted irradiated RAG1^{-/-} recipient mice with the transduced bone marrow cells to produce retrogenic mice expressing the NR23.4 TCR. By ~10 wk following bone marrow transplantation, CD8⁺ T cells, but not CD4⁺ T cells, were efficiently selected in the thymus of the retrogenic mice (Fig. 3A). This result was in contrast to a C57BL/6 thymus, where both CD4⁺ and CD8⁺ cells were efficiently selected. In the peripheral blood of the retrogenic mice, there was a significant population of H2-D^b/CrpA₆₃₋₇₁ tetramer-binding GFP⁺ cells that did not bind a control H2-D^b/GP₃₃₋₄₁ tetramer (Fig. 3B). Together, these results showed that we successfully generated retrogenic mice that can be exploited as an abundant source of CrpA₆₃₋₇₁-specific T cells for use in analyzing *Chlamydia*-specific T cell responses in vivo.

CrpA₆₃₋₇₁-specific CD8⁺ retrogenic T cells proliferate following systemic *Chlamydia* infection in vivo

To test whether the CrpA₆₃₋₇₁-specific retrogenic T cells responded to infection in vivo, we tested whether they proliferated following systemic *Chlamydia* infection. We labeled the CrpA₆₃₋₇₁-specific retrogenic T cells with the fluorescent dye SNARF and adoptively

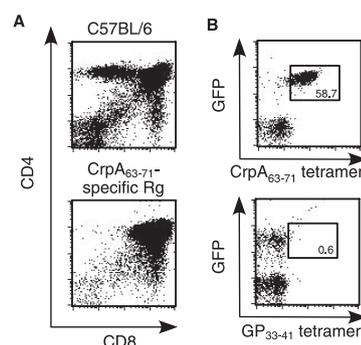


FIGURE 3. CrpA₆₃₋₇₁-specific retrogenic mice have a high frequency of CrpA₆₃₋₇₁-specific T cells. **A**, Thymocytes from C57BL/6 mice or the retrogenic (Rg) mice expressing the NR23.4 TCR were analyzed for CD4 and CD8 expression. Results for retrogenic thymocytes were gated on live GFP⁺ cells to identify cells that had been transduced. **B**, Peripheral blood from the retrogenic mice was stained with a CrpA₆₃₋₇₁-specific or a GP₃₃₋₄₁-specific tetramer.

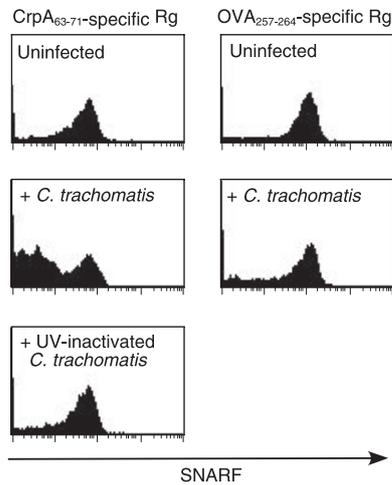


FIGURE 4. CrpA₆₃₋₇₁-specific retrogenic T cells proliferate in response to systemic *C. trachomatis* infection in vivo. One to two million of the CrpA₆₃₋₇₁- or OVA₂₅₇₋₂₆₄-specific SNARF-labeled retrogenic (Rg) T cells were transferred into C57BL/6 recipients. One day later, groups of mice were infected i.v. with 10^7 IFU of live *C. trachomatis* or with an equivalent dose of UV-inactivated *C. trachomatis*. Splenocytes were harvested 3 days later to determine the extent to which retrogenic T cells had proliferated. Retrogenic T cells were identified by gating on live GFP⁺ cells.

transferred $1-2 \times 10^6$ labeled cells into C57BL/6 recipients. SNARF is a fluorescent dye that partitions equally among daughter cells as they divide, so cells that have proliferated have a lower fluorescence intensity than cells that have not proliferated. One day following the transfer, recipient mice were infected i.v. with 10^7 IFU of *C. trachomatis* L2. As shown in Fig. 4, infected mice had a significant population of retrogenic cells with low levels of SNARF, suggesting that these cells had proliferated in response to infection. In addition, proliferation of the CrpA₆₃₋₇₁ retrogenic T cells appeared to be Ag-specific as OVA₂₅₇₋₂₆₄-specific retrogenic T cells failed to proliferate following *Chlamydia* infection. Lastly, UV-inactivated *C. trachomatis* did not stimulate the CrpA₆₃₋₇₁-specific retrogenic cells to proliferate significantly. These results are consistent with the inability of UV-inactivated *C. trachomatis* to develop into the replicating developmental form, thereby limiting production of CrpA and/or access of CrpA to the MHC class I processing pathway.

Following genital infection with C. trachomatis, CrpA₆₃₋₇₁-specific CD8⁺ T cells proliferate, produce IFN- γ , and migrate to the infected genital tract

Because *C. trachomatis* is primarily a pathogen of the genital mucosa, our goal was to determine whether CrpA₆₃₋₇₁-specific CD8⁺ T cells were stimulated during genital infection with *C. trachomatis*. We therefore tested whether intraperitoneal inoculation with *C. trachomatis* induced CrpA₆₃₋₇₁-specific retrogenic T cells to proliferate. To monitor proliferation, we labeled CrpA₆₃₋₇₁-specific retrogenic T cells with the fluorescent dye CFSE that, like SNARF, also is diluted in cell populations as they proliferate. CFSE-labeled retrogenic T cells, which expressed CD90.2, were transferred into CD90.1 congenic recipients so that transferred cells could be readily identified by staining for CD90.2. Recipients were then either mock infected or infected in the uterus with 10^6 IFU of *C. trachomatis*. At various times postinfection, cells from the DLN were harvested and analyzed by flow cytometry. No proliferation was observed 3 and 4 days postinfection, whereas by 5 days postinfection retrogenic T cells in the DLN began proliferating (data not shown). By 6 days postinfection, extensive proliferation

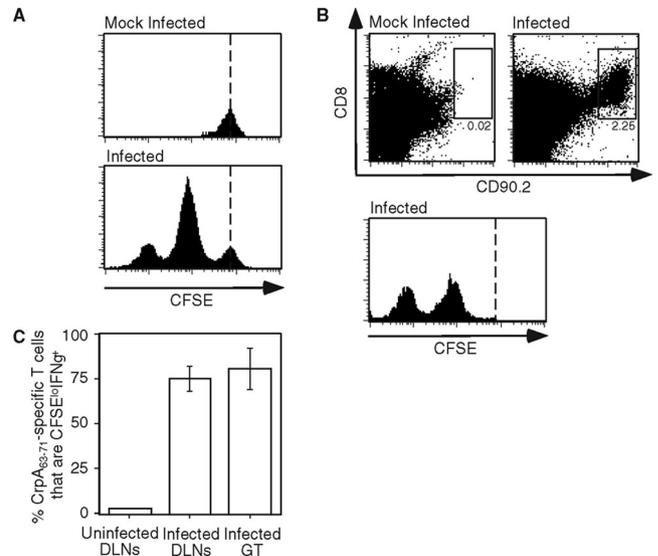


FIGURE 5. CrpA₆₃₋₇₁-specific retrogenic T cells proliferate, produce IFN- γ , and migrate to the genital mucosa of mice infected in the uterus with *C. trachomatis*. One to two million CFSE-labeled CD90.2⁺ CrpA₆₃₋₇₁-specific retrogenic T cells were transferred into CD90.1⁺ female recipients, which were then mock infected or infected in the uterus with 10^6 IFU of *C. trachomatis* L2. *A*, Retrogenic cells from the DLNs of mock infected or infected recipients were examined for CFSE fluorescence. The dashed line marks the average CFSE fluorescence intensity of cells that did not proliferate. Results were gated on live, CD90.2⁺CD8⁺ cells to identify the CrpA₆₃₋₇₁-specific retrogenic T cells. *B*, Genital tract tissue from mock-infected (*top left*) or infected (*top right*) recipients was examined for the presence of the retrogenic cells. Results were gated on live cells. Retrogenic T cells from the infected genital tract (*bottom*) were examined for CFSE fluorescence. The dashed line marks the average CFSE fluorescence intensity of cells in the DLNs that did not proliferate. Results were gated on live, CD90.2⁺ CD8⁺ cells to identify the CrpA₆₃₋₇₁-specific retrogenic T cells. *C*, Retrogenic T cells from the DLNs or genital tract (GT) tissue of mock infected or infected recipients were examined for IFN- γ production following PMA/ionomycin stimulation. IFN- γ production in the genital tract of uninfected recipients was not determined due to the absence of retrogenic T cells in this tissue. Results were gated on live, CD90.2⁺ CD8⁺ cells.

had occurred in the DLN of infected but not mock-infected mice (Fig. 5*A*). These results suggested that the CrpA₆₃₋₇₁-specific retrogenic T cells were stimulated in the regional lymph nodes following infection of the genital tract with *C. trachomatis*.

To determine whether *Chlamydia*-specific CD8⁺ T cells that had proliferated in the DLN migrated into the genital mucosa, we examined whether the CrpA₆₃₋₇₁-specific retrogenic T cells were present in the genital epithelium of infected mice. Retrogenic T cells were first observed in the genital mucosa 5 days postinfection (data not shown). By 6 days postinfection, significant numbers of the retrogenic T cells were present in the genital mucosa of the infected but not the mock-infected mice (Fig. 5*B*, *top panels*). Furthermore, although both cells that had not proliferated and cells that had proliferated were present in the DLN of infected mice (Fig. 5*A*), only cells that had proliferated were present in the infected genital mucosa (Fig. 5*B*, *bottom panel*). These results suggested that only activated CrpA₆₃₋₇₁-specific CD8⁺ T cells migrated to the site of infection.

Because IFN- γ is a crucial effector cytokine in the control of *Chlamydia* infection (23, 25, 26), we tested whether the proliferating CrpA₆₃₋₇₁-specific T cells produced IFN- γ . CFSE-labeled CrpA₆₃₋₇₁-specific T cells were isolated from the DLN or genital tracts of mock infected or infected recipients and then stimulated *ex vivo* with PMA and ionomycin. Proliferating IFN- γ -producing

cells were first observed in the DLN and genital tract 5 days postinfection (data not shown) and further accumulated by 6 days postinfection (Fig. 5C). In summary, following introduction of *C. trachomatis* into the genital epithelia, *Chlamydia*-specific CD8⁺ T cells were primed, developed effector function, and migrated into the infected genital mucosa.

Discussion

Although CD8⁺ T cells can contribute to controlling *Chlamydia* genital infection (4), it has been unclear whether *Chlamydia*-specific CD8⁺ T cells actually migrate into the genital tract in response to infection. CD8⁺ T cells have been identified in the genital mucosa following *C. trachomatis* infection (1, 2), but it has been unclear whether these cells were specific for *Chlamydia* Ags or whether they were nonspecifically recruited by the inflammatory environment. To develop a system to identify and track the activity of *Chlamydia*-specific CD8⁺ T cells in vivo, we focused on CrpA₆₃₋₇₁, a *Chlamydia*-specific CD8⁺ T cell Ag that we recently identified (8). We first generated the CrpA₆₃₋₇₁-specific T cell clone NR23.4 and demonstrated its specificity in vitro. We then generated retrogenic mice expressing the CrpA₆₃₋₇₁-specific TCR from NR23.4. By transferring the retrogenic cells into recipient mice, we determined that these CrpA₆₃₋₇₁-specific T cells proliferated in the DLN in response to genital infection with *C. trachomatis*. Furthermore, the cells that had proliferated were readily detected in the infected genital tract, demonstrating that *Chlamydia*-specific CD8⁺ T cells migrate into the genital mucosa in response to infection.

CrpA₆₃₋₇₁-specific CD8⁺ T cells were not detected in the uninfected genital tract, consistent with the notion that the genital mucosa lacks organized lymphoid elements and that naive T cells do not reside in this tissue (27). Naive T cells are, however, present in regional lymph nodes, and it is likely that Ags from the genital tract must drain to these nodes to activate T cells against genital pathogens. The activated T cells must subsequently migrate back to the site of infection. This appears to contrast with the intestinal mucosa, where Peyer's patches situated within the intestinal wall can serve as a reservoir of naive T cells. It has been demonstrated that following oral infection with *Salmonella*, *Salmonella*-specific CD4⁺ T cells in the Peyer's patches are activated within hours (28). Although the *Salmonella* study examined the response of CD4⁺ T cells while our study examined *Chlamydia*-specific CD8⁺ T cells, we have also recently demonstrated that activation of *Chlamydia*-specific CD4⁺ T cells appears to occur outside of the genital tract (29). Therefore, architectural differences between the genital and intestinal mucosa may result in distinct mechanisms for inducing immunity against pathogens that infect these tissues.

The CrpA₆₃₋₇₁-specific CD8⁺ T cells that were recruited to the infected genital tract were able to produce the effector cytokine IFN- γ . Several groups have demonstrated that IFN- γ is required for resolution of *Chlamydia* infection (25, 30), and work in our laboratory suggests that IFN- γ secretion is the key mechanism by which CD8⁺ T cells control *Chlamydia* replication (23). One way that IFN- γ can control *Chlamydia* infection in vivo is by enhancing the ability of phagocytes to eliminate the organisms. In addition, depending on the type of host cell, IFN- γ can up-regulate a number of proteins that can lead to inhibition of *Chlamydia* replication within infected cells. In human cells, the induction of IDO may deprive *Chlamydia* of intracellular tryptophan (31). In contrast, studies in mice and murine cell lines have suggested that induction of inducible NO synthase may restrict *Chlamydia* growth (32–34). More recent evidence suggests that following infection of murine cells with human serovars of *C. trachomatis*, IFN- γ may control infection primarily through the induction of a

p47 GTPase Iigp1 (35). IFN- γ secretion by *Chlamydia*-specific CD8⁺ T cells in the genital mucosa may affect clearance of *Chlamydia* through a combination of these pathways.

Although retrogenic mice have been used to study autoimmunity (20) and thymic selection (15), this technology has not been applied to study T cell responses to pathogens. The retrogenic mice described in this study have proven useful as we can now study the initial encounter of CD8⁺ T cells with *Chlamydia* in vivo. Because retrogenic mice require fewer resources to produce relative to conventional TCR transgenic mice, we envision that retrogenic mice will be useful for studying and comparing T cell responses to a variety of *Chlamydia* Ags. Because many *Chlamydia* proteins are developmentally regulated (36), T cell responses against different Ags may differ depending on the expression pattern of the particular Ag. By comparing T cell responses to multiple Ags we will obtain a better understanding of the overall *Chlamydia*-specific CD8⁺ T cell response following genital infection.

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Disclosures

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