Monitoring the T cell response to genital tract infection

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To date, it has not been possible to study antigen-specific T cell responses during primary infection of the genital tract. The low frequency of pathogen-specific T cells in a naïve mouse makes it difficult to monitor the initial events after antigen encounter. We developed a system to examine the response of pathogen-specific T cells in the genital mucosa after intrauterine infection. We identified the protective CD4$^+$ T cell antigen Cta1 from *Chlamydia trachomatis* and generated T cell receptor (TCR) transgenic (tg) mice with specificity for this protein. By transferring TCR tg T cells into naïve animals, we determined that *Chlamydia*-specific T cells were activated and proliferated in the lymph nodes draining the genital tract after primary intrauterine infection. Activated T cells migrated into the genital mucosa and secreted IFN-γ. The development of *Chlamydia*-specific TCR tg mice provides an approach for dissecting how pathogen-specific T cells function in the genital tract.

Chlamydia | adaptive immunity | T cell receptor transgenic

Most pathogens enter a mammalian host by penetrating mucosal surfaces such as the lung, intestine, or genitourinary tract. The prevalence of sexually transmitted diseases has prompted studies to understand how infection is established in the genital tract and how pathogens are cleared from this site. Despite the importance of T cells in controlling a number of genital pathogens, the behavior of pathogen-specific T cells in the genital mucosa is not well characterized. The major obstacle to studying primary T cell responses is the low frequency of antigen-specific T cells in naïve mice. Because it is difficult to identify and track endogenous pathogen-specific T cells before infection, T cell receptor (TCR) transgenic (tg) animals have been developed that express pathogen-specific TCRs. T cells from TCR tg animals can be labeled and transferred into recipient mice, boosting the number of naïve, pathogen-specific T cells so that they can be monitored during infection. Although TCR tg mice have been useful in the study of organisms that infect animals systemically or the tracking of the response of T cells to pathogens that infect the lung or intestine (1–5), they have not been used to examine infection of genital tissues.

To develop a system for studying pathogen-specific T cell responses in the genital mucosa, we developed TCR tg mice specific for *Chlamydia trachomatis*. *C. trachomatis* is a major cause of sexually transmitted disease and the leading cause of preventable blindness worldwide. In the genital tract, chronic and often undiagnosed infection stimulates a marked inflammatory response that contributes to *Chlamydia*-associated sequelae such as ectopic pregnancy and infertility (6, 7). Both protective immunity against *C. trachomatis* and the pathologies associated with *Chlamydia* infection are mediated by T cells. In humans, T cells respond to *C. trachomatis* at the site of infection and appear to contribute to pathology (8). In mice, depletion of T cells results in loss of protective immunity, and adoptive transfer of *Chlamydia*-specific T cells into naïve animals enhances resistance (9–13). Despite the importance of T cells in controlling *Chlamydia* infection, it has been challenging to study the initial encounter of T cells with *Chlamydia* antigens in the genital tract during primary infection. Little is known about when and where *Chlamydia*-specific T cells respond to infection and the extent to which they migrate into infected tissues. It is also unknown when effector cytokines are produced by *Chlamydia*-specific T cells and where the effector functions are deployed.

Here we monitor the response of *Chlamydia*-specific T cells in the genital tract by transferring *Chlamydia*-specific TCR tg T cells into recipient animals. Because no murine CD4$^+$ T cell antigens had been previously identified that were recognized during *C. trachomatis* infection, the development of TCR tg mice required the identification of an antigen, *Chlamydia*-specific T cell antigen 1 (Cta1). TCR tg mice specific for Cta1 were generated and used in adoptive transfer studies to track *Chlamydia*-specific T cell responses *in vivo*. In mice that received the Cta1-specific TCR tg T cells, we demonstrated that genital infection with *C. trachomatis* resulted in activation and proliferation of the T cells in the lymph nodes draining the genital tract. We were also able to show that the Cta1-specific TCR tg T cells migrated into the genital mucosa and secreted the inflammatory cytokine IFN-γ in response to *C. trachomatis* infection. The development of TCR tg tools that allow the examination of T cell responses in genital tissues will lead to a better understanding of how T cells respond at this anatomic site and how T cells contribute to protective immunity and pathology associated with human pathogens.

Results

Adoptive Transfer of a *Chlamydia*-Specific CD4$^+$ T Cell Clone Protects Against Challenge with *C. trachomatis*. The identification of a *Chlamydia*-specific TCR was necessary for the generation of TCR tg mice specific for a *C. trachomatis* antigen. We cultured a *Chlamydia*-specific CD4$^+$ T cell line from a mouse infected with *C. trachomatis*, and an individual clone we designated NR9.2 was isolated by limiting dilution. To confirm that NR9.2 was *Chlamydia*-specific, we tested whether it recognized bone marrow-derived macrophages (BMMs) infected with *C. trachomatis*. NR9.2 secreted significant levels of IFN-γ in response to the infected cells (Fig. 1*a*), suggesting that the T cell clone was specific for a *C. trachomatis* antigen.

We then tested whether adoptive transfer of this T cell clone into naïve mice protected against *Chlamydia* challenge. We transferred 10^7 NR9.2 T cells into naïve C57BL/6 mice and then challenged the mice i.v. with 10^7 inclusion-forming units (IFU) of *C. trachomatis*. Three days later, the animals were killed, and the number of *C. trachomatis* IFU was measured in the spleen. Mice that received the adoptive transfer of NR9.2 T cells had *Chlamydia* spleen titers significantly lower than mice that did not receive NR9.2 (Fig. 1*b*). The level of protection afforded by the transfer of T cells was similar to the level of protection observed in mice that had recovered from prior infection. These results

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Abbreviations: TCR, T cell receptor; tg, transgenic; Cta1, *Chlamydia*-specific T cell antigen 1; BMM, bone marrow-derived macrophage; IFU, inclusion-forming units; CFSE, carboxyfluorescein diacetate succinimidyl ester; ILN, iliac lymph node; NDLN, nondraining lymph node.

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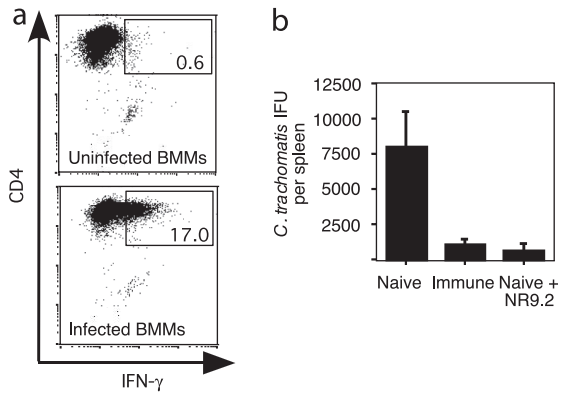

Fig. 1. CD4$^+$ T cell clone NR9.2 is *Chlamydia*-specific and protective. (*a*) CD4$^+$ T cell clone NR9.2 was cultured with *Chlamydia*-infected or uninfected BMMs, and IFN-γ secretion was assessed by intracellular cytokine staining. Results are gated on live cells. (*b*) Naïve mice, *C. trachomatis* immune mice, and naïve mice that received 10^7 NR9.2 T cells were challenged i.v. with 10^7 IFU of *C. trachomatis*. Immune mice had recovered from infection 3 weeks before challenge. Spleen titers were determined 3 days after challenge.

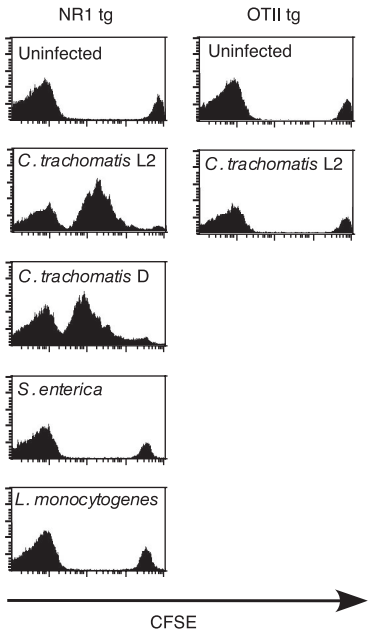

Fig. 2. NR1 TCR tg cells proliferate in response to *Chlamydia* infection *in vivo*. CFSE-labeled NR1 (*Left*) or OTII (*Right*) cells were transferred into C57BL/6 recipients. One day later, mice were infected i.v. with the indicated pathogen, and spleens were harvested 3 days later. Results were gated on live CD4$^+$ Vα2$^+$ cells to detect the NR1 TCR tg cells.

suggested that the antigen recognized by NR9.2 was expressed at a sufficient level during infection to allow recognition by protective CD4$^+$ T cells.

To identify the *C. trachomatis* antigen recognized by NR9.2, we generated a library of *Escherichia coli* clones expressing each of the *Chlamydia* ORFs identified in the published *C. trachomatis* genome sequence (14). The clones comprising the library were incubated with BMMs in individual wells of an assay dish, and the T cell clone NR9.2 was added to each well. Activation of the NR9.2 T cells was assessed by IFN-γ ELISA. Of the 894 ORFs we screened, only *E. coli* expressing the protein encoded by CT788 stimulated NR9.2 to secrete significant levels of IFN-γ (Fig. 6*a*, which is published as supporting information on the PNAS web site). CT788 was annotated in the published *C. trachomatis* genome as a predicted periplasmic protein of unknown function (14), and its sequence shares little homology with proteins outside of the *Chlamydia* genus. Because the product encoded by CT788 was recognized by T cells, we designated the protein Cta1. To more closely map the epitope within Cta1 recognized by the NR9.2 T cells, we screened a series of overlapping 20-mer peptides covering the Cta1 sequence for their ability to stimulate NR9.2 to secrete IFN-γ. The 20-mer peptide Cta1$_{133–152}$ (KGIDPQELWVWKKGMPNWEK) induced NR9.2 to secrete significant levels of IFN-γ (Fig. 6*b*), confirming the specificity of these *C. trachomatis*-specific T cells to an epitope within these 20 aa.

Cta1-Specific TCR tg Cells Proliferate in Response to Chlamydia Infection *in Vivo*. To generate *Chlamydia*-specific TCR tg mice, we cloned the rearranged genomic TCR-α and TCR-β sequences from NR9.2 into expression vectors and injected these constructs into C57BL/6 fertilized oocytes. Pseudopregnant female recipients were then implanted with the oocytes, and individual pups born from the foster mothers were screened by using primers specific for the NR9.2 TCR. We identified a TCR tg founder line that we designated NR1. To confirm that the NR9.2 TCR was expressed on the tg cells in NR1, we tested whether cells from the peripheral blood of these animals expressed the Vα2 and Vβ8.3 TCR elements. Vα2 and Vβ8.3 were the variable chains expressed by the original NR9.2 T cell clone (data not shown). A significant percentage of CD4$^+$ T cells from the peripheral blood of the tg mice expressed Vα2 and Vβ8.3 (Fig. 7*a*, which is published as supporting information on the PNAS web site), demonstrating that both the TCR-α and TCR-β transgenes from NR9.2 were efficiently expressed. The tg cells were also CD69^{lo}, CD25^{lo}, CD62L^{hi}, CD44^{lo}, and CTLA4^{lo}, indicating that they were naïve T cells (data not shown).

To determine whether the NR1 tg T cells were specific and responsive to Cta1, we tested the proliferation of tg spleen cells in response to Cta1$_{133–152}$. Spleen cells from naïve NR1 mice showed a strong proliferative response to Cta1$_{133–152}$ (Fig. 7*b*). NR1 spleen cells also secreted high levels of IFN-γ in response to this peptide (data not shown). In contrast, spleen cells from NR1 did not proliferate in response to a control peptide from ovalbumin, OVA$_{323–336}$ (data not shown).

We then tested whether NR1 tg T cells proliferated in mice in response to *C. trachomatis* infection. Thirty million cells from the spleen and peripheral lymph nodes of NR1 mice were labeled with the fluorescent dye carboxyfluorescein diacetate succinimidyl ester (CFSE) and transferred into C57BL/6 recipients. Because $\approx$10% of NR1 cells were CD4$^+$ T cells expressing the Cta1-specific TCR (data not shown), the transferred population contained $\approx 3 \times 10^6$ Cta1-specific T cells. CFSE-labeled NR1 tg cells retained high levels of CFSE after transfer into uninfected recipient mice, showing that the tg cells did not divide in the absence of infection. In other C57BL/6 animals that had received the CFSE-labeled tg cells, we infected the animals i.v. with 10^7 IFU of *C. trachomatis*. The *C. trachomatis* organisms used for infection were serovar L2, a serovar associated with lymphogranuloma venereum in humans, or serovar D, a serovar associated with typical human genital tract infection. We observed that within 3 days of infection with either *C. trachomatis* serovar the tg cells had proliferated extensively (Fig. 2). We also observed that the proliferation of NR1 cells was specific for *C. trachomatis* infection. When animals that had received NR1 cells were infected i.v. with *Salmonella enterica* or *Listeria monocytogenes*, the tg T cells were not stimulated to proliferate. As an additional control to demonstrate that TCR tg T cells with other specificities would not respond to *C. trachomatis* infection in the recipient mice, we showed that ovalbumin-specific OTII tg T cells proliferated in response to ovalbumin protein with adjuvant

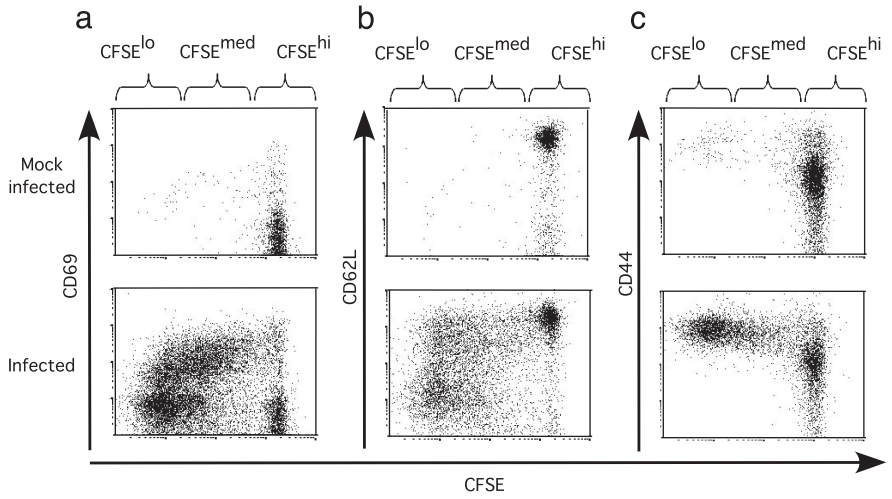

Fig. 3. NR1 cells are activated and proliferate in the ILNs after intrauterine infection with *C. trachomatis*. CFSE-labeled NR1 cells were transferred into CD90.1 recipients, which were then mock-infected or infected in the uterus with 10^6 IFU of *C. trachomatis* serovar L2. Cells from the ILNs of infected CD90.1 recipients were harvested 5 (*a*) or 7 (*b* and *c*) days after infection. The levels of CD69 (*a*), CD62L (*b*), and CD44 (*c*) expression were measured on $CD4^+$ NR1 cells. Results were gated on live $CD90.2^+$ $CD4^+$ cells to specifically detect the NR1 TCR tg cells.

(data not shown) but not in response to *C. trachomatis* infection (Fig. 2).

NR1 Cells Are Activated and Proliferate in the Iliac Lymph Nodes (ILNs) After Intrauterine Infection with *C. trachomatis*. Because the natural site of *C. trachomatis* infection in humans is the genital mucosa, we wanted to determine whether the NR1 TCR tg T cells would respond to mouse infection of the genital tract. We tested whether NR1 cells were activated and proliferated after intrauterine inoculation of female mice with *C. trachomatis* serovar L2. Up-regulation of the T cell activation marker CD69 was used to assess early T cell activation, and proliferation was monitored by a loss in CFSE fluorescence of labeled tg cells. In these experiments, CD90.1 congenic mice were given 3×10^7 CFSE-labeled NR1 cells. The mice were then infected in the uterus with 10^6 IFU of *C. trachomatis* serovar L2. Mock-infected control animals were inoculated with buffer. ILNs, which drain antigen from the genital tract (15–17), were removed 5 days after infection, and NR1 T cells in the nodes were examined for CD69 up-regulation and loss of CFSE fluorescence. As shown in Fig. 3*a*, a significant number of NR1 T cells from infected animals showed progressive dilution of CFSE, suggesting that extensive proliferation had occurred. Furthermore, recently divided ($CFSE^{med}$) NR1 T cells expressed high levels of CD69, indicating that these cells also had been recently activated. Once NR1 cells had undergone extensive proliferation ($CFSE^{lo}$), they expressed lower levels of CD69. These results are consistent with the notion that CD69 is an early T cell activation marker and is only transiently up-regulated after antigen encounter (18, 19). Cells from the ILNs of mock-infected recipients were $CFSE^{hi}$ and had not up-regulated CD69, suggesting that they were not activated. Interestingly, there was a similar population of $CFSE^{hi}CD69^{lo}$ NR1 cells in the infected recipients. These could be cells that did not encounter antigen or cells that were not expressing the appropriate Cta1-specific TCR because of endogenous TCR rearrangements (20, 21).

Other characteristics of activated T cells are down-regulation of the naive marker CD62L and up-regulation of the activation molecule CD44. To further verify that NR1 cells were activated after *Chlamydia* genital infection, we analyzed the expression of CD62L and CD44 on transferred CFSE-labeled NR1 T cells from the ILNs of recipient mice. Seven days after infection, a subset of NR1 T cells that had proliferated extensively ($CFSE^{lo}$) had reduced expression of CD62L (Fig. 3*b*). In contrast, undivided ($CFSE^{hi}$) NR1 cells in both mock-infected and infected recipients were mostly $CD62L^{hi}$. In addition to displaying a $CD62L^{lo}$ phenotype, effector T cells typically express high levels of the activation marker CD44. As demonstrated in Fig. 3*c*, proliferating NR1 T cells from the ILNs of infected recipients were exclusively $CD44^{hi}$. Collectively, these results further confirm that NR1 cells were activated and proliferated in the ILNs of mice after genital infection with *Chlamydia*.

Extensive Proliferation of NR1 Cells Occurs Preferentially in the ILNs. To test whether the activation and proliferation of NR1 cells in the ILNs were the result of antigen draining from the genital tract to these nodes, we compared the response of NR1 cells in the ILNs to the response in the lymph nodes that do not drain the genital tract (nondraining lymph nodes, NDLNs). We monitored T cell activation and proliferation over time to ensure that we would observe any activity that may have occurred in the NDLNs over the course of infection.

In these experiments we transferred 3×10^7 CFSE-labeled NR1 cells into CD90.1 mice. The mice were then infected in the uterus with 10^6 IFU *C. trachomatis* serovar L2, and lymph nodes were harvested at various times after infection. In the ILNs, up-regulation of CD69 was seen on NR1 cells beginning 3 days after infection. In addition, down-regulation of CD62L and up-regulation of CD44 appeared 4 days after infection. Acquisition of the activation markers occurred preferentially in NR1 cells from the ILNs and not in NR1 cells from the NDLNs (data not shown). These results suggested that NR1 cells were specifically activated in the ILNs after genital infection with *Chlamydia*.

To further confirm that NR1 cells preferentially encountered antigen in the ILNs, we monitored proliferation of the transferred NR1 cells at various times after infection. In the ILNs, NR1 cells were predominantly $CFSE^{hi}$ 2 and 3 days after infection, suggesting that these cells had not proliferated at these early time points (Fig. 4). In contrast, NR1 cells in the ILNs had proliferated extensively within 4 days of infection. A small number of NR1 cells had also proliferated in the NDLNs within 4 days of infection, but this number was significantly less than that seen in the ILNs. The proliferating NR1 cells in the NDLNs contained lower levels of CFSE and did not express significant levels of CD69 relative to those in the ILNs (data not shown), suggesting that these cells may have migrated to the NDLNs

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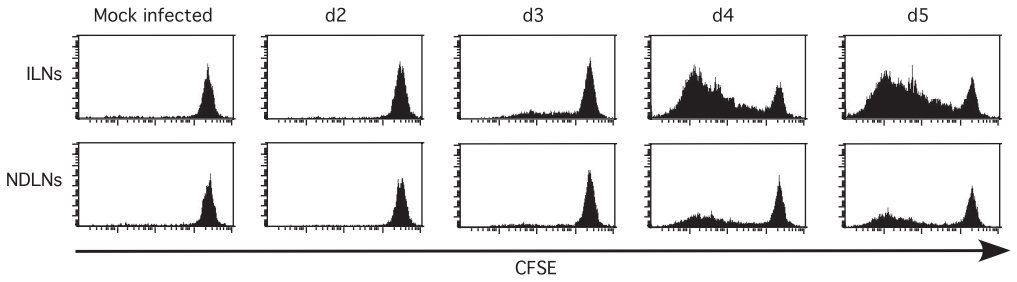

Fig. 4. NR1 cells proliferate preferentially in the ILNs after intrauterine infection with *C. trachomatis*. CFSE-labeled NR1 cells were transferred into CD90.1 recipients, which were then mock-infected or infected in the uterus with 10^6 IFU of *C. trachomatis* serovar L2. ILNs (*Upper*) and NDLNs (*Lower*) were harvested at the indicated times after infection, and proliferation of $CD4^+$ NR1 cells was examined. Results were gated on live $CD90.2^+$ $CD4^+$ $V\alpha2^+$ cells to specifically detect the NR1 TCR tg cells.

from other sites after activation. We did not observe significant expansion of NR1 T cells in the NDLNs even 1 week after infection (data not shown), again demonstrating that proliferation of NR1 cells occurred preferentially in the ILNs.

NR1 Cells in the ILNs Develop the Ability to Secrete IFN-γ. To examine the differentiation of antigen-activated NR1 cells into effector T cells, we determined whether proliferating NR1 cells secreted effector cytokines in response to *C. trachomatis* infection. We transferred 3×10^7 CFSE-labeled NR1 cells into CD90.1 congenic mice and then infected these mice in the uterus with 10^6 IFU of *C. trachomatis* serovar L2. The ILNs were removed 6 days later, and the NR1 T cells were analyzed by flow cytometry for production of cytokines. Proliferating NR1 cells ($CFSE^{lo}$) secreted IFN-γ, whereas nonproliferating cells ($CFSE^{hi}$) did not secrete IFN-γ (Fig. 8, which is published as supporting information on the PNAS web site). The NR1 cells did not secrete IL-4 (data not shown). These results suggested that NR1 cells differentiated preferentially into effector T cells of the T helper 1 phenotype after genital infection with *Chlamydia*.

Antigen-Experienced NR1 Cells Traffic to the Genital Tract. After activation, effector T cells are typically recruited to the site of infection where they contribute to the elimination of the pathogen (22, 23). To explore whether *Chlamydia*-specific NR1 cells migrated to the site of genital infection in mice, we transferred 3×10^7 CFSE-labeled NR1 cells into CD90.1 congenic mice and then infected these recipients in the uterus with 10^6 IFU of *C. trachomatis* serovar L2. We then isolated genital tract tissue and determined whether the transferred *Chlamydia*-specific T cells were present. We were able to detect significantly more NR1 cells in the genital mucosa of animals infected with *C. trachomatis* than in animals that were mock-infected (Fig. 5*a*). Furthermore, tg cells that were recruited to the genital tract had the phenotype of antigen-experienced cells ($CFSE^{lo}$ $CD62L^{lo}$ $CD44^{hi}$) and secreted IFN-γ (Fig. 5 *b* and *c*). In summary, we have shown that activated NR1 TCR tg T cells that secrete IFN-γ are recruited to the genital mucosa in response to *C. trachomatis* infection.

Discussion

Although pathogen-specific TCR tg T cells have been used in other infectious disease models (1–5), the NR1 mice described here are unique in that they are specific for a pathogen that infects the genital tract. Previously, it had not been possible to examine T cell responses to genital pathogens such as *C. trachomatis* because it was difficult to identify and track naïve T cells specific for genital antigens *in vivo*. In particular, the inability to genetically modify *C. trachomatis* to express a heterologous T cell epitope and the lack of a well defined *Chlamydia*-specific $CD4^+$ T cell antigen had made it difficult to study T cell responses to this genital pathogen.

Because murine $CD4^+$ T cell antigens recognized during *Chlamydia* infection had not been defined previously to our knowledge, analysis of primary *Chlamydia*-specific T cell responses was limited to examining polyclonal T cell responses to undefined antigens (16). With these experiments, the investigators could not differentiate true *Chlamydia*-specific T cell responses from bystander T cell activation resulting from infection. In a number of other infectious disease models, bystander T cell responses have been shown to contribute to the overall response (24, 25). In this report we identified a $CD4^+$ T cell antigen, Cta1, allowing us to examine an antigen-specific T cell response stimulated during *C. trachomatis* infection. This antigen was

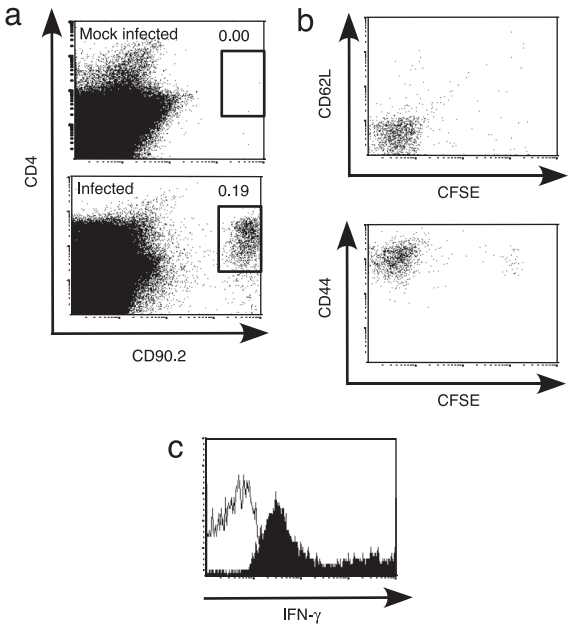

Fig. 5. Antigen-experienced NR1 cells migrate to the genital tract tissue after intrauterine infection with *Chlamydia*. CFSE-labeled NR1 cells were transferred into CD90.1 recipients and then mock-infected or infected in the uterus with 10^6 IFU of *C. trachomatis* serovar L2. Seven days after infection, the genital tracts were removed from the mice and analyzed for the presence of the transferred NR1 cells. (*a*) The presence of $CD90.2^+$ $CD4^+$ NR1 cells was compared in the genital tracts of mock-infected and infected recipients. Results were gated on live cells. (*b*) The levels of CD62L, CD44, and CFSE on NR1 cells were examined in the genital tracts of mock-infected and infected mice. Results were gated on live $CD90.2^+$ $CD4^+$ cells to specifically detect the NR1 TCR tg cells. (*c*) NR1 cells from the genital tract were stimulated with phorbol 12-myristate 13-acetate/ionomycin and analyzed by flow cytometry to detect production of IFN-γ. Results are gated on live $CD90.2^+$ $CD4^+$ cells to specifically detect the NR1 TCR tg cells. Solid line indicates isotype control; filled histogram indicates IFN-γ.

predicted to be a periplasmic protein because of an N-terminal signal sequence, but its function is unknown (14). We have found that Cta1 is conserved in all of the *C. trachomatis* serovars that have been sequenced, including those that cause genital tract infection, ocular infection, and lymphogranuloma venereum. We demonstrated in this study that Cta1 stimulated protective T cells after natural infection, suggesting that it may play a significant role in immunity against *C. trachomatis*.

Another difficulty in studying the initial encounter of T cells with antigen is the low precursor frequency of pathogen-specific T cells in an unimmunized animal. Other investigators have attempted to increase the frequency of *Chlamydia*-specific T cells by transferring T cell clones of unknown specificity into *Chlamydia*-infected mice (17, 26). However, because the transferred T cells are antigen-experienced and have been propagated through multiple rounds of restimulation, the response of these T cells cannot be used to model the initial encounter of naïve T cells with *C. trachomatis*. To overcome the limitations of previous approaches and study the initial *Chlamydia*-specific T cell response, we developed NR1, a TCR tg mouse line specific for Cta1. Here we used adoptive transfer of NR1 cells into unimmunized mice to increase the frequency of naïve, *Chlamydia*-specific T cells while still maintaining the polyclonal T cell environment in the recipient animals (27).

In this study we focused on the response of *Chlamydia*-specific T cells to the infected murine genital tract. We successfully used an infection model where we inoculated the uterus of the mouse with the human *C. trachomatis* serovar L2. This route of infection has previously been used to prime *Chlamydia*-specific T cells that could subsequently be cultured from the spleen (28) and has also been demonstrated to cause salpingitis in mice (29, 30). Other studies have used a different *Chlamydia* species, *Chlamydia muridarum*, as a mouse model of infection. *C. muridarum* is not known to infect humans but causes an ascending infection when inoculated into the vaginal vault of female mice. However, we were unable to use this model as our Cta1-specific T cells did not recognize *C. muridarum*-infected cells (data not shown), only those infected with *C. trachomatis*. These data suggest that the Cta1 epitope in *C. trachomatis* is not conserved in the homolog of Cta1 in *C. muridarum*.

Using intrauterine infection with a human serovar of *C. trachomatis*, we established that *Chlamydia*-specific T cells exhibited a T helper 1 response in the genital tract in response to infection. These cells secreted IFN-γ while still in the ILNs, and secretion continued after migration into the infected genital tract. IFN-γ has long been implicated as a critical effector in *Chlamydia* clearance, but may also lead to the tissue pathology associated with infection. *In vitro*, IFN-γ enhances the ability of phagocytes to control *Chlamydia* replication (31). *In vivo*, susceptibility to *Chlamydia* infection is increased in IFN-$\gamma-/-$ mice, and *Chlamydia*-specific T cells transferred into mice only appear to be protective if they secrete IFN-γ (32). Besides its role in protection, IFN-γ induces *Chlamydia* to develop into a persistent state *in vitro* (31, 33), and there is evidence that organisms persist in some human infections (34). It is thought that persistence or repeated infection with *Chlamydia* contributes to tissue scarring *in vivo* (35). Consistent with the hypothesis that IFN-γ may promote tissue pathology, lymphocytes from patients with *Chlamydia*-associated tubal factor infertility secreted high levels of IFN-γ in response to *Chlamydia* relative to lymphocytes from control patients (36). Although we have now demonstrated that naïve *Chlamydia*-specific T cells develop into T helper 1 effector cells in response to infection, it remains unclear what factors influence whether these T cells will be beneficial or detrimental to the host. Additional work using the NR1 T cells in infection models may lead to a better understanding of the dynamics of the response that tip the balance between protection and pathology.

In our model, up-regulation of CD69 on NR1 T cells in the ILNs did not occur until 3 days after genital infection and proliferation did not occur until 4 days after the infection. The period between intrauterine *Chlamydia* inoculation and activation of NR1 cells may define the amount of time required for *Chlamydia* antigens to travel into the draining lymph nodes where the antigen can activate naïve T cells. Elements of the immune system in the genital tissues are less well characterized than those in intestinal tissues, but differences between these two mucosal surfaces are nonetheless apparent. Unlike the intestinal lumen, the genital mucosa lacks organized lymphoid elements. While the intestinal lumen is equipped with Peyer's patches that are poised to immediately sample luminal contents, the initiation of T lymphocyte responses against genital pathogens must occur outside the genital mucosa, perhaps in the ILNs, which drain antigen from the genital tract (15–17). The amount of time it takes *Chlamydia* antigens to migrate from the genital surface to the ILNs may explain the lack of NR1 activation before 3 days postinfection.

The genital and intestinal mucosa also differ in cell surface adhesion molecules responsible for recruiting lymphocytes. Whereas interaction of the $\alpha 4\beta 7$ integrin on lymphocytes with the MAdCAM-1 adhesion molecule on the intestinal endothelium mediates recruitment of T lymphocytes to the intestinal mucosa, such an interaction does not appear to play a significant role in recruitment to the genital mucosa (37, 38). Our capacity to track *Chlamydia*-specific T cells in the genital tissues of *Chlamydia*-infected mice will now allow us to identify and characterize the homing molecules responsible for directing pathogen-specific T cells to the genital mucosa.

Materials and Methods

Mice. C57BL/6J (H-2^b), B6.PL-thy1^a0Cy (CD90.1 congenic), and OTII mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Tissue Culture. Bone marrow-derived dendritic cells and BMMs were cultured as described (39, 40).

Growth, Isolation, and Detection of Bacteria. Elementary bodies of *C. trachomatis* serovar L2 434/Bu and *C. trachomatis* serovar D (UW-3/Cs) were propagated and quantitated as described (41). *S. enterica* serovar Typhimurium ATCC 14028 was grown at 37°C in LB medium. *L. monocytogenes* 10403s was grown at 30°C in brain heart infusion medium (Difco/Becton Dickinson, Sparks, MD).

Generation of the NR9.2 T Cell Clone. Splenocytes from mice were isolated 21 days after infection with *C. trachomatis* serovar L2 and cultured with irradiated (2,000 rad) bone marrow-derived dendritic cells, UV-inactivated *C. trachomatis* serovar L2, and naïve syngeneic splenocytes in RP-10 (RPMI medium 1640 supplemented with 10% FCS, L-glutamine, Hepes, 50 μM 2-β-2-mercaptoethanol, 50 units/ml penicillin, and 50 μg/ml streptomycin) with α-methyl mannoside and 5% supernatant from Con A-stimulated rat spleen cells. $CD8^+$ T cells were depleted from the culture by using Dynabeads Mouse CD8 (Invitrogen, Carlsbad, CA). The $CD4^+$ T cells were restimulated every 7 days with *C. trachomatis*-pulsed bone marrow-derived dendritic cells. Once a *C. trachomatis*-specific $CD4^+$ T cell line was established, the T cell clone NR9.2 was isolated by limiting dilution.

Identification of the T Cell Antigen Cta1. An expression library of genomic sequences from *C. trachomatis* serovar D was inserted into a modified form of the pDEST17 vector (Invitrogen) and transformed into the *Stbl2* strain of *E. coli* (Invitrogen). After induction of protein expression, the bacteria were fixed in 0.5% paraformaldehyde and incubated with BMMs. NR9.2 T cells

were then added, and after 24 h, the supernatant was tested for the level of IFN-γ by ELISA (Endogen, Rockford, IL). To identify the reactive peptide epitope within Cta1, synthetic 20-mer peptides (MIT Biopolymers Lab, Cambridge, MA) were used at a concentration of 25 μM in an IFN-γ ELISA (Endogen).

Flow Cytometry and Antibodies. Antibodies specific for CD4, CD90.2, Vα2, Vβ8.3, CD69, CD25, CD44, CD62L, CTLA-4, IFN-γ, and IL-4 were purchased from BD Biosciences (San Diego, CA). Data were collected on a BD Biosciences FACS-Calibur flow cytometer and analyzed with CellQuest software. Intracellular cytokine staining was performed by incubating NR9.2 T cells with *Chlamydia*-infected BMMs (multiplicity of infection 5:1) in the presence of GolgiPlug (BD Biosciences). Intracellular cytokine staining of NR1 tg cells was performed by stimulating cells for 4 h in the presence of phorbol 12-myristate 13-acetate (50 ng/ml; MP Biomedicals, Solon, OH), ionomycin (1 μg/ml; Sigma, St. Louis, MO), and GolgiPlug (BD Biosciences). Cells were permeabilized with the Cytofix/Cytoperm Plus kit (BD Biosciences). Phycoerythrin-conjugated rat IgG1 (BD Biosciences) was used as an isotype control antibody.

Generation of NR1 TCR tg Mice. The rearranged TCR from NR9.2 uses the Vα2Jα16 and Vβ8.3DJβ1.2 receptor chains. The genomic TCR sequences were cloned and inserted into the TCR vectors pTα and pTβ at the recommended restriction sites (42). Prokaryotic DNA sequences were then removed from both vectors before injection into the pronuclei of fertilized C57BL/6J oocytes. TCR tg founders were identified by PCR. Routine screening to identify tg mice was carried out by staining samples of orbital blood from the mice with antibodies specific for Vα2 and Vβ8.3, followed by flow cytometry.

Adoptive Transfer of NR1 Cells, Infection of Mice, and Preparation of Tissues from Mice. Spleen and peripheral lymph nodes were isolated from NR1 TCR tg mice and labeled with the dye CFSE (5 μM; Molecular Probes, Eugene, OR). Recipient mice were injected i.v. with 3×10^7 NR1 cells. Mice were infected 1 day after transfer of the cells. Where indicated, mice were infected i.v. with 10^7 IFU of *C. trachomatis*, 3×10^3 colony-forming units (CFU) of *L. monocytogenes*, or 5×10^3 CFU of *S. enterica*. To infect the genital tract, mice were treated with 2.5 mg of medroxyprogesterone acetate s.c. 1 week before infection to synchronize the mice into a diestrus state (43, 44). Intrauterine infection was carried out by inoculating the uterine horns with 10^6 IFU of *C. trachomatis* serovar L2. At various times after infection, single-cell suspensions of spleen, ILNs, or NDLNs taken from the axillary and cervical lymph nodes were prepared, stained, and analyzed by flow cytometry as described above. To isolate lymphocytes from the genital mucosa, genital tracts (oviduct, uterus, and cervix) were removed from mice and digested with collagenase (type XI; Sigma) for 1 h before staining and flow cytometry.

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