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Integrin $\alpha_4\beta_1$ Is Necessary for CD4$^+$ T Cell–Mediated Protection against Genital Chlamydia trachomatis Infection

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Chlamydia trachomatis infection is the most common sexually transmitted bacterial infection in the United States and a significant health burden worldwide. Protection from Chlamydia infection in the genital mucosa is dependent on IFN-\(\gamma\) derived from CD4$^+$ Th1 cells. These CD4$^+$ T cells must home successfully to the genital tract to exert their effector function and decrease C. trachomatis burden. Although adhesion receptors expressed by CD4$^+$ T cells in the genital tract have been characterized, the integrin receptor required for Chlamydia-specific CD4$^+$ T cell–mediated protection has not been explored. In this study, we demonstrate that C. trachomatis infection of the upper genital tract results in recruitment of Chlamydia-specific CD4$^+$ T cells robustly expressing the integrin $\alpha_4\beta_1$. Interfering with $\alpha_4\beta_1$, but not $\alpha_7\beta_7$, function resulted in defective CD4$^+$ T cell trafficking to the uterus and high bacterial load. We conclude that integrin $\alpha_4\beta_1$ is necessary for CD4$^+$ T cell–mediated protection against C. trachomatis infection in the genital mucosa. By identifying homing molecules required for successful CD4$^+$ T cell trafficking to C. trachomatis–infected tissues, we will be better equipped to design vaccines that elicit sterilizing, long-lasting immunity without inducing immune pathologies in the upper genital tract. The Journal of Immunology, 2014, 192: 4284–4293.

C. trachomatis is the most common cause of bacterial sexually transmitted infection in the United States and the leading cause of preventable blindness worldwide (1). C. trachomatis is an obligate intracellular pathogen that infects conjunctival and genital tract epithelial cells. In the upper genital tract, complications from C. trachomatis infection include pelvic inflammatory disease, ectopic pregnancy, and infertility (2, 3). The high frequency of infection, low incidence of acquired immunity, and lack of an effective vaccine make C. trachomatis a continuing public health concern.

Protection of the genital mucosa from C. trachomatis is dependent on the production of IFN-\(\gamma\) (4). IFN-\(\gamma\) protects through the upregulation of IDO, NO synthase, and immunity-related GTPases that interfere with various aspects of the pathogen’s developmental cycle and reduce growth (5–8). Mice that are deficient in IFN-\(\gamma\) production have delayed resolution of infection in the genital mucosa (9). CD4$^+$ T cells must produce IFN-\(\gamma\) to mediate protection, as transfer of Chlamydia-specific CD4$^+$ T cells only protects naïve mice against challenge when IFN-\(\gamma\) is produced by those T cells. It is also critical that Ag-specific, IFN-\(\gamma\)–secerting CD4$^+$ T cells efficiently traffic to the genital mucosa in response to Chlamydia infection to drive protective immunity (10, 11).

Homing receptors mediate the migration of immune cells toward specific signals to exit the circulation and enter target tissues (12). Integrins are a family of adhesion receptors consisting of $\alpha$ and $\beta$ heteroduplexes that direct signaling from both outside and inside of the cell membrane (13). The role of certain integrin members on leukocytes has been studied extensively. For example, LFA-1 has been shown to play a crucial function in the arrest of leukocytes in the blood vessels at the site of inflammation (14, 15). Other integrin heterodimers, namely $\alpha_4\beta_1$ and $\alpha_\gamma\beta_7$, provide tissue specificity to T cells when homing to different areas of the body. Descriptions of how lymphocytes traffic to the gastrointestinal tract and CNS have been reported. Lymphocyte recruitment to the gastrointestinal tract is largely mediated by the chemokine receptor CCR9 and the integrin receptor $\alpha_4\beta_7$ (16). Alternatively, integrin $\alpha_4\beta_1$ regulates trafficking to the CNS. In these models, interfering with $\alpha_4\beta_1$ and $\alpha_4\beta_7$ profoundly impairs immune cell recruitment to the respective tissues (17, 18). In fact, integrin-specific Abs are used clinically to block immune cell infiltration and provide relief from autoimmune diseases such as ulcerative colitis and multiple sclerosis (19, 20). Unfortunately, our understanding of how CD4$^+$ T cells traffic to the genital mucosa has been limited, including what combination of adhesion receptors is required for successful migration.

In this study, we interrogated the importance of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ integrin heterodimers in promoting Chlamydia-specific CD4$^+$ T cell recruitment to the genital mucosa and protecting mice from C. trachomatis infection. We show that integrin $\alpha_4\beta_1$ is dramatically increased on the surface of both polyclonal and Chlamydia-specific CD4$^+$ T cells in the uterus following infection. We find that blocking or deleting integrin $\alpha_4\beta_1$, but not $\alpha_4\beta_7$, on pathogen-specific CD4$^+$ T cells results in the impairment of trafficking to the uterus and a decrease in the protective capacity of CD4$^+$ T cells. We conclude that integrin $\alpha_4\beta_1$ is necessary for CD4$^+$ T cell–mediated protection against C. trachomatis. Identifying the receptors required for CD4$^+$ T cell trafficking to the genital tract in response to C. trachomatis is important in designing a vaccine that elicits sterilizing, long-lasting immunity against the pathogen while limiting the extent of tissue pathology.
Materials and Methods

Mice

C57BL/6, B6.PL-Thy1.2 (CD90.1 congenic), B6.SJL-Pep3b/Jig (CD45.1 congenic), B6.Cg-Tg (Lck-cre/Ifg7tm1Efu Jig) and B6;129-Ifg7tm1Efu/Jig mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and NR1 mice were previously described (21). B6;129-Ifg7tm1Efu/Jig mice were crossed with C57BL6. 

C. trachomatis inoculation and infection

C57BL6/J female mice were used in all experiments. A minimum of four mice per group were used in each experiment.

Statistical analysis

Statistical significance between groups was determined using an unpaired two-tailed t test and depicted within figures.

Results

C. trachomatis infection leads to robust integrin αβ1 surface expression on bulk CD4+ T cells in the uterus

Previous reports differ regarding the levels of αβ1 and αβ7 expression on T cells in the uterus during Chlamydia infection (23-25). As a first step to resolve these discrepancies, we examined the surface expression of αβ1, αβ7, and β1 on CD4+ T cells responding to C. trachomatis infection in the genital mucosa. To test differences in integrin expression, mice were infected transcervically with C. trachomatis (10). Seven days later, the uterus and draining (iliaic) lymph nodes were isolated and examined for the surface expression of integrins on endogenous CD4+ T cells by flow cytometry. We found that αβ1 and β1 were dramatically upregulated on the surface of CD4+ T cells in the uterus relative to those present in the draining lymph nodes of infected mice (Fig. 1A). In contrast, the surface expression of β7 was only modestly increased on CD4+ T cells in the genital mucosa compared with αβ1 and β1. We next quantified the absolute number of αβ1+β1+ and αβ7+β1+ CD4+ T cells in the genital tract (Fig. 1B). Very few CD4+ T cells were found in the uterus during steady-state in naïve mice. These extremely low numbers precluded conclusive interpretations about integrin staining differences in naïve mice. Following infection, the absolute number of αβ1+β1+ CD4+ T cells in the upper genital tract significantly increased whereas the number of αβ7+β1+ cells did not. We next compared the number of activated αβ1+β1+ or αβ7+β1+ CD4+ T cells responding to the genital mucosa by gating for populations expressing high levels of CD44. Interestingly, both the number of activated αβ1+β1+ and αβ7+β1+ CD4+ T cells were significantly increased in infected animals relative to naïve controls (Fig. 1C). Nonetheless, there was a more robust recruitment of activated αβ1+β1+ CD4+ T cells to infected uteri compared with αβ7+β1+ CD4+ T cells. Our results show that whereas both αβ1+β1+ and αβ7+β1+ CD4+ T cells are found in the infected genital mucosa, β1 is more highly expressed. These observations on endogenous T cells suggest that αβ1 is the primary integrin driving CD4+ T cell recruitment to the genital mucosa in response to C. trachomatis infection.

Infection leads to increased αβ1 surface expression on C. trachomatis--specific CD4+ T cells in the uterus

During Chlamydia infection, Ag-specific T cells are primed and recruited specifically to the genital tract. However, inflammatory cytokines can also activate bystander T cells at the site of infection independently of Ag specificity (26). Previous work characterizing
shown). In agreement with our observations with polyclonal CD4+ T cells, NR1 cells did not have major differences prior to transfer (data not shown). Comparing the geometric mean fluorescence intensity (gMFI) ratio of CD4+ T cells localized in the genital mucosa to those in the draining lymph node. (Fig. 2B) The absolute numbers of α4β1+ and α4β7+ CD4+ T cells were quantified in the genital tract of naive or infected mice with C. trachomatis for 7 d. (C) The absolute numbers of activated α4β1+ and α4β7+ CD4+ T cells were quantified in the genital tract of naive or infected mice; activation was determined by CD44 staining. Shown are representative results from one of two independent experiments. *p < 0.05, **p < 0.01, ****p < 0.0001.

Because we identified a strong upregulation of α4β1 on the surface of CD4+ T cells responding to C. trachomatis infection, we next assessed the functional role of β1 and β7 in promoting protective immunity. Our group has previously shown that transfer of in vitro–activated Th1-skewed NR1 cells into naive mice confers significant protection from C. trachomatis infection compared with mice that receive no T cells (10). We hypothesized that if specific integrins are required for recruitment to the genital mucosa, blocking these integrins on activated NR1 cells may alter their protective capacity. To test this and determine the relative
contributions of $\beta_1$ and $\beta_7$ to CD4$^+$ T cell–mediated protection, we selectively blocked integrin receptors using Abs (Fig. 3). NR1 cells from naive mice were harvested and polarized in vitro to a Th1 phenotype. IFN-$\gamma$ production by the NR1 cells was assayed to confirm their Th1 phenotype by flow cytometry prior to transfer (data not shown). One million NR1 Th1 cells were pretreated with Ab that blocked $\alpha_4$ ($\alpha_4\beta_1$ and $\alpha_4\beta_7$), $\alpha_4\beta_1$, or an isotype control and then transferred i.v. into host mice that were then transcorvically infected with $C. trachomatis$. The integrin surface expression on NR1 cells was analyzed by comparing the geometric mean fluorescence intensity (gMFI) ratio of NR1 cells in uterus to those localized in the draining lymph nodes. (A) Eight days after $C. trachomatis$ infection, the integrin surface expression on NR1 cells was analyzed by comparing the geometric mean fluorescence intensity (gMFI) ratio of NR1 cells in uterus to those localized in the draining lymph nodes. (B) The absolute numbers of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ NR1 cells were quantified 3 and 8 d after transcorvical infection in the uterus (left) and the draining lymph nodes (right). (C) Quantification of the trafficking kinetics of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ NR1 cells in the uterus (left) and draining lymph nodes (right) at the indicated time points. Shown are representative results from one of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. 

FIGURE 2. $C. trachomatis$ infection leads to robust $\alpha_4\beta_1$ surface expression on $C. trachomatis$–specific CD4$^+$ T cells responding to the genital tract. One million CD90.1$^+$ NR1 cells were transferred i.v. into CD90.2$^+$ recipient mice. The following day mice were infected transcervically with $10^8$ IFU $C. trachomatis$. The uterus and draining lymph nodes were harvested at the indicated time points following infection and prepared for flow cytometry. For integrin surface staining analysis, cells were pregated as live V$\alpha_2^+CD4^+CD90.1^+$ cells and then examined for the surface expression of $\alpha_4$, $\beta_1$, and $\beta_7$. (A) Eight days after $C. trachomatis$ infection, the integrin surface expression on NR1 cells was analyzed by comparing the geometric mean fluorescence intensity (gMFI) ratio of NR1 cells in uterus to those localized in the draining lymph nodes. (B) The absolute numbers of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ NR1 cells were quantified 3 and 8 d after transcorvical infection in the uterus (left) and the draining lymph nodes (right). (C) Quantification of the trafficking kinetics of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ NR1 cells in the uterus (left) and draining lymph nodes (right) at the indicated time points. Shown are representative results from one of three independent experiments. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. 

A reduction in CD4$^+$ T cells is responsible for the higher $C. trachomatis$ burden in anti-$\alpha_4$ Ab–treated mice. Although blocking $\alpha_4$ was sufficient to prevent CD4$^+$ T cell–mediated protection in the genital mucosa, we had not determined the mechanism responsible for higher burden. We predicted that the loss of protection seen in mice treated with $\alpha_4$ blocking Ab was due
treatment did not impact NR1 cell recruitment to the uterus, as found that the migration index was profoundly decreased in mice NR1 cells in the uteri to draining lymph nodes within the same suggesting no general defect in trafficking of NR1 cells following lymph nodes were not significantly different between the groups significantly decreased only in the group. We confirmed the efficiency of Ab blockade of FIGURE 3. Ab blockade of αβ but not αβ-exacerbates C. trachomatis burden in the genital mucosa. NR1 cells were skewed in vitro to a Th1 phenotype for 5 d. NR1 cells were pretreated with the indicated Abs and then transferred i.v. into naive recipients. The following day, mice were infected transversally with 5 × 10⁶ IFU C. trachomatis. Groups were injected with the respective integrin or isotype control Ab 1 and 3 d following infection. Five days postinfection, the genital tract was isolated and genomic DNA was purified. The levels of Chlamydia 16S DNA relative to the levels of host GADPH were quantified using qPCR. Shown are representative results from one of two independent experiments. *p < 0.05, **p < 0.01.
to diminished recruitment of NR1 cells to the infected genital mucosa. To test this possibility, we monitored the trafficking of NR1 cells to the infected genital tract after integrin blockade. NR1 cells were skewed to the Th1 phenotype in vitro and then transferred into congenically mismatched host mice. The next day mice were infected transversally with C. trachomatis. Prior to transfer, NR1 cells were pretreated with individual integrin blocking Abs or an isotype control. Mice were also treated with the same blocking Ab or isotype control 1 and 3 d postinfection. Five days postinfection, we examined the number of NR1 cells present in the genital tract and draining lymph nodes by flow cytometry. We noted that the number of NR1 cells in the genital mucosa was significantly diminished following treatment with the αβ blocking Ab relative to the isotype-treated control mice (Fig. 4A, 4B). In contrast, αβ Ab treatment did not impact NR1 cell recruitment to the uterus, as absolute numbers were similar between isotype- and αβ-treated groups. The absolute numbers of NR1 cells present in the draining lymph nodes were not significantly different between the groups suggesting no general defect in trafficking of NR1 cells following Ab treatment. We also calculated a migration index (the ratio of live NR1 cells in the uteri to draining lymph nodes within the same animal) to normalize for mouse-to-mouse variability (Fig. 4C). We found that the migration index was profoundly decreased in mice treated with αβ blocking Ab compared with isotype-treated mice. These results show that blocking αβ prevents efficient CD4⁺ T cell trafficking from the draining lymph nodes to the uteri following C. trachomatis infection. The migration index of mice treated with αβ Ab was not statistically different from isotype control–treated mice, demonstrating that αβ plays a limited role in NR1 cell trafficking to the genital mucosa in response to C. trachomatis infection.

We next examined how Ab treatment directly alters the recruitment of effector CD4⁺ T cell populations in the genital mucosa. NR1 cells were stained for CD44 and CD62L to evaluate the presence of effector CD4⁺ T cells at the site of the infection. The absolute number of CD44⁺CD62Llow NR1 cells in the uterus was significantly decreased only in the αβ Ab–treated group compared with isotype-treated mice (Fig. 4D). Moreover, αβ Ab treatment did not significantly decrease the absolute number of effector NR1 cells in the genital tract relative to the isotype-treated group. We confirmed the efficiency of αβ Ab blockade in vitro (data not shown). These results show that disrupting the integrin αβ but not αβ on NR1 cells is sufficient to eliminate CD4⁺ T cell–mediated protection provided following C. trachomatis infection in the genital tract.

Integrin β₇-deficient C. trachomatis–specific CD4⁺ T cells are unable to protect the uterus

To complement the Ab blocking experiments showing that αβ is required for Chlamydia-specific CD4⁺ T cells to home to and protect the genital mucosa, we generated TCR transgenic mice in which the T cells are deficient in either integrin β₁ or β₇. Because loss of β₁ results in embryonic lethality (28), we used a CRE-Flox system to generate NR1 cells conditionally deficient in β₁. NR1 transgenic mice were crossed to Lck-CRE and Itgb7flo/flo animals such that only the lymphocytes were deficient in β₁. We also crossed NR1 transgenic mice with Itgb7⁻/⁻ mice to generate Chlamydia-specific CD4⁺ T cells deficient in β₇. We first confirmed that integrin surface expression was significantly altered for each knockout T cell genotype (Supplemental Fig. 1). Interestingly, loss of β₁ led to a concomitant increase of surface β₁ on NR1 cells similar to a previous report showing that αβ heterodimers form more readily in the absence of the β₁-chain (29). The loss of β₇ also led to an increase of the percentage of β₁⁺ NR1 cells after in vitro activation. LFA-1 was robustly upregulated on NR1 cells from the three different genotypes. However, no compensatory LFA-1 expression was observed between integrin-deficient T cells. Additionally, NR1 cells do not express CD103 upon in vitro activation (data not shown). We next confirmed that NR1 cells deficient in individual integrins proliferated normally. Integrin-deficient NR1 cells were harvested from the knockout mice and polarized in vitro for 5 d to a Th1 phenotype. We found no significant difference between groups in the total number of recovered NR1 cells 5 d following activation, demonstrating that all the genotypes are viable (Fig. 5A). We next examined the activation and cytokine production for each group. For all genotypes examined, NR1 cells were robustly activated as determined by staining for the activation markers CD25 and CD44 (Fig. 5B). When we assayed cytokine profiles using intracellular cytokine staining, we found similar levels of IFN-γ and TNF-α in all groups, demonstrating that loss of integrin β-chains does not negatively impact Th1 differentiation (Fig. 5C). Therefore, the absence of either β₁ or β₇ does not interfere with expansion, activation, and Th1 cytokine production of NR1 cells in vitro.

Upon showing that the various genotypes of NR1 cells had normal effector phenotypes, we then tested whether deficiency in either the β₁- or β₇-chain would adversely affect the protective capacity of Chlamydia-specific CD4⁺ T cells in vivo. Based on our previous results from Ab blocking experiments, we hypothesized that β₁-deficient NR1 cells would be unable to protect the genital mucosa from C. trachomatis infection. We transferred 10⁷ wild-type, β₁⁻/⁻, or β₇⁻/⁻ Th1-skewed NR1 cells into naive mice 1 d prior to transversal infection with C. trachomatis (Fig. 5D). Five days postinfection, we harvested the genital tract and quantified the levels of C. trachomatis using qPCR. As expected, mice that received wild-type NR1 cells showed a significant reduction in C. trachomatis burden compared with mice that received no transfer. Mice that received β₁⁻/⁻ NR1 cells were not protected against C. trachomatis infection, as indicated by bacterial burdens similar to mice receiving no transferred NR1 cells. In line with our previous findings using Ab blockade, we also found that mice receiving β₇⁻/⁻ NR1 cells were significantly protected against C. trachomatis infection relative to mice that received no transfer, and trended toward being even more protective than wild-type NR1 cells. These findings definitively show that integrin β₁ on NR1
cells is dispensable for protecting the uterus from *C. trachomatis* infection. In summary, integrin β1, but not β7, is necessary for *Chlamydia*-specific CD4+ T cells to protect against infection in the genital mucosa.

**Integrin β1 deficiency impairs *C. trachomatis*-specific CD4+ T cell homing to the uterus**

We next sought to understand the mechanisms responsible for the loss of protective capacity in β1−/− *Chlamydia*-specific CD4+ T cells. Our previous data using Ab blocking showed that T cell trafficking to the genital mucosa was inhibited and therefore provided limited protection. In this study we used a competitive homing assay to test the trafficking potential of integrin-deficient *Chlamydia*-specific CD4+ T cells. We directly compared the migration of integrin-sufficient and -deficient NR1 cells under identical conditions within the same infected host. We transferred an equal number of CD45.2/CD90.2β1−/−, β7−/−, or wild-type NR1 cells and CD45.2/CD90.1 wild-type NR1 cells into congenically mismatched CD45.1 recipients. The next day, we infected mice transcervically with *C. trachomatis*. Seven days postinfection, we isolated and processed tissues to quantify the numbers of both NR1 populations using flow cytometry. We found that β1−/− NR1 cells were far less efficient

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**FIGURE 4.** Blockade of α4 but not α4β7 impairs *C. trachomatis*-specific CD4+ T cell trafficking to the genital tract following infection. NR1 cells were skewed in vitro to a Th1 phenotype for 5 d. NR1 cells were pretreated with the indicated Abs and then 10^6 CD90.1+ NR1 cells were transferred i.v. into CD90.2+ host mice. The following day mice were infected transcervically with 5 × 10^6 IFU *C. trachomatis*. Groups were injected with the respective integrin or control Ab 1 and 3 d following infection. The uterus and draining lymph nodes were harvested 5 d following infection and prepared for flow cytometry. For quantification of NR1 cell trafficking, we gated on live Vα2+CD4+CD90.1+ cells. (A) Representative flow cytometry plots indicate the percentage of NR1 cells following the indicated Ab treatment in the draining lymph nodes (top) and uterus (bottom). (B) The absolute numbers of NR1 cells were quantified in the uterus (left) and draining lymph nodes (right). (C) A migration index for each Ab treatment was calculated by comparing the absolute number of live NR1 cells in the uterus directly to the number of NR1 cells in draining lymph nodes within each animal. A lower migration index ratio indicates decreased NR1 cell recruitment to the uterus. (D) The absolute number of effector NR1 cells was determined by examining the CD44+CD62Llow gated population in the genital mucosa. Shown are representative results from one of two independent experiments. *p < 0.05, **p < 0.01, ***p < 0.001.
than wild-type NR1 cells in trafficking to the genital mucosa, whereas $\beta_1^{--}$ NR1 cells outcompeted their wild-type counterparts (Fig 6A). None of the experimental groups showed defects in their migration to the draining lymph node. Interestingly, we noted a higher percentage of $\beta_1^{--}$ NR1 cells in the lymph nodes compared with the wild-type NR1 cells, the inverse of what we observed in the genital mucosa. These results are likely due to a decreased ability of $\beta_1^{--}$ NR1 cells to leave the circulation to enter the infected genital mucosa. To normalize for mouse-to-mouse variation in absolute T cell numbers, we calculated a migration index for each mouse by comparing the ratio of the two transferred NR1 cell populations in the uterus (percentage integrin-deficient CD90.2/percentage integrin-sufficient CD90.1) to the ratio of the transferred populations in the draining lymph nodes or spleen within the same animal (Fig. 6B). A smaller migration index indicated less efficient trafficking of integrin-deficient NR1 cells specifically to the uterus relative to the circulation. We found a dramatically lower migration index for $\beta_1^{--}$ NR1 cells, demonstrating that trafficking to the uterus during infection was significantly impaired relative to wild-type NR1 cells. Intriguingly, the migration index for $\beta_7^{--}$ NR1 cells was significantly higher than wild-type, demonstrating enhanced homing of Chlamydia-specific CD4$^+$ T cells to the uterus in the absence of integrin $\beta_7$. These results suggest that integrin $\beta_7$ is not only dispensable, but that deficiency of $\beta_7$ enhances Ag-specific CD4$^+$ T cell migration to the genital tract during C. trachomatis infection. Our findings collectively reveal that integrin $\beta_1$ plays a crucial role in trafficking of CD4$^+$ T cells to the genital mucosa and that absence of $\beta_1$ negatively affects the protective capacity of C. trachomatis–specific CD4$^+$ T cells.

**Discussion**

The orchestration of events required for a successful T cell response determines whether an intracellular pathogen will be eliminated from the host. In the case of C. trachomatis infection, a robust CD4$^+$ Th1 cell population that homes to the genital tract provides the vigorous IFN-\(\gamma\) response necessary for bacterial clearance
FIGURE 6. β1-deficient Chlamydia-specific CD4+ T cells are unable to traffic efficiently to the genital tract following infection. An equivalent number of CD45.2/CD90.1 integrin wild-type NR1 cells and CD45.2/CD90.2 wild-type, β1+/−, or β1−/− NR1 cells were transferred i.v. into CD45.1/CD90.2 host mice. The next day, mice were infected transcervically with 10^6 IFU C. trachomatis. The genital tract, draining lymph nodes, and spleen were harvested 7 d postinfection and prepared for flow cytometry. We examined the recruitment of NR1 cells by preying on live Vax2+CD4+CD52+ cells. We then differentiated the competing NR1 populations by examining the number of CD90.1+ or CD90.2+ cells. (A) Shown are representative plots of integrin-sufficient and -deficient NR1 cells in the draining lymph node (top) and genital mucosa (bottom). (B) The migration index within each group was calculated by comparing the percentage of CD90.2+ to CD90.1+ NR1 cells in the uterus to the CD90.2+ to CD90.1+ NR1 cells in the draining lymph nodes (left) or spleen (right). A lower migration index indicates less efficient trafficking of integrin-deficient NR1 cells specifically to the uterus relative to the circulation. Shown are representative results from one of three independent experiments. **p < 0.01, ***p < 0.001, ****p < 0.0001.
mice. Ruane et al. (33) found that a subset of residing dendritic cells (DCs) can imprint lung T cells to express αββ. The T cells expressing αββ do not mediate protection in the lungs but rather provide gut mucosal immunity. Given that a subset of lung DCs can imprint a population of T cells to express αββ, we speculate that a similar process also occurs in the genital tract. Although most CD4+ T cells are primed to express αββ, which mediates immunity in the genital mucosa during Chlamydia infection, perhaps a subset of uterine DCs also imprints αββ on a fraction of the CD4+ T cell population that may provide systemic mucosal immunity. This hypothesis will need to be examined in future experiments.

It is well established that integrin αββ adheres to the addressin VCAM-1 and the extracellular matrix protein fibronectin (34, 35). Surface VCAM-1 increases on endothelial cells lining microvessels following infection (27). For example, patients with CNS autoimmune disorders are often treated with natalizumab, an anti–α4 Ab that blocks αββ and αββ interactions with their ligands. It is thought that natalizumab treatment decreases undesirable inflammation in the CNS by interfering with αββ-mediated immune cell recruitment to this sensitized area in the body (36). Previous studies have also shown that surface VCAM-1 becomes abundant on murine and human genital mucosa following Chlamydia infection. In contrast, the expression of the mucosal addressin cell adhesion molecule-1, the binding partner for αββ, has been reported to be expressed robustly in the gut but only modestly in the genital tract (25, 37).

The noticeable increase of surface VCAM-1 in the genital mucosa following Chlamydia infection corresponds to the upregulation of surface αββ on Chlamydia-specific CD4+ T cells in the uterus observed in this study.

Several other signals stimulate the rapid increase of VCAM-1 on vaginal epithelial cells, including IFN-γ treatment and HSV infection (38). Given that VCAM-1 can be selectively upregulated on both endothelial and nonendothelial cells, responding CD4+ T cells may require αββ signaling for multiple steps to provide protection. Previous studies have characterized the importance of αββ to slow/arrest lymphocytes in the blood vessel, but αββ may also mediate the interactions between effector CD4+ T cells and infected epithelial cells as has been suggested to occur during Chlamydia infection (39). It remains unknown whether Chlamydia–specific CD4+ T cells directly interact with infected epithelial cells in the genital mucosa or whether their antimicrobial effects occur by altering the cytokine milieu at the site of infection. In this study we observed that integrin ββ and βγ were dispensable for proliferation and differentiation to a Th1 phenotype (Fig. 5A–C). Consequently, we conclude that the function of βγ in mediating protection (Fig. 5D) is to allow successful Chlamydia-specific CD4+ T cell trafficking to the uterus (Fig. 6) rather than playing a role in activation or production of IFN-γ.

Future studies must further explore the interaction between IFN-γ-producing CD4+ T cells and the infected epithelium to determine whether βγ is required for cellular interactions in vivo within the genital mucosa. Additional studies will need to determine whether T cell recruitment and effector activity, mediated by αββ, contribute to genital tract pathology following Chlamydia infection. Although integrin βγ is found primarily in complex with α4 on T cells, our findings do not entirely eliminate the possibility that βγ can form additional heterodimers on CD4+ T cells and that these heterodimers may play a role in the retention of T cells in the genital tract following infection.

The results obtained through this study further elucidate the essential homing receptors required for an effective CD4+ T cell defense in the genital mucosa. It remains to be determined whether CD4+ T cells responding to other pathogens in the genital tract also require the homing receptors we have identified (CXCR3, CCR5, and αββ) as required for successful trafficking during Chlamydia infection. Generating a robust and long-lived protective T cell response is crucial to clear infection and avoid recurrent cycles of inflammation and associated pathology. Vaccine efforts against intracellular pathogens should examine whether the appropriate T cell population, with the necessary homing molecules, is being generated to ensure protection and minimize pathology. In addition to the current treatments for autoimmune diseases in the gut and the CNS, integrin- and chemokine receptor–targeted therapies could be used to selectively shape the recruitment of desired T cells to other mucosal tissues.

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Disclosures
The authors have no conflicts of interest.

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