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

Sergio J. Davila,[1] Andrew J. Olive,[1] and Michael N. Starnbach

***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-γ 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 $\alpha_4\beta_7$, 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.**

Chlamydia 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-γ (4). IFN-γ 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-γ production have delayed resolution of infection in the genital mucosa (9). $CD4^+$ T cells must produce IFN-γ to mediate protection, as transfer of *Chlamydia*-specific $CD4^+$ T cells only protects naive mice against challenge when IFN-γ is produced by those T cells. It is also critical that Ag-specific, IFN-γ–secreting $CD4^+$ 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 α and β 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 $\alpha_4\beta_7$, 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.

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Abbreviations used in this article: DC, dendritic cell; IFU, inclusion-forming unit; qPCR, quantitative PCR.

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Materials and Methods

Mice

C57BL/6, B6.PL-*Thy1*^{a} (CD90.1 congenic), B6.SJL-*Ptprc*^{a} *Pep3*/BoyJ (CD45.1 congenic), B6.Cg-Tg (*Lck-CRE*)548Jxm/J, C57BL/6-*Itgb7*^{tm1Cgn}/J (*Itgb7*$^{-/-}$), and B6;129-*Itgb1*^{tm1Efu}/J (*Itgb1*$^{flox/flox}$) mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and NR1 mice were previously described (21). B6;129-*Itgb1*^{tm1Efu}/J (*Itgb1*$^{flox/flox}$) mice were crossed with C57BL/6 for >10 generations. *C. trachomatis*–specific integrin-deficient mice were generated by breeding NR1 mice to either *Lck-CRE* and *Itgb1*$^{flox/flox}$ or *Itgb7*$^{-/-}$ mice. All mice were maintained in facilities managed by the Harvard Medical School Center for Animal Resources and Comparative Medicine. To normalize for the murine estrous cycle, mice were treated s.c. with 2.5 mg medroxyprogesterone 7 d prior to infection. Harvard's Institutional Animal Care and Use Committee approved all of the experiments described. A minimum of four mice per group were used in each experiment.

Growth, isolation, and detection of C. trachomatis

C. trachomatis serovar L2 (434/Bu) was propagated using McCoy cell monolayers grown in Eagle's MEM (Invitrogen, Grand Island, NY) plus 10% FCS, 1.5 g/l sodium bicarbonate, 0.1 M nonessential amino acids, and 1 mM sodium pyruvate. Infected McCoy cells were detached from plates using sterile glass beads and then sonicated to disrupt *C. trachomatis* inclusions. Density gradient centrifugation was used to purify elementary bodies (22). Aliquots were stored at −80°C in a medium containing 250 mM sucrose, 10 mM sodium phosphate, and 5 mM L-glutamic acid.

Transfer of NR1 cells, infection of mice, and tissue preparation

C. trachomatis–specific CD4$^+$ T cells were isolated from the lymph nodes and spleens of naive donor NR1 mice. Recipient mice received 10^6 NR1 cells and were infected the following day with 10^6 *C. trachomatis* inclusion forming units (IFU) in 10 μl sucrose-phosphate-glutamate media. We used the NSET device (ParaTechs) to bypass the cervix and directly infect the uterine horns (10). The uterus was harvested and disaggregated by digestion with 1 mg/ml type XI collagenase (Sigma-Aldrich, St. Louis, MO) and 50 Kunitz/ml DNase (Sigma-Aldrich) for 30 min at 37°C. Single-cell suspensions from tissues were obtained by mechanical disaggregation prior to staining. Suspensions of splenocytes were treated with a hypotonic buffer to lyse RBCs prior to use.

Flow cytometry

Single-cell suspensions were stained immediately for activation markers or stimulated for 5 h with 100 ng/ml PMA (Alexis Biochemicals) and 1 μg/ml ionomycin (Calbiochem) in brefeldin A (BD Biosciences) for intracellular cytokine staining. Cells were treated with anti-FcγR (BioXCell) before staining with combinations of the following Abs: anti-β_1 Pacific Blue, anti-β7 FITC, anti-TCRvα2 allophycocyanin, anti-CD90.1 PerCP, anti-CD45.2 PE, anti-CD90.2 FITC, anti–IFN-γ PE, anti–TNF-α PE-Cy7, anti-CD25 PE, anti-CD44 PE or Pacific Blue, anti-CD62L FITC (BioLegend), anti-CD3ε allophycocyanin, anti-α_4 PE (BD Biosciences), anti-CD4 Qdot605, and a Live/Dead dead cell stain kit (Invitrogen). The efficacy of all Abs used in this study was confirmed extensively in vitro and compared with isotype control Abs. For cytokine staining, cells were permeabilized using a Cytofix/Cytoperm Plus kit following the manufacturer's instructions (BD Biosciences). Cell number was determined with AccuCheck counting beads (Invitrogen). Flow cytometry data were collected on a modified FACSCalibur (Cytek Development) or an LSR II (BD Biosciences) and analyzed using FlowJo (Tree Star).

Th1 polarization and protection against C. trachomatis

CD4$^+$ T cells were harvested from the lymph nodes and spleens of naive NR1 mice and enriched with a mouse CD4 negative isolation kit (Invitrogen) following the manufacturer's protocol. CD4$^+$ T cells were cultured in media consisting of RPMI 1640 (Invitrogen), 10% FCS, L-glutamine, HEPES, 50 μM 2-ME, 50 U/ml penicillin, and 50 mg/ml streptomycin. NR1 cells were activated by coculture with irradiated or mitomycin-treated splenocytes pulsed with 5 μM Cta1$_{133–152}$ peptide at a stimulator/T cell ratio of 4:1. Th1 polarization was achieved by supplying cultures with 10 ng/ml IL-12 (PeproTech, Rocky Hill, NJ) and 10 μg/ml anti–IL-4 Ab (BioLegend). After 5 d of stimulation, NR1 Th1 cells were transferred i.v. into naive recipient mice. In integrin Ab blocking experiments, NR1 cells were treated with 100 μg anti-α_4, anti-$\alpha_4\beta_7$, or isotype Ab (BioXCell) for 1 h at room temperature prior to transfer into recipients. The next day, mice were challenged with 5×10^6 *C. trachomatis* IFU and the upper genital tract was analyzed for burden 5 d postinfection. Mice were treated i.p. with 200 μg of the respective Ab at 1 and 3 d postinfection for integrin blocking experiments.

Competitive homing

For this assay an equivalent number of integrin wild-type NR1 cells (CD45.2/CD90.1) and congenic wild-type, *Lck-CRE*/*Itgb1*$^{flox/flox}$, or *Itgb7*$^{-/-}$ NR1 cells (CD45.2/CD90.2) were combined and transferred into congenically mismatched CD45.1 hosts. The following day, mice were transcervically infected with 5×10^6 *C. trachomatis* IFU. Tissues were harvested and analyzed 7 d postinfection.

Quantitative PCR

Bacterial burden was evaluated by quantifying *C. trachomatis* 16S DNA relative to mouse GADPH DNA (21). The uterus was homogenized and DNA was extracted using the DNeasy blood and tissue kit (Qiagen). DNA was analyzed using *C. trachomatis*– and mouse-specific primer pairs and dual-labeled probes. Threshold values were detected by an ABI Prism 7000 sequence system (Applied Biosystems). The ratio of *C. trachomatis* to host DNA was obtained using a standard curve.

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 $\alpha_4\beta_1$ surface expression on bulk CD4$^+$ T cells in the uterus*

Previous reports differ regarding the levels of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ 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 α_4, β_1, and β_7 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 (iliac) lymph nodes were isolated and examined for the surface expression of integrins on endogenous CD4$^+$ T cells by flow cytometry. We found that α_4 and β_1 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 β_7 was only modestly increased on CD4$^+$ T cells in the genital mucosa compared with α_4 and β_1. We next quantified the absolute number of $\alpha_4^+\beta_1^+$- and $\alpha_4^+\beta_7^+$-expressing CD4$^+$ T cells in the genital tract (Fig. 1B). Very few CD4$^+$ T cells were found in the uterus during steady-state in naive mice. These extremely low numbers precluded conclusive interpretations about integrin staining differences in naive mice. Following infection, the absolute number of $\alpha_4^+\beta_1^+$ CD4$^+$ T cells in the upper genital tract significantly increased whereas the number of $\alpha_4^+\beta_7^+$ cells did not. We next compared the number of activated $\alpha_4^+\beta_1^+$ or $\alpha_4^+\beta_7^+$ CD4$^+$ T cells responding to the genital mucosa by gating for populations expressing high levels of CD44. Interestingly, both the number of activated $\alpha_4^+\beta_1^+$ and $\alpha_4^+\beta_7^+$ CD4$^+$ T cells were significantly increased in infected animals relative to naive controls (Fig. 1C). Nonetheless, there was a more robust recruitment of activated $\alpha_4^+\beta_1^+$ CD4$^+$ T cells to infected uteri compared with $\alpha_4^+\beta_7^+$ CD4$^+$ T cells. Our results show that whereas both $\alpha_4^+\beta_1^+$ and $\alpha_4^+\beta_7^+$ CD4$^+$ T cells are found in the infected genital mucosa, β_1 is more highly expressed. These observations on endogenous T cells suggest that $\alpha_4\beta_1$ is the primary integrin driving CD4$^+$ T cell recruitment to the genital mucosa in response to *C. trachomatis* infection.

Infection leads to increased $\alpha_4\beta_1$ 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

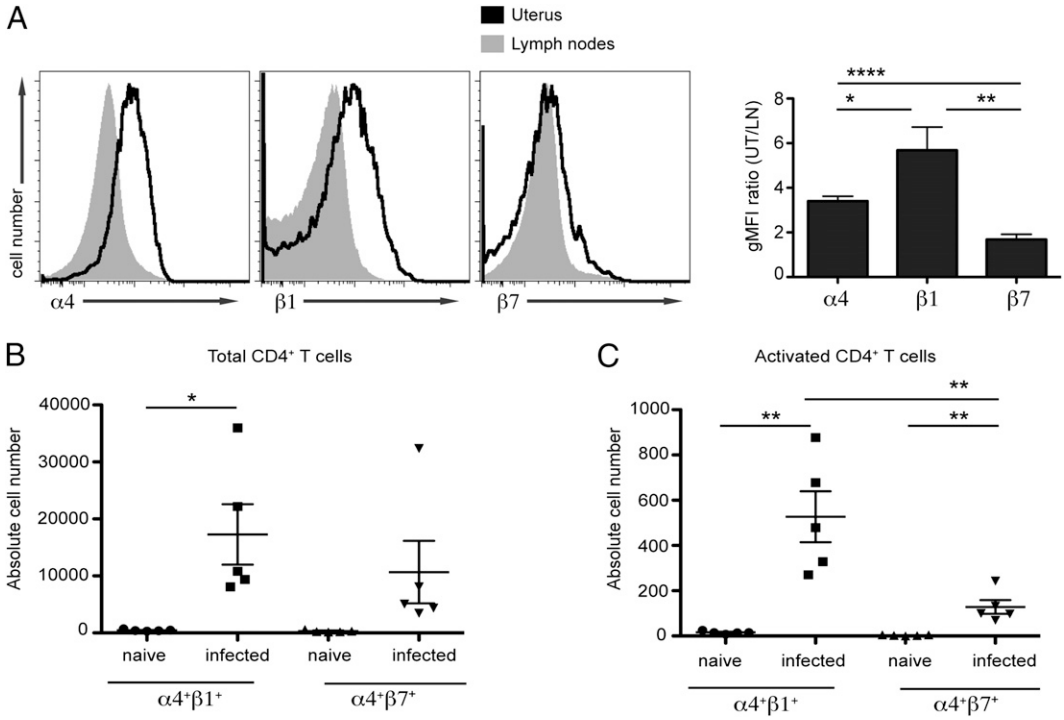

FIGURE 1. *C. trachomatis* infection leads to robust integrin $\alpha_4\beta_1$ surface expression on polyclonal $CD4^+$ T cells in the genital tract. C57BL/6 mice were transcervically infected with 10^6 IFU *C. trachomatis*. Seven days following infection the indicated tissues were harvested and prepared for flow cytometry. After gating on live cells that were $CD3^+CD4^+$ the surface expression of α_4, β_1, and β_7 was quantified. (**A**) The integrin surface expression was analyzed by comparing the geometric mean fluorescence intensity (gMFI) ratio of $CD4^+$ T cells localized in the genital mucosa to those in the draining lymph node. (**B**) The absolute numbers of $\alpha_4^+\beta_1^+$ and $\alpha_4^+\beta_7^+$ $CD4^+$ T cells were quantified in the genital tract of naive or mice infected with *C. trachomatis* for 7 d. (**C**) The absolute numbers of activated $\alpha_4^+\beta_1^+$ and $\alpha_4^+\beta_7^+$ $CD4^+$ 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. $^*p < 0.05$, $^{**}p < 0.01$, $^{****}p < 0.0001$.

integrin receptors required for T cell recruitment to the genital mucosa focused exclusively on memory $CD4^+$ T cell lines (23), polyclonal $CD4^+$ T cells (24), or bulk $CD4^+$ T cells (25) but never examined naive *C. trachomatis*–specific $CD4^+$ T cells. However, only by using naive Ag-specific T cells is it possible to properly model primary infection. To directly interrogate the integrin profile of *Chlamydia*-specific $CD4^+$ T cells, we took advantage of *C. trachomatis*–specific TCR transgenic $CD4^+$ T cells that are locked into specificity for the *Chlamydia* Ag Cta1 (referred to subsequently as NR1 cells) (21). Based on the results described above, we hypothesized that NR1 cells would display significantly increased surface β_1, rather than β_7, upon trafficking to infected uteri. To test this prediction, we transferred 10^6 NR1 cells i.v. into congenic naive animals that were then infected transcervically the following day with *C. trachomatis*. The integrin profile of naive NR1 cells did not have major differences prior to transfer (data not shown). In agreement with our observations with polyclonal $CD4^+$ T cells (Fig. 1), 8 d postinfection there was a >10-fold increase in the surface expression of integrin β_1 on NR1 cells in the uterus compared with NR1 cells in the draining lymph nodes (Fig. 2A). To better understand the dynamics of integrin expression we monitored the surface expression of integrins on NR1 cells in the genital tract and draining lymph nodes at three time points following *C. trachomatis* infection. Three, 8, and 13 d postinfection were chosen to coincide with T cell activation, peak of infiltration to the site of infection, and contraction, respectively (Fig. 2B, 2C). There was a surge of both $\alpha_4^+\beta_1^+$ and $\alpha_4^+\beta_7^+$ NR1 cells in the genital tract 8 d postinfection compared with what was observed with these populations 3 d postinfection (Fig. 2B). Even though both NR1 populations increased during infection, the magnitude of infiltration of the $\alpha_4^+\beta_1^+$ NR1 cells to the uterus was far more robust ($^{***}p < 0.001$) than for the $\alpha_4^+\beta_7^+$ population ($^*p < 0.05$) (Fig. 2B, *left*). Surface integrin profiles of NR1 cells in the draining lymph nodes were statistically indistinguishable between these two time points (Fig. 2B, *right*). Thirteen days postinfection the decline of NR1 cells (Fig. 2C) corresponded to the resolution of *C. trachomatis* infection in the genital mucosa (10). Even at this late time point, $\alpha_4^+\beta_1^+$ NR1 cells were found significantly more frequently than $\alpha_4^+\beta_7^+$ NR1 cells in the genital tract. These observations suggest that $\alpha_4^+\beta_1^+$ NR1 cells are either recruited or retained in the uterus for a longer period of time than other T cell populations. We confirmed that the limited β_7 expression observed in Figs. 1 and 2 was not due to poor Ab staining. Robust β_7 staining was detected on NR1 cells activated in vitro in the presence of retinoic acid, a metabolite previously shown to induce $\alpha_4\beta_7$ expression on T cells (data not shown). Taken together, these findings demonstrate that both polyclonal and *Chlamydia*-specific $CD4^+$ T cells preferentially express α_4 and β_1 on their surface in the murine genital mucosa in response to *C. trachomatis* infection.

Blocking integrin α_4 but not $\alpha_4\beta_7$ increases C. trachomatis *burden*

Because we identified a strong upregulation of $\alpha_4\beta_1$ on the surface of $CD4^+$ T cells responding to *C. trachomatis* infection, we next assessed the functional role of β_1 and β_7 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

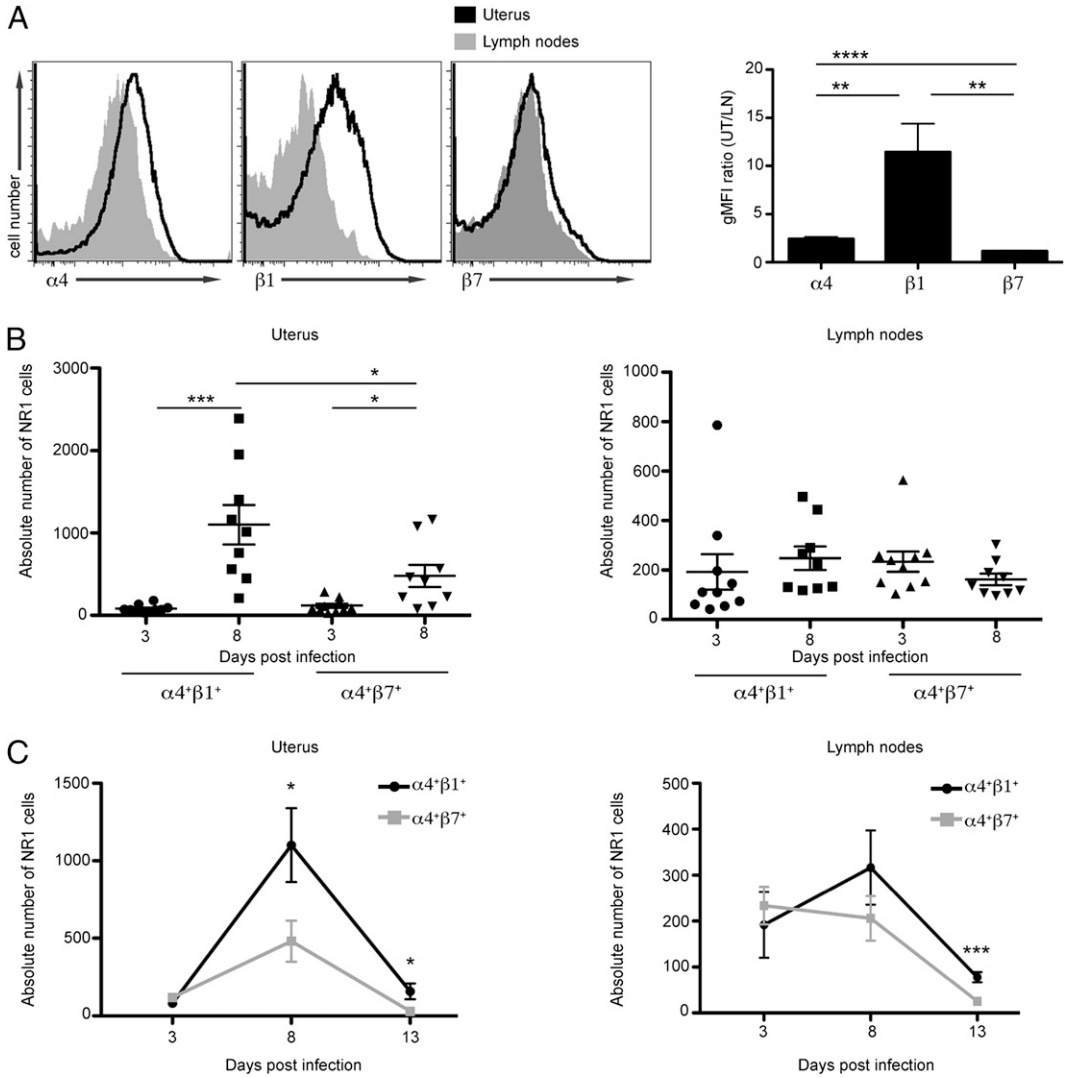

FIGURE 2. *C. trachomatis* infection leads to robust $\alpha_4\beta_1$ surface expression on *C. trachomatis*–specific $CD4^+$ T cells responding to the genital tract. One million $CD90.1^+$ NR1 cells were transferred i.v. into $CD90.2^+$ recipient mice. The following day mice were infected transcervically with 10^6 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 $V\alpha2^+CD4^+CD90.1^+$ cells and then examined for the surface expression of α_4, β_1, and β_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 transcervical 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. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

contributions of β_1 and β_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-γ 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 α_4 (blocks both $\alpha_4\beta_1$ and $\alpha_4\beta_7$), $\alpha_4\beta_7$, or an isotype control and then transferred i.v. into host mice that were then transcervically infected with *C. trachomatis* the following day. To ensure robust blockade of integrins, each group of mice was also treated with Ab 1 and 3 d following infection. Five days postinfection, *C. trachomatis* burden in the uterus was measured using quantitative PCR (qPCR). Mice that were treated with blocking Ab against α_4 had a higher *C. trachomatis* burden in the genital tract than did isotype control–treated animals, similar to bacterial levels found in mice that received no NR1 cells at all. Alternatively, mice treated with $\alpha_4\beta_7$ blocking Ab had significantly lower *C. trachomatis* levels than the no transfer group and similar bacterial burden to the isotype Ab–treated mice. Despite the lack of an Ab that specifically blocks $\alpha_4\beta1$, we can infer the importance of $\alpha_4\beta_1$ indirectly. Because blocking α_4 with Ab prevents both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ signaling, the differences observed in protection between α_4 and $\alpha_4\beta_7$ Ab treatment groups point toward a function of $\alpha_4\beta_1$. Because the integrin α_4-chain dimerizes with either β_1 or β_7 (27), the results shown in Fig. 3 indirectly confirm that only $\alpha_4\beta_1$ is required for $CD4^+$ T cell–mediated protection against *C. trachomatis* in the genital mucosa.

A reduction in $CD4^+$ T cells is responsible for the higher C. trachomatis *burden in anti-α_4 Ab–treated mice*

Although blocking α_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 α_4 blocking Ab was due

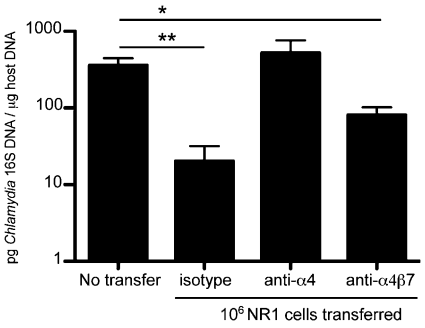

FIGURE 3. Ab blockade of α_4 but not $\alpha_4\beta_7$ exacerbates *C. trachomatis* burden in the genital mucosa. NR1 cells were skewed in vitro to a Th1 phenotype for 5 d. NR1 cells were pretreated with the indicated Abs and then transferred i.v. into naive recipients. The following day, mice were infected transcervically with 5×10^6 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 transcervically 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 α_4 blocking Ab relative to the isotype-treated control mice (Fig. 4A, 4B). In contrast, $\alpha_4\beta_7$ Ab treatment did not impact NR1 cell recruitment to the uterus, as absolute numbers were similar between isotype- and $\alpha_4\beta_7$-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 α_4 blocking Ab compared with isotype-treated mice. These results show that blocking α_4 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 $\alpha_4\beta_7$ Ab was not statistically different from isotype control–treated mice, demonstrating that $\alpha_4\beta_7$ 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 $CD44^+CD62L^{low}$ NR1 cells in the uterus was significantly decreased only in the α_4 Ab–treated group compared with isotype-treated mice (Fig. 4D). Moreover, anti–$\alpha_4\beta_7$ 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 $\alpha_4\beta_7$ Ab blockade in vitro (data not shown). These results show that disrupting the integrin $\alpha_4\beta_1$ but not $\alpha_4\beta_7$ on NR1 cells is sufficient to eliminate $CD4^+$ T cell–mediated protection provided following *C. trachomatis* infection in the genital tract.

Integrin β_1–deficient C. trachomatis*–specific $CD4^+$ T cells are unable to protect the uterus*

To complement the Ab blocking experiments showing that $\alpha_4\beta_1$ 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 β_1 or β_7. Because loss of β_1 results in embryonic lethality (28), we used a CRE-Flox system to generate NR1 cells conditionally deficient in β_1. NR1 transgenic mice were crossed to *Lck-CRE* and *Itgb1*$^{flox/flox}$ animals such that only the lymphocytes were deficient in β_1. We also crossed NR1 transgenic mice with *Itgb7*$^{-/-}$ mice to generate *Chlamydia*-specific $CD4^+$ T cells deficient in β_7. We first confirmed that integrin surface expression was significantly altered for each knockout T cell genotype (Supplemental Fig. 1). Interestingly, loss of β_1 led to a concomitant increase of surface β_7 on NR1 cells similar to a previous report showing that $\alpha_4\beta_7$ heterodimers form more readily in the absence of the β_1-chain (29). The loss of β_7 also led to an increase of the percentage of β_1^+ 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 β_1 or β_7 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 β_1- or β_7-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 β_1-deficient NR1 cells would be unable to protect the genital mucosa from *C. trachomatis* infection. We transferred 10^5 wild-type, $\beta_1^{-/-}$, or $\beta_7^{-/-}$ Th1-skewed NR1 cells into naive mice 1 d prior to transcervical 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 $\beta_1^{-/-}$ 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 $\beta_7^{-/-}$ 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 β_7 on NR1

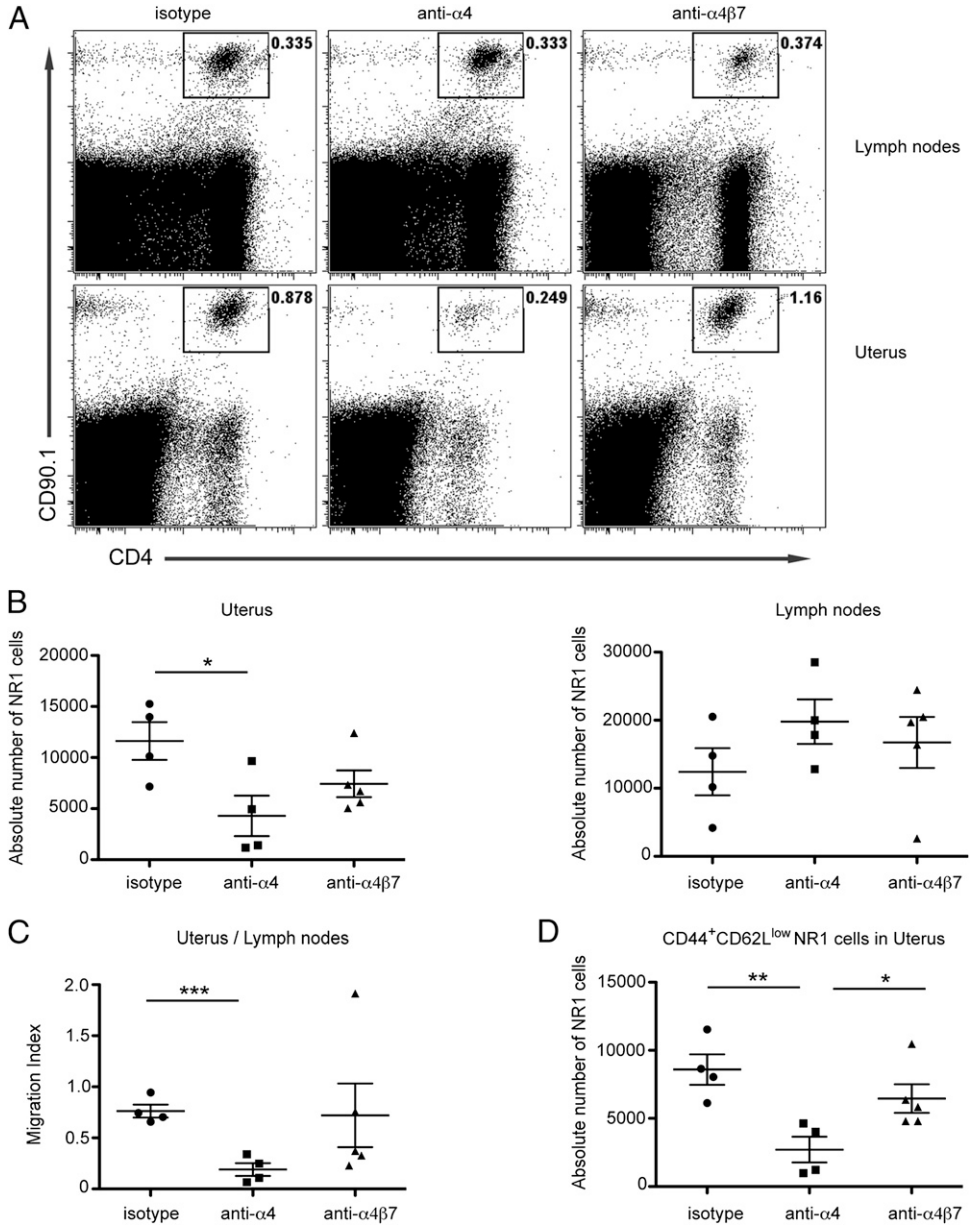

FIGURE 4. Blockade of α_4 but not $\alpha_4\beta_7$ impairs *C. trachomatis*–specific $CD4^+$ T cell trafficking to the genital tract following infection. NR1 cells were skewed in vitro to a Th1 phenotype for 5 d. NR1 cells were pretreated with the indicated Abs and then 10^6 $CD90.1^+$ NR1 cells were 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 $V\alpha2^+CD4^+CD90.1^+$ 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 $CD44^+CD62L^{low}$ gated population in the genital mucosa. Shown are representative results from one of two independent experiments. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

cells is dispensable for protecting the uterus from *C. trachomatis* infection. In summary, integrin β1, but not β_7, is necessary for *Chlamydia*-specific $CD4^+$ T cells to protect against infection in the genital mucosa.

Integrin β_1 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 $\beta_1^{-/-}$ *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/CD90.2 $\beta_1^{-/-}$, $\beta_7^{-/-}$, 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 $\beta_1^{-/-}$ NR1 cells were far less efficient

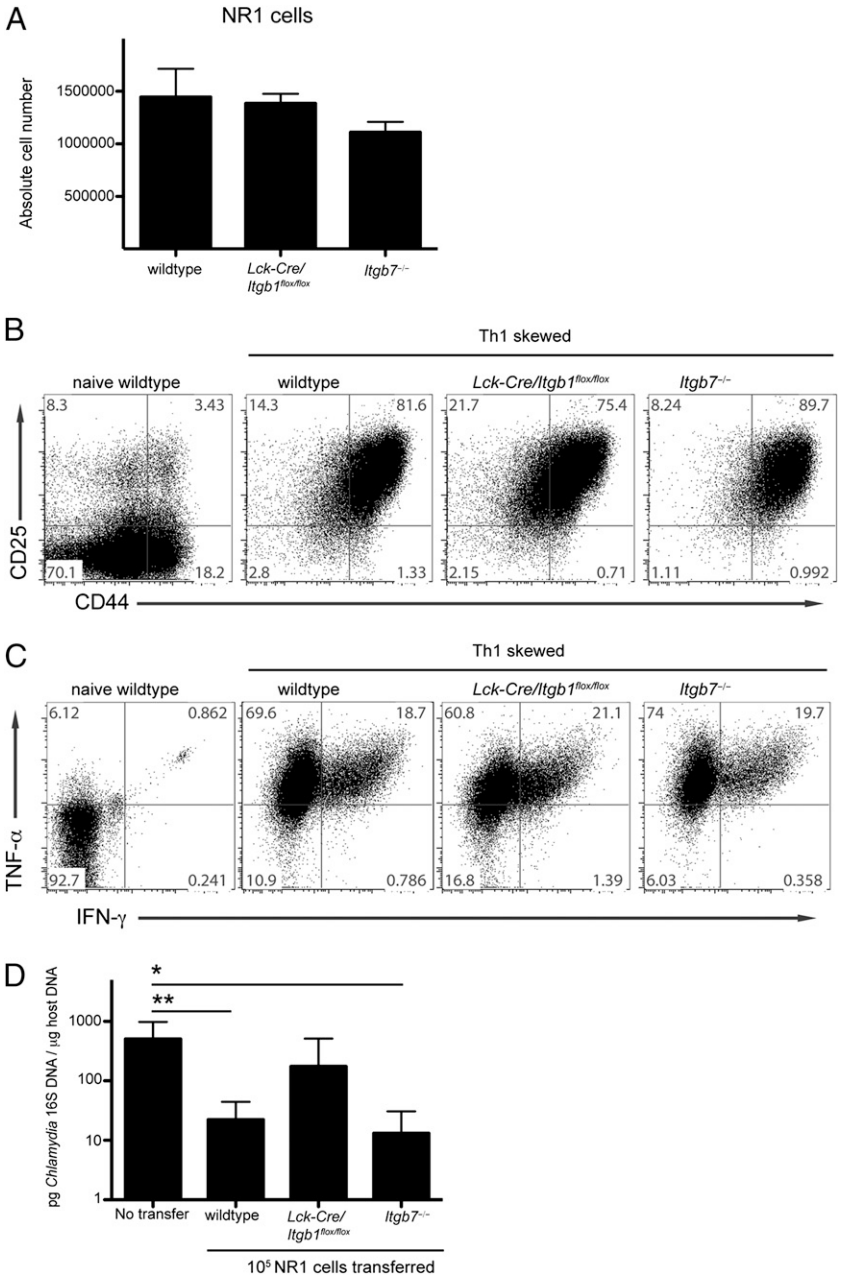

FIGURE 5. *Chlamydia*-specific $CD4^+$ T cells deficient in integrin β_1 are unable to protect mice from *C. trachomatis* infection. The indicated NR1 genotypes were skewed in vitro to a Th1 phenotype for 5 d. (**A**) The expansion of NR1 cells was compared for the three genotypes. (**B**) The activation of live NR1 cells was assessed by gating for $CD25^+CD44^+$ cells. (**C**) TNF-α and IFN-γ production was examined using intracellular cytokine staining. (**D**) After 5 d of in vitro stimulation, 10^5 NR1 cells were transferred i.v. into naive hosts. The following day, mice were transcervically infected with 5×10^6 IFU *C. trachomatis*. Five days postinfection, the genital tract was harvested 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 three independent experiments. $*p < 0.05$, $**p < 0.01$.

than wild-type NR1 cells in trafficking to the genital mucosa, whereas $\beta_7^{-/-}$ 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 β_7. These results suggest that integrin β_7 is not only dispensable, but that deficiency of β_7 enhances Ag-specific $CD4^+$ T cell migration to the genital tract during *C. trachomatis* infection. Our findings collectively reveal that integrin β_1 plays a crucial in trafficking of $CD4^+$ T cells to the genital mucosa and that absence of β_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-γ response necessary for bacterial clearance

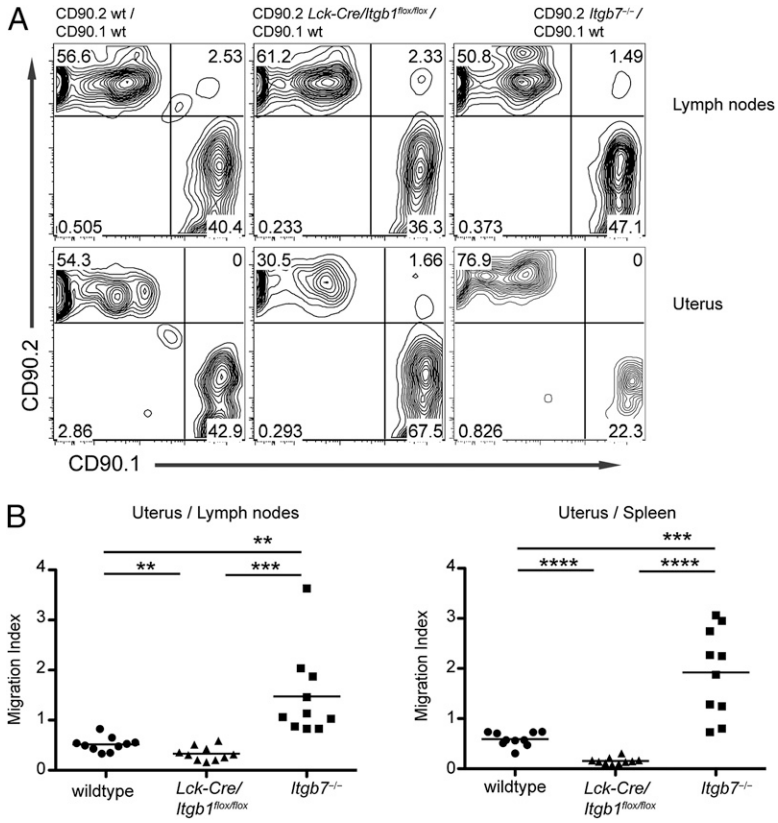

FIGURE 6. β_1-deficient *Chlamydia*-specific $CD4^+$ T cells are unable to traffic efficiently to the genital tract following infection. An equivalent number of CD45.2/CD90.1 integrin wild-type NR1 cells and CD45.2/CD90.2 wild-type, $\beta_1^{-/-}$, or $\beta_7^{-/-}$ NR1 cells were transferred i.v. 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 pregating on live $V\alpha2^+CD4^+CD45.2^+$ 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. $^{**}p < 0.01$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

(10). The integrin receptors important for *C. trachomatis*–specific $CD4^+$ T cell–mediated protection have not been previously described. Using *C. trachomatis*–specific $CD4^+$ T cells, we demonstrated that perturbing integrin $\alpha_4\beta_1$ but not $\alpha_4\beta_7$, through Ab blockade or genetic deletion, results in impaired T cell trafficking to the genital tract and a loss of protective capacity following *C. trachomatis* infection. Taken together, these observations demonstrate that integrin $\alpha_4\beta_1$ is necessary for $CD4^+$ T cell–mediated protection against *C. trachomatis* infection in the murine genital tract.

We previously demonstrated that the chemokine receptors CXCR3 and CCR5 are required for *Chlamydia*-specific $CD4^+$ T cells to home to and protect the genital mucosa following infection with *C. trachomatis* (22). In the present study we extended these studies to identify integrin receptors that are important for $CD4^+$ T cell–mediated protection. When we examined the surface expression of β_1 and β_7 on both bulk and Ag-specific $CD4^+$ T cells, we found that relative β_1 expression was dramatically increased on most $CD4^+$ T cells found in the uterus following *C. trachomatis* infection (Figs. 1A, 2A). These findings are in line with Perry et al. (25) who demonstrated that surface β_1 is upregulated on bulk lymphocytes localized in the genital tract but not in the intestinal mucosa following infection with the mouse-adapted species *Chlamydia muridarum*. Although most $CD4^+$ T cells were expressing high levels of surface integrin β_1, that report also noted a minor percentage of β_7^+ $CD4^+$ T cells in the genital tract (25). Separate reports suggested that $\alpha_4\beta_7$ was the dominant integrin receptor expressed on $CD4^+$ T cells in the genital tract. However, those studies had significant experimental constraints that limit their interpretation. For example, Kelly and Rank (30) did not interrogate the integrin profile during the primary response or differentiate between Ag-specific and bystander $CD4^+$ T cells in the genital tract. Another report examined the surface integrin receptors on a memory $CD4^+$ T cell line in culture following stimulation but did not assess in vivo integrin dynamics (23). Memory $CD4^+$ T cells can exert effector activities with greater ease than primary T cells (31), and therefore examining memory cells is not indicative of the trafficking properties of T cells in a primary response. Because those studies did not examine naive, Ag-specific $CD4^+$ T cells they were unable to recapitulate the initial activation events during primary *C. trachomatis* infection. Additionally, those reports did not examine how perturbation of distinct integrin complexes alters the protective capacity of $CD4^+$ T cells responding to genital *C. trachomatis* infection.

The results presented in the present study show that $\alpha_4\beta_1$ drives *Chlamydia*-specific $CD4^+$ T cells to the infected genital mucosa (Figs. 4, 6) and is required to mediate protective immunity following *C. trachomatis* infection (Figs. 3, 5). Whereas $\alpha_4\beta_1$ was the dominant integrin in the genital mucosa, we did identify a second β_7^+ population that when perturbed did not alter immunity to *Chlamydia* infection. The existence of two NR1 cell populations with distinct integrin profiles and identical TCR specificity shows that integrin levels are not hardwired but rather imprinted during T cell activation, as has been suggested previously (32). Our results also suggest that only $CD4^+$ T cells with the correct integrin profile can extravasate into the infected genital mucosa. We speculate that although $\alpha_4\beta_1$-expressing T cells can enter the infected tissue efficiently, the $\alpha_4\beta_7^+$ $CD4^+$ T cells remain in the circulation and fail to provide protection in the genital mucosa. Because the entire uterus, including its associated vasculature, was harvested in all experiments it was not possible to distinguish between those T cells present in blood vessels and those that had completed transendothelial migration. It is possible that the $\alpha_4\beta_7^+$ $CD4^+$ T cell population still contributes to immunity elsewhere in the mouse, but this remains to be tested. Interestingly, a recent report uncovered a mechanism that drives distinct integrin-expressing T cell populations in the lungs of

mice. Ruane et al. (33) found that a subset of residing dendritic cells (DCs) can imprint lung T cells to express $\alpha_4\beta_7$. The T cells expressing $\alpha_4\beta_7$ 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 $\alpha_4\beta_7$, we speculate that a similar process also occurs in the genital tract. Although most $CD4^+$ T cells are primed to express $\alpha_4\beta_1$, which mediates immunity in the genital mucosa during *C. trachomatis* infection, perhaps a subset of uterine DCs also imprints $\alpha_4\beta_7$ 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 $\alpha_4\beta_1$ adheres to the addressin VCAM-1 and the extracellular matrix protein fibronectin (34, 35). Surface VCAM-1 increases on endothelial cells lining microvessels following inflammation (27). For example, patients with CNS autoimmune disorders are often treated with natalizumab, an anti–α_4 Ab that blocks $\alpha_4\beta_1$ and $\alpha_4\beta_7$ interactions with their ligands. It is thought that natalizumab treatment decreases undesirable inflammation in the CNS by interfering with $\alpha_4\beta_1$-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 $\alpha_4\beta_7$, 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 $\alpha_4\beta_1$ 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 $\alpha_4\beta_1$ signaling for multiple steps to provide protection. Previous studies have characterized the importance of $\alpha_4\beta_1$ to slow/arrest lymphocytes in the blood vessel, but $\alpha_4\beta_1$ 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 *C. trachomatis*–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 β_1 and β_7 were dispensable for proliferation and differentiation to a Th1 phenotype (Fig. 5A–C). Consequently, we conclude that the function of β_1 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 β_1 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 $\alpha_4\beta_1$, contribute to genital tract pathology following *Chlamydia* infection. Although integrin β_1 is found primarily in complex with α_4 on T cells, our findings do not entirely eliminate the possibility that β_1 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 $\alpha_4\beta_1$) as required for successful trafficking during *C. trachomatis* 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.

References

1. Brunham, R. C., and J. Rey-Ladino. 2005. Immunology of *Chlamydia* infection: implications for a *Chlamydia trachomatis* vaccine. *Nat. Rev. Immunol.* 5: 149–161.
2. Cohen, C. R., and R. C. Brunham. 1999. Pathogenesis of *Chlamydia* induced pelvic inflammatory disease. *Sex. Transm. Infect.* 75: 21–24.
3. Roan, N. R., and M. N. Starnbach. 2008. Immune-mediated control of *Chlamydia* infection. *Cell. Microbiol.* 10: 9–19.
4. Perry, L., H. Su, K. Feilzer, R. Messer, S. Hughes, W. Whitmire, and H. Caldwell. 1999. Differential sensitivity of distinct *Chlamydia trachomatis* isolates to IFN-γ-mediated inhibition. *J. Immunol.* 162: 3541–3548.
5. Thomas, S., L. Garrity, C. Brandt, C. Schobert, G. Feng, M. Taylor, J. Carlin, and G. Byrne. 1993. IFN-γ-mediated antimicrobial response. Indoleamine 2,3-dioxygenase-deficient mutant host cells no longer inhibit intracellular *Chlamydia* spp. or *Toxoplasma* growth. *J. Immunol.* 150: 5529–5534.
6. Mayer, J., M. L. Woods, Z. Vavrin, and J. B. Hibbs, Jr. 1993. Gamma interferon-induced nitric oxide production reduces *Chlamydia trachomatis* infectivity in McCoy cells. *Infect. Immun.* 61: 491–497.
7. Nelson, D. E., D. P. Virok, H. Wood, C. Roshick, R. M. Johnson, W. M. Whitmire, D. D. Crane, O. Steele-Mortimer, L. Kari, G. McClarty, and H. D. Caldwell. 2005. Chlamydial IFN-γ immune evasion is linked to host infection tropism. *Proc. Natl. Acad. Sci. USA* 102: 10658–10663.
8. Coers, J., I. Bernstein-Hanley, D. Grotsky, I. Parvanova, J. Howard, G. Taylor, W. Dietrich, and M. Starnbach. 2008. *Chlamydia muridarum* evades growth restriction by the IFN-γ-inducible host resistance factor Irgb10. *J. Immunol.* 180: 6237–6245.
9. Gondek, D., N. Roan, and M. Starnbach. 2009. T cell responses in the absence of IFN-γ exacerbate uterine infection with *Chlamydia trachomatis*. *J. Immunol.* 183: 1313–1319.
10. Gondek, D., A. Olive, G. Stary, and M. Starnbach. 2012. $CD4^+$ T cells are necessary and sufficient to confer protection against *Chlamydia trachomatis* infection in the murine upper genital tract. *J. Immunol.* 189: 2441–2449.
11. Johansson, M., and N. Lycke. 2001. Immunological memory in B-cell-deficient mice conveys long-lasting protection against genital tract infection with *Chlamydia trachomatis* by rapid recruitment of T cells. *Immunology* 102: 199–208.
12. Springer, T. A. 1994. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* 76: 301–314.
13. Richard, O. H. 2002. Integrins: bidirectional, allosteric signaling machines. *Cell* 110: 683–687.
14. Makgoba, M. W., M. E. Sanders, G. E. Ginther Luce, M. L. Dustin, T. A. Springer, E. A. Clark, P. Mannoni, and S. Shaw. 1988. ICAM-1 a ligand for LFA-1-dependent adhesion of B, T and myeloid cells. *Nature* 331: 86–88.
15. Springer, T. A., M. L. Dustin, T. K. Kishimoto, and S. D. Marlin. 1987. The lymphocyte function-associated LFA-1, CD2, and LFA-3 molecules: cell adhesion receptors of the immune system. *Annu. Rev. Immunol.* 5: 223–252.
16. Mora, J. R., M. R. Bono, N. Manjunath, W. Weninger, L. L. Cavanagh, M. Rosemblatt, and U. H. Von Andrian. 2003. Selective imprinting of gut-homing T cells by Peyer's patch dendritic cells. *Nature* 424: 88–93.
17. Yednock, T. A., C. Cannon, L. C. Fritz, F. Sanchez-Madrid, L. Steinman, and N. Karin. 1992. Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha_4\beta_1$ integrin. *Nature* 356: 63–66.
18. Bauer, M., C. Brakebusch, C. Coisne, M. Sixt, H. Wekerle, B. Engelhardt, and R. Fässler. 2009. Beta1 integrins differentially control extravasation of inflammatory cell subsets into the CNS during autoimmunity. *Proc. Natl. Acad. Sci. USA* 106: 1920–1925.
19. Feagan, B. G., G. R. Greenberg, G. Wild, R. N. Fedorak, P. Paré, J. W. McDonald, R. Dubé, A. Cohen, A. H. Steinhart, S. Landau, et al. 2005.

Treatment of ulcerative colitis with a humanized antibody to the $\alpha_4\beta_7$ integrin. *N. Engl. J. Med.* 352: 2499–2507.
20. Steinman, L. 2005. Blocking adhesion molecules as therapy for multiple sclerosis: natalizumab. *Nat. Rev. Drug Discov.* 4: 510–518.
21. Roan, N. R., T. M. Gierahn, D. E. Higgins, and M. N. Starnbach. 2006. Monitoring the T cell response to genital tract infection. *Proc. Natl. Acad. Sci. USA* 103: 12069–12074.
22. Olive, A. J., D. C. Gondek, and M. N. Starnbach. 2011. CXCR3 and CCR5 are both required for T cell-mediated protection against *C. trachomatis* infection in the murine genital mucosa. *Mucosal Immunol.* 4: 208–216.
23. Hawkins, R. A., R. G. Rank, and K. A. Kelly. 2000. Expression of mucosal homing receptor $\alpha_4\beta_7$ is associated with enhanced migration to the *Chlamydia*-infected murine genital mucosa in vivo. *Infect. Immun.* 68: 5587–5594.
24. Kelly, K., A. Chan, A. Butch, and T. Darville. 2009. Two different homing pathways involving integrin β_7 and E-selectin significantly influence trafficking of CD4 cells to the genital tract following *Chlamydia muridarum* infection. *Am. J. Reprod. Immunol.* 61: 438–445.
25. Perry, L., K. Feilzer, J. Portis, and H. Caldwell. 1998. Distinct homing pathways direct T lymphocytes to the genital and intestinal mucosae in *Chlamydia*-infected mice. *J. Immunol.* 160: 2905–2914.
26. Di Genova, G., N. Savelyeva, A. Suchacki, S. M. Thirdborough, and F. K. Stevenson. 2010. Bystander stimulation of activated $CD4^+$ T cells of unrelated specificity following a booster vaccination with tetanus toxoid. *Eur. J. Immunol.* 40: 976–985.
27. von Andrian, U. H., and C. R. Mackay. 2000. T-cell function and migration. Two sides of the same coin. *N. Engl. J. Med.* 343: 1020–1034.
28. Fässler, R., and M. Meyer. 1995. Consequences of lack of β_1 integrin gene expression in mice. *Genes Dev.* 9: 1896–1908.
29. DeNucci, C., A. Pagán, J. Mitchell, and Y. Shimizu. 2010. Control of $\alpha_4\beta_7$ integrin expression and CD4 T cell homing by the β_1 integrin subunit. *J. Immunol.* 184: 2458–2467.
30. Kelly, K. A., and R. G. Rank. 1997. Identification of homing receptors that mediate the recruitment of CD4 T cells to the genital tract following intravaginal infection with *Chlamydia trachomatis*. *Infect. Immun.* 65: 5198–5208.
31. Croft, M., L. Bradley, and S. Swain. 1994. Naive versus memory CD4 T cell response to antigen. Memory cells are less dependent on accessory cell costimulation and can respond to many antigen-presenting cell types including resting B cells. *J. Immunol.* 152: 2675–2685.
32. Iwata, M., A. Hirakiyama, Y. Eshima, H. Kagechika, C. Kato, and S.-Y. Song. 2004. Retinoic acid imprints gut-homing specificity on T cells. *Immunity* 21: 527–538.
33. Ruane, D., L. Brane, B. S. Reis, C. Cheong, J. Poles, Y. Do, H. Zhu, K. Velinzon, J.-H. Choi, N. Studt, et al. 2013. Lung dendritic cells induce migration of protective T cells to the gastrointestinal tract. *J. Exp. Med.* 210: 1871–1888.
34. Mariano, J. E., O. Laurelee, T. Yoshikazu, C. Carol, L. Stefan, E. H. Martin, and R. L. Roy. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell* 60: 577–584.
35. Alon, R., P. D. Kassner, M. W. Carr, E. B. Finger, M. E. Hemler, and T. A. Springer. 1995. The integrin VLA-4 supports tethering and rolling in flow on VCAM-1. *J. Cell Biol.* 128: 1243–1253.
36. von Andrian, U. H., and B. Engelhardt. 2003. α_4 Integrins as therapeutic targets in autoimmune disease. *N. Engl. J. Med.* 348: 68–72.
37. Johansson, E. L., A. Rudin, L. Wassén, and J. Holmgren. 1999. Distribution of lymphocytes and adhesion molecules in human cervix and vagina. *Immunology* 96: 272–277.
38. Parr, M. B., and E. L. Parr. 2000. Interferon-γ up-regulates intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 and recruits lymphocytes into the vagina of immune mice challenged with herpes simplex virus-2. *Immunology* 99: 540–545.
39. Jayarapu, K., M. Kerr, S. Ofner, and R. M. Johnson. 2010. *Chlamydia*-specific CD4 T cell clones control *Chlamydia muridarum* replication in epithelial cells by nitric oxide-dependent and -independent mechanisms. *J. Immunol.* 185: 6911–6920.