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Expression of Mucosal Homing Receptor α4β7 Is Associated with Enhanced Migration to the *Chlamydia*-Infected Murine Genital Mucosa In Vivo

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The CD4 T helper cell type 1 (Th1) response is essential for the resolution of chlamydial genital infection in mice. However, not all Th1 clones are equally protective in eradicating the infection. Since oral immunization regimens produce protective immunity, we evaluated the role of the mucosa-associated homing receptor, α4β7, in trafficking to the genital mucosa. Using a panel of CD4, Th1 cell lines and clones, we compared the lymphocyte homing patterns of a *Chlamydia*-specific, protective clone (P-MoPn), a nonprotective clone (N-MoPn), and a keyhole limpet hemocyanin (KLH)-specific cell line (KLH-1). T cells were labeled with the fluorescent dye PKH-26, adoptively transferred into *Chlamydia*-infected mice, and monitored at different time points throughout the course of a genital infection. We found that clones P-MoPn and N-MoPn migrated to similar extents to the genital tract and in significantly greater numbers than the KLH-specific T-cell line. Both clones and the KLH-1 line expressed similar levels of the adhesion molecules α4, β1, CD44, and CD11a. However, clones P-MoPn and N-MoPn expressed higher levels of the mucosal homing receptor, α4β7. Also, clones P-MoPn and N-MoPn but not the KLH-1 line migrated to the mesenteric lymph node, suggesting a mucosal recirculation pattern. Moreover, blocking α4β7 adhesion interaction in vivo significantly reduced the recruitment of P-MoPn but not KLH-1 to the genital tract. These findings show that the mucosal homing receptor α4β7 is utilized by a subset of CD4 cells during migration to the *Chlamydia*-infected genital tract.

Chlamydial genital infection is the most common cause of bacterial sexually transmitted disease in the United States, accounting for approximately 4 million new cases annually (2). Due to the insidious nature of the infection (40), a reliable prophylactic intervention, such as vaccine administration, has been advocated for preventing the spread of disease and subsequent morbidity (40, 45, 46). Indeed, prior studies have indicated that cell-mediated immunity is essential for the resolution of a current murine genital infection as well as protection from a subsequent challenge with the mouse pneumonitis agent of *Chlamydia trachomatis* (MoPn) (20, 25, 36). In further studies, $CD4^+$ T cells were shown to mediate the protective immune response following chlamydial genital infection (30, 42), with the CD4 helper cell type 1 (Th1) subset dominating the response (10). In addition, eradication of *Chlamydia* within the genital mucosa coincides with the recruitment of CD4 cells (24). However, the retention of CD4 cells within the genital tract (GT) (24) and protective immunity are transient both in animal models of infection (20, 36) and in humans (7, 22), an important aspect to consider in the design of an effective vaccine.

The potency and longevity of the anamnestic response within the genital mucosa appears to be influenced by the site of primary immune stimulation. For example, mice immunized via mucosal (oral, intranasal, and vaginal) routes were more resistant to a vaginal MoPn challenge (25) and demonstrated increased protection against infertility following reinfection (31) than mice immunized parenterally. In addition, immunization via mucosal routes resulted in a greater production of gamma interferon (IFN-γ) in the GT following a vaginal challenge (21). These data suggested that *Chlamydia*-specific T cells, stimulated in secondary lymphoid tissues that drain mucosal surfaces, are more proficient at trafficking to the genital mucosa. However, controversy surrounds the adhesive interactions that govern the recruitment of protective T-cell populations to the GT (24, 34) due to a lack of functional evidence.

Based on the propensity of mesenteric lymph node (MLN) cells to traffic to the gut, GT, and mammary glands, these mucosal tissues were proposed to belong to a common mucosal immune system (29). The discovery of the mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1) provided a mechanism for the distinct homing patterns between mucosal and nonmucosal tissues (41). Although MAdCAM-1 is expressed predominately in the intestine and associated lymphoid tissues, limited expression has been observed in the nasal-associated lymphoid tissue (12), pancreas (18), and GT (24). Intriguingly, oral immunization resulted in a less effective protection compared to intranasal immunization (21, 25). These and other data (34) suggest that the intestinal and genital mucosa differ in adhesive interactions that regulate lymphocyte recruitment to these sites. In this study, we provide functional data that support a role for the mucosa-associated integrin receptor α4β7 in lymphocyte recruitment to the genital mucosa during *Chlamydia* infection.

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MATERIALS AND METHODS

Antibodies. The following rat anti-mouse monoclonal antibodies (MAbs) were used in flow cytometry analysis at concentrations ranging from 10 to 25 μg/ml and were purchased from Pharmingen (San Diego, Calif.): anti-CD49d (9C10, immunoglobulin G2a [IgG2a]), anti-CD29 (9E67, IgG2a), anti-CD44 (IM7,

IgG2b), anti-β7 (M293, IgG2a), anti-CD11a (M17/4, IgG2a), and anti-LPAM-1 (DATK-32, IgG2a). A rat IgG2bκ myeloma protein (IR863) and the rat monoclonal IgG2a (R35-95) (Pharmingen) were used as negative controls. Antibodies used for in vivo blocking experiments were anti-LPAM-1 and anti-CD49d (R1-2, IgG2b; Pharmingen) at the specified concentrations (see below). Negative selection of CD4 cells was accomplished using tissue culture supernatants from the hybridoma clones Lyt-2, B220, and MAC-1, purchased from the American Type Culture Collection (Manassas, Va.).

Experimental animals. Female BALB/c mice 6 to 8 weeks of age were purchased from Harlan-Sprague Dawley (Indianapolis, Ind.) and housed in an Association for Assessment and Accreditation of Laboratory Animal Care-approved facility. Animals were given access to food and water as desired and housed (groups of five mice per cage) in an environmentally controlled area with a cycle of 12 h of light and 12 h of darkness.

Infection of mice. Seven days prior to inoculation, mice received 2.5 mg of progesterone (Depo-Provera; Upjohn, Kalamazoo, Mich.) to maintain them in a state of anestrus (32, 36). Under pentobarbital anesthesia, each mouse was infected by intravaginal inoculation of 10^7 infection-forming units (IFU) of MoPn grown in McCoy cells (50% infective dose = 2.5×10^3 IFU) in 30 μl of sucrose-phosphate-glutamate buffer.

Chlamydial antigen preparation. MoPn elementary bodies (EB) were grown in HeLa cells (American Type Culture Collection) and were purified from the above stocks by density gradient centrifugation over a discontinuous density gradient of 40, 44, and 54% Hypaque 60 (Sanofi-Winthrop Pharmaceuticals, New York, N.Y.). Chlamydial suspensions were layered and then centrifuged at 4°C for 1 h at 43,000 × *g*. The EB within the 44 and 54% layers were extracted, washed, and resuspended in phosphate-buffered saline. After the protein concentration was adjusted to 500 μg/ml, the EB antigen preparation was inactivated by UV light. The antigen was then aliquoted and stored at −70°C until used.

Assessment of infection. Vaginal swabs were prepared and collected as previously described (20). The resulting isolation solution was inoculated (200 μl) on McCoy cells grown in individual wells of 96-well plates and then centrifuged at 1,900 × *g* for 1 h. Following centrifugation, the plates were incubated for 2 h at 37°C, after which the isolation medium was replaced with fresh cycloheximide medium and the plates were incubated for an additional 32 h. After methanol fixation, MoPn inclusions were detected by addition of anti-MoPn immune sera followed by anti-mouse IgG conjugated to fluorescein isothiocyanate (ICN Immunobiological, Irvine, Calif.). The monolayers were counterstained with Evans blue, and the inclusion bodies were enumerated by counting 20 fields (40×) under a fluorescence microscope and calculating IFU per milliliter.

Derivation and maintenance of MoPn-specific clones. Clones P-MoPn and N-MoPn were derived from a T-cell line previously described (19) and maintained using HeLa-grown UV-inactivated EB. The keyhole limpet hemocyanin (KLH)-specific Th1 line KLH-1 was derived from BALB/c mice that were administered KLH (50 μg) in complete Freund's adjuvant via intradermal injection at the base of the tail. The mice were boosted 2 weeks later with same dose of KLH emulsified in incomplete Freund's adjuvant. Ten days later, single-cell suspensions from the inguinal lymph nodes were enriched for $CD4^+$ cells by incubating the lymphocytes with anti-CD8, B220, and MAC-1, followed by anti-rat IgG conjugated to microbeads (Miltenyi Biotec Inc., Auburn, Calif.). The cell preparation was passed over magnetic columns, resulting in a population that contained 95% CD4 cells. The CD4 cells were then restimulated in vitro in complete medium composed of RPMI 1640 (Gibco Laboratories, Grand Island, N.Y.) supplemented with 15 mM HEPES (Gibco), 1.0 mM sodium pyruvate (Gibco), 0.1 mM nonessential amino acids (Gibco), 2 mM glutamine (Gibco), 100 U of penicillin (Gibco) per ml, 100 μg of streptomycin (Gibco) per ml, 10% heat-inactivated fetal calf serum (Atlanta Biological, Norcross, Ga.), 2×10^{-5} M 2-mercaptoethanol (Sigma, St. Louis, Mo.), 12.5% (vol/vol) concanavalin A-conditioned medium, and 5 μg of KLH antigen per ml; 5×10^7 γ-irradiated splenocytes served as antigen-presenting cells. After 4 days of culture, the T cells were expanded and maintained in vitro as a primary line by culture with KLH-1, antigen-presenting cells, and 12.5% (vol/vol) concanavalin A conditioned medium. The antigen specificity of the line was confirmed using a blast transformation assay as previously described (10), and the KLH-specific line did not respond to MoPn. Line KLH-1 was designated Th1 after determining that the ratio of IFN-γ-producing to interleukin-4 (IL-4)-producing cells was approximately 3:1, using an enzyme-linked immunospot assay as previously described (24).

Flow cytometry. T-cell clones and lines were suspended in Dulbecco modified Eagle medium (Gibco) containing 1% bovine serum albumin (Sigma) and 0.1% sodium azide (staining buffer) according to the microplate technique previously described (25). Cells were initially incubated with rat anti-mouse cell surface markers for 25 min on ice and then washed twice with Dulbecco modified Eagle medium containing 10% bovine serum albumin. Next the cells were resuspended on ice for 25 min in goat anti-rat IgG-conjugated fluorescein isothiocyanate (BioSource International, Camarillo, Calif.) at a concentration of 20 μg/ml containing 10% autologous mouse serum, followed by washing as described above. The cells were kept at 4°C after resuspension in a solution of 1% paraformaldehyde in phosphate-buffered saline. Flow cytometry analysis was done with a fluorescence-activated cell-sorting analyzer equipped with a 488-nm argon laser and Lysis II software (FACScan; Becton Dickinson, Mountain View, Calif.). The instrument was calibrated with beads (CaliBRITE; Becton Dickinson) and use of AutoCOMP software. These settings were not changed for the duration of the study. Exclusion of dead cells was done based on forward angle and 90° light scatter; 10,000 gated cells were analyzed per sample.

Fluorescence labeling experiments. Cells were stained with PKH-26 (Sigma) or BODIPY (Molecular Probes, Eugene, Oreg.) according to the manufacturer's protocol. The labeled samples were checked for viability and fluorescence intensity on the flow cytometer prior to adoptive transfer as described in the figure legends. Control, unlabeled clone cells were transferred to account for possible background autofluorescence of the cells. Eighteen hours after transfer into uninfected or MoPn-infected recipient mice, the tissues were harvested and processed as described previously (24). The number of fluorescent cells within each tissue was determined by collecting 10,000 fluorescence-labeled cells by flow cytometry while tabulating the total number of cells analyzed. The frequency of labeled cells within each tissue was expressed as the number of labeled cells per 100,000 total cells analyzed by flow cytometry. The numbers of lymphocytes per MLN, cervical lymph node (CLN), and spleen (7×10^6, 7×10^6, and 5×10^7, respectively), did not change throughout the course of infection. The numbers of cells within the iliac lymph node (ILN) and GT varied based on day of infection for days 0, 7, 14, 21, and 35 as follows: ILN, 1.5×10^6, 9×10^6, 10^7, 4×10^6, and 2×10^6, respectively; GT, 1.5×10^6, 1.5×10^7, 10^7, 5×10^6, and 2×10^6, respectively. Gating was adjusted to include only mononuclear cells. For dual-labeling experiments, a 50/50 mixture of PKH-26- and BODIPY-labeled clone cells was transferred into recipient mice. Compensation between the FL1 (BODIPY) and FL2 (PKH-26) channels was adjusted using a mixture of labeled cells. For blocking experiments in which anti-integrin receptor antibodies were used recipient mice were injected intraperitoneally with the desired antibodies. Four hours later, 5×10^6 PKH-26-labeled cells were adoptively transferred to these mice. Additionally, each cell population was incubated at 4°C for 30 min with one of the above antibodies at a concentration of 6.7 μg/10^6 cells prior to adoptive transfer. The antibodies used in the blocking studies were tested for cytotoxicity by incubating the antibodies (50 μg/10^6 cells) and clone cells with rabbit complement (Cedarlane) for 60 min at 37°C. The percentage of viable cells after the incubation period was determined by trypan blue exclusion. Anti-asialo-GM1 (Wako, Richmond, Va.) was used as a positive control antibody with known cytotoxic effects. None of the antibodies used in this study displayed significant complement-mediated cytotoxicity.

Statistics. One-way analysis of variance was used to detect statistical differences in cell surface molecule expression or number of fluorescent cells among groups. Statistical differences between the control (unlabeled clone) and experimental transfer groups were determined by unpaired *t* test for each tissue. The paired *t* test was used for determining whether differences existed between PKH-26- and BODIPY-labeled cells within mice. A difference was considered significant when P was <0.05.

RESULTS

Homing receptor expression patterns. As a model for studying *Chlamydia*-specific memory T-cell recruitment to the GT, we evaluated a panel of T-cell lines and clones to identify potential homing receptor-adhesion molecule pairs used in this process. We chose two CD4 Th1 clones specific for MoPn and one control CD4 Th1 cell line specific for KLH. The two MoPn-specific clones represent a selected memory cell population since they were derived from spleens of nude mice that had eradicated a genital MoPn infection after receiving lymphocytes from MoPn-immune mice. However, these clones differed in the ability to clear chlamydiae from the GT of infected nude mice following adoptive transfer (19). We first characterized cell surface molecules with a potential for mediating T-cell recirculation or homing using flow cytometry. We found that many of the T-cell surface markers on the clones and cell line transiently increased during the first 2 weeks after antigen stimulation. Therefore, we performed the flow cytometry analysis and cell transfers 3 to 4 weeks after antigen stimulation, at a time when cell surface markers of interest were expressed at a consistent level (data not shown).

As shown in Fig. 1, flow cytometric analysis revealed that both the protective MoPn-specific clone P-MoPn and the nonprotective clone N-MoPn expressed higher levels of the mucosal homing receptor, indicated by the expression of β7 as well as the epitope, LPAM-1, (α4β7), than the KLH-1 line (Fig. 1). However, both clones and the KLH-1 line expressed similar levels of α4, β1, CD11a, and CD44. Also note that the expression levels of these cell surface markers are consistent

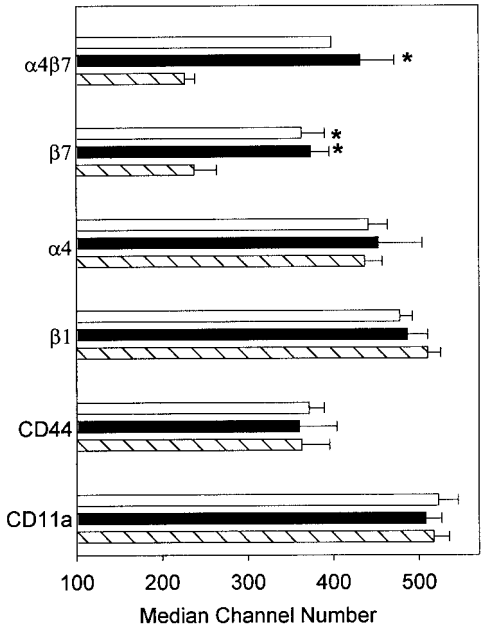

FIG. 1. Homing receptor expression on clone P-MoPn (■), clone N-MoPn (□), and line KLH-1 (▨). Single-cell suspensions of clone P-MoPn, clone N-MoPn, and line KLH-1 were stained with the indicated rat anti-mouse cells surface markers 3 to 4 weeks after antigen stimulation. Data are expressed as the average median channel number from two separate experiments ± SD. *, significantly greater compared with KLH-1 ($P < 0.05$; t test).

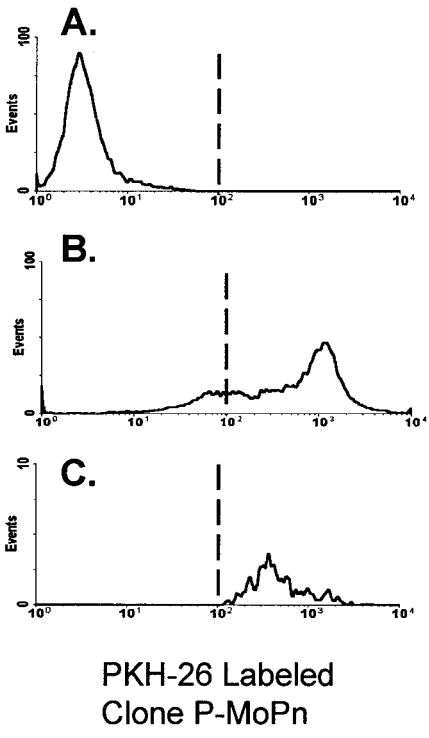

FIG. 2. In vivo detection of PKH-26-labeled P-MoPn. (A) Relative fluorescence intensity of clone P-MoPn prior to labeling with PKH-26. (B) Relative fluorescence intensity after 18 h in culture following PKH-26 labeling. (C) Representative histogram of labeled cells detected in the GT at day 7 after MoPn infection. PKH-26-labeled P-MoPn cells (5×10^6) were adoptively transferred 7 days following MoPn vaginal infection and harvested 18 h later. The isolated GT cells were examined by flow cytometry. Cells with a fluorescence intensity greater than 10^2 were considered positive.

over time, with small standard deviations (SD) observed when separate batches of clone cells are measured. In addition, these clones and cell line also expressed the phenotype of memory T cells ($CD62L^{lo}$ and $CD45RB^{hi}$ [data not shown]). Previous studies have shown that β7 expression is involved in the tissue-specific migration of memory T cells (47), delineating subpopulations which home to mucosal sites from those which home to nonmucosal sites. Therefore, the MoPn-specific clones would be expected to possess a mucosal homing pattern.

Parameters of in vivo recirculation of a protective MoPn-specific Th1 clone in response to MoPn infection. We first characterized the in vivo trafficking pattern of the protective MoPn-specific CD4 clone by monitoring its distribution pattern following adoptive transfer into MoPn-infected recipients. We chose to use the fluorescent reporter molecule PKH-26 since it had been shown to provide stable long-term labeling with little deleterious effect on cellular functions, particularly in regard to lymphocyte migration (39). Prior to any in vivo investigation, we examined the efficiency of PKH-26 labeling of clone P-MoPn in vitro. As shown in Fig. 2A and B, PKH-26 remained associated with over 95% of clone P-MoPn for at least 18 h following in vitro labeling. After the labeled cells were transferred in vivo, some of the label was lost, but the cells were readily identifiable in target tissues (Fig. 2C).

To determine the optimal number of clone P-MoPn to be used in adoptive transfers, various numbers (4×10^7, 10^7, and 5×10^6) of labeled clone cells were intravenously infused into mice that had been vaginally infected 7 days previously. Although fewer labeled clone cells were recovered when 5×10^6 cells were transferred per mouse, we could readily detect labeled clone at this dose (data not shown). Using 5×10^6 cells, we also found that labeled clone appeared in the spleen (38.5 ± 16.9; mean of labeled cells per 10^5 total cells ± SD) and peripheral blood (18.8 ± 8.1) 1 h after transfer. In contrast, labeled clone cells did not appear in the GT (97 ± 35.6), ILN (6.8 ± 5.5), and MLN (2.4 ± 1.5) until 4 h after transfer. However, in comparison, the frequency of labeled cells markedly increased by 18 h after transfer (Fig. 3, day 7). Based on these data, subsequent adoptive transfer experiments used 5×10^6 labeled clone with an 18-h time of harvest.

In vivo recirculation patterns of clone P-MoPn during the course of MoPn genital infection. The capacity of protective T-cell subpopulations to home to the genital mucosa and associated lymphoid tissues in response to infection has yet to be fully described. Prior studies indicated that adhesion molecules were expressed for a limited time in the genital mucosa following chlamydial genital infection (24). Among the infection-induced adhesion molecules found were vascular cell adhesion molecule 1 (VCAM-1) and MAdCAM-1 (24), which facilitate T-cell recirculation to perivascular sites of inflammation (27) and mucosal sites (43), respectively. To correlate the expression of vascular addressins with recruitment of the MoPn-specific Th1 clone to the GT, we transferred the labeled clone to recipients at various times following MoPn genital infection. As shown in Fig. 3, the transfer of labeled clone P-MoPn to uninfected mice (day 0) did not result in the appearance of cells within the GT. However, by day 7 after genital infection (time of maximum chlamydial burden) (4, 24), recruitment to the GT was clearly taking place. Recruitment of clone P-MoPn to the GT was also seen when the cells were transferred on days 14 and 21 after vaginal inoculation. However, the number of positive cells appearing in the GT was less than the number

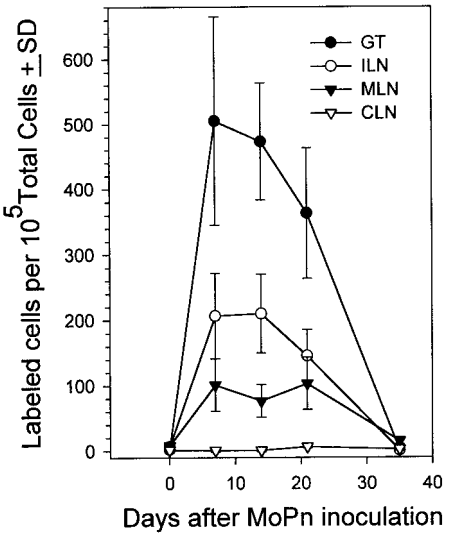

FIG. 3. Differential ability of clone P-MoPn to traffick to the genital tract at various times throughout the course of MoPn infection. Clone P-MoPn was labeled with the fluorescent dye PKH-26. Labeled or unlabeled (control) clone P-MoPn (5×10^6/mouse) was adoptively transferred to mice throughout the course of MoPn infection (day 0 = uninfected mice). Tissues were harvested 18 h posttransfer, and single-cell suspensions were prepared for flow cytometric analysis. Data are expressed as the mean number of labeled cells detected on the flow cytometer from six mice ± SD. Negligible numbers of false-positive cells (none to five) were detected in a parallel group of control mice that received unlabeled clone. This mean was subtracted from the experimental group values. Significantly higher numbers of labeled cells were detected in the GT, ILN, and MLN on days 7, 14, and 21 after infection compared to CLN ($P < 0.01$; t test).

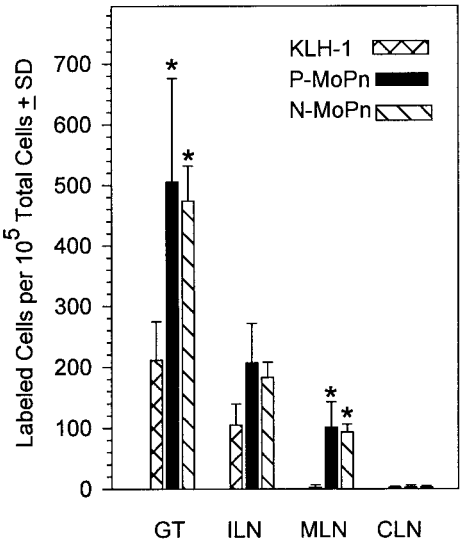

FIG. 4. Homing patterns of KLH-1, P-MoPn, and N-MoPn 7 days after MoPn genital infection. KLH-1, P-MoPn, and N-MoPn cells were labeled and transferred into recipient mice as described for Fig. 3. Tissues were harvested 18 h later. Negligible numbers of label-positive cells (none to seven) were detected in control mice, and these values were subtracted from those obtained from mice which received labeled cells. Data are expressed as the mean number of labeled cells from three to five mice each for KLH-1, P-MoPn, and N-MoPn ± SD. *, significantly higher compared to KLH-1 ($P < 0.01$; t test).

observed when the transfer was performed on day 7 after inoculation. By day 35 postinfection, at which time viable chlamydiae have been cleared from the GT (24), PKH-26-labeled cells in the GT were not significantly more numerous than in control mice which had received unlabeled clone. Correspondingly, adhesion molecule expression in the GT was shown to subside following resolution of infection (24). These data indicate that clone P-MoPn is recruited to the GT during active chlamydial infection, a finding that coincides with the induction of relevant adhesion molecules on the GT endothelium.

In addition to GT homing, we examined the ability of clone P-MoPn to traffic to the ILN, which directly drains the genital mucosa (33), as well as the MLN, which is associated with the mucosal lymphoid system. Previous studies have indicated that MoPn-specific T cells appeared in these tissues following MoPn genital infection (10, 25). As a control, trafficking to the CLN was also evaluated because this lymph node does not directly drain the genital mucosa (44). As shown in Fig. 3, labeled clone cells were not detected in any tissue examined when transferred to uninfected mice. However, when the labeled clone cells were transferred at 7 days after genital infection, the cells were detected in the ILN and MLN but not the CLN. Although not statistically different, the mean number of labeled clone cells detected in the ILN was consistently greater in relation to the MLN on days 7, 14, and 21 during infection. In contrast, the CLN remained negative throughout the course of infection. At 21 days after genital infection, the ILN and MLN still contained significantly higher numbers of the labeled clone compared to controls. However, after resolution of infection (day 35), the number of labeled cells in all tissues examined was not significantly different in experimental versus control groups. Taken together, these data indicate that the recruitment of a protective, MoPn-specific Th1 clone to the GT of *Chlamydia*-infected mice coincides with the expression of adhesion molecules on the local endothelium. In addition, this clone recirculates to lymph nodes associated with *Chlamydia*-specific responses during infection but not in uninfected mice.

The in vivo homing of a nonprotective MoPn-specific Th1 clone. The trafficking capacity of clone P-MoPn appears to be regulated by addressin expression within the GT. Based on this finding, it is plausible that the efficiency with which MoPn-specific memory cells migrate to the infected GT may also influence its ability to protect against a challenge infection. To evaluate the GT homing efficiency of clone P-MoPn, we compared its trafficking pattern with that of another CD4 Th1 clone, N-MoPn. Although isolated from the same T-cell line as clone P-MoPn, this clone was not able to confer protection upon adoptive transfer into genitally infected nude mice (19). As shown in Fig. 4, the recirculation pattern observed for clone N-MoPn did not differ significantly from that of clone P-MoPn. Moreover, both clones displayed a mucosal pattern of migration in that they migrated similarly to the MLN. These data indicate that the ability of a clone to affect the outcome of MoPn genital infection also depends on its degree of antichlamydial activity in addition to its capacity to home to the GT.

Migration of lymphocytes to perivascular sites of inflammation is controlled by specialized interactions between homing receptors and complementary endothelial ligands; therefore, antigen specificity influences but does not limit the homing of memory/effector T cells to these sites. Since the KLH-specific line expressed lower levels of $\alpha 4\beta 7$ in comparison to the clones, we also evaluated its GT homing potential. Although KLH-1 is not specific for MoPn, we could detect significant numbers of labeled KLH-1 in the GT and ILN compared to control mice 7 days after MoPn infection (Fig. 4). However, we found that approximately 50% fewer cells from line KLH-1

migrated to the GT compared to clone P-MoPn. Moreover, clone P-MoPn was found in the MLN 7 days after MoPn genital infection, whereas the KLH-1 line could not be detected in the MLN, further indicating differences in the capacity of clone P-MoPn and line KLH-1 to migrate to mucosal sites. Thus, we report a positive relationship between α4β7 expression and trafficking to the GT of clone P-MoPn, suggesting that α4β7 is at least one homing receptor that may participate in GT recruitment during chlamydial infection.

Effect of antigen specificity on the GT homing potential in vivo. The recognition of cognate antigen has been shown to effect the transendothelial migration of T cells (23, 28). To confirm that the enhanced recruitment of clone P-MoPn is not due solely to retention of antigen-reactive cells, we directly compared the migration of clone P-MoPn and line KLH-1 at the same time within single mice shortly after transfer. To identify each cell population, we labeled P-MoPn with PKH-26 and the KLH-1 line with the green fluorescent molecule BODIPY. Equal numbers of each clone were mixed and adoptively transferred to mice on day 7 postinfection. At 6 h after transfer, the GT contained a significantly greater number of red fluorescent P-MoPn cells than green fluorescent KLH-1 cells (Fig. 5). By 18 h posttransfer, it was clear that the rate of migration to the GT was greater for clone P-MoPn than for the KLH-1 line. By 36 h, the recruitment of both clones had stabilized. The increased GT homing potential of clone P-MoPn was not due to the effects of the dye on lymphocyte migration since switching the labels produced the same result (Fig. 5).

A similar pattern of migration was observed for both cell populations in the ILN (Fig. 5). In contrast, we detected significantly more cells from the KLH-1 line than clone P-MoPn in the spleen at all time points posttransfer (Fig. 5), further suggesting that P-MoPn is actively recruited to the GT and ILN. Again, switching the labels resulted in similar profiles in the ILN and spleen (data not shown). Although we cannot rule out that the differences in recruitment between P-MoPn and KLH-1 at 36 h posttransfer are to some extent a reflection of antigen-specific retention and proliferation, the significant differences observed at 6 and 18 h illustrate an increased rate of recruitment for clone P-MoPn to the infected GT compared to line KLH-1. Accordingly, we cannot exclude the influence of antigen specificity in increasing the migration rate of clone P-MoPn, but it is clear from these data that clone P-MoPn exhibits an increased rate of transendothelial migration across the genital mucosa compared to line KLH-1.

Effect of anti-homing receptor antibodies on the GT migration of clone P-MoPn and line KLH-1. To determine whether α4β7 plays a role in T-cell trafficking to the GT, we examined the ability of antibodies directed against α4 and α4β7 to block in vivo homing to the GT. We used the anti-α4β7 MAb DATK-32 because it has been shown to bind to a conformational epitope formed by the α4β7 heterodimer (LPAM-1) (1) and to inhibit homing to mucosal sites such as Peyer's patches and intestines in vivo (17). Conversely, the anti-α4 MAb R1-2 does not inhibit homing to mucosal tissues (17) but is involved in the recruitment of lymphocytes to nonmucosal sites of inflammation (16). As shown in Fig. 6A, mice that were given anti-α4β7 exhibited significantly reduced recruitment of clone P-MoPn to the GT at 18 h posttransfer compared to the group given an isotype-matched control antibody and the group given the anti-α4 antibody. However, administration of the anti-α4 antibody reduced the recirculation of clone P-MoPn to the GT to some extent compared to mice given the control antibody ($P < 0.05$, t test). Additionally, anti-α4β7, but not anti-α4, inhibited the recruitment of clone P-MoPn to the ILN and MLN. In contrast, the homing of line KLH-1 to the GT was not

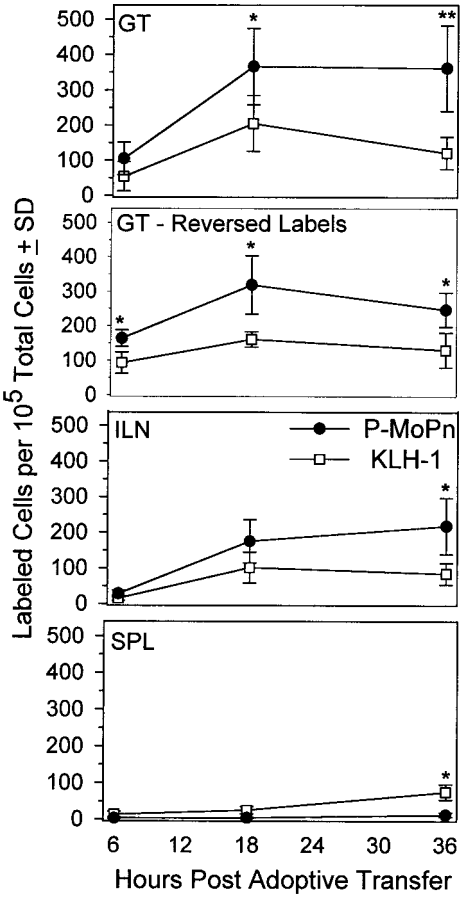

FIG. 5. Homing kinetics of P-MoPn and KLH-1 in the same mouse. On day 7 after MoPn infection, each mouse received 5×10^6 clone P-MoPn (PKH-26-labeled) cells and an equal number of line KLH-1 (BODIPY-labeled) cells. Cell suspensions were isolated from GT, ILN, and spleen at indicated times after transfer and analyzed by flow cytometry. No unlabeled cells appeared as false-positive cells in a control group of mice. Data are expressed as the mean number of labeled cells detected from six mice ± SD. GT, GT homing of P-MoPn and KLH-1. *, significantly greater compared with KLH-1 ($P < 0.01$; t test); **, significantly greater compared with KLH-1 ($P < 0.005$; t test). GT-Reversed Labels, GT homing of P-MoPn (BODIPY) and KLH-1 (PKH-26). *, significantly greater compared with KLH-1 ($P < 0.05$; t test). ILN, homing of P-MoPn and KLH-1. *, significantly greater compared with KLH-1 ($P < 0.05$; t test). SPL, splenic homing of P-MoPn and KLH-1. *, significantly higher compared with P-MoPn ($P < 0.05$; paired t test).

reduced in mice treated with anti-α4β7 (Fig. 6B). The reduced ability of clone P-MoPn to migrate to the GT in mice given anti-α4β7 did not appear to be due to clearance of the cells by bound antibody, since no in vitro cytotoxicity was detected (see Materials and Methods) and the anti-α4β7 that bound to the KLH-1 line (Fig. 1) did not reduce homing of these cells to the GT or ILN. Thus, these data indicate that T cells migrating to the inflamed GT can utilize multiple ligands, but expression of α4β7 appears to be associated with more efficient transendothelial migration. Thus, natural infection may favor the selection of a population of memory T cells that utilize α4β7 for homing to the GT.

DISCUSSION

Previous studies of lymphocyte trafficking to the *Chlamydia*-infected GT have resulted in different conclusions regarding

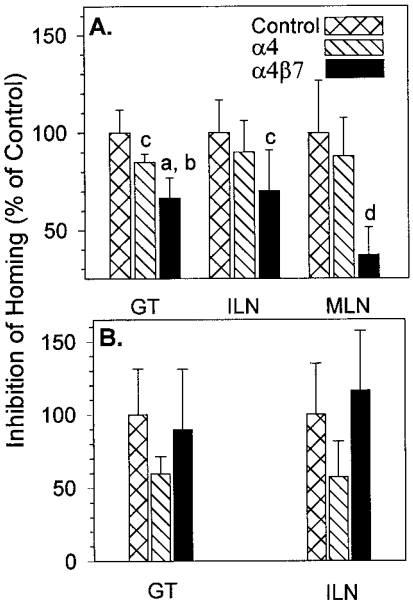

FIG. 6. Ability of MAb directed against α4 and α4β7 (LPAM-1) to alter in vivo homing of clone P-MoPn and line KLH-1. On day 7 after MoPn infection, each mouse was injected intraperitoneally with anti-α4β7 or anti-α4 1 h prior to adoptive transfer of PKH-26-labeled cells. Additionally, cells were incubated with one of the above antibodies prior to transfer. Control mice were given control antibody and received cells treated with control antibody. Single-cell suspensions were isolated from the indicated tissues 18 h after transfer and analyzed by flow cytometry. (A) Effect of anti-homing receptor MAb on the GT recruitment of clone P-MoPn. Data are expressed as the mean number of labeled cells detected from six mice each ± SD. [a], significantly lower compared with control ($P < 0.001$; t test); [b], significantly lower compared with anti-α4 mice ($P < 0.01$; t test); [c], significantly lower compared with control ($P < 0.05$; t test); [d], significantly lower compared to control ($P < 0.01$; t test). (B) Effect of anti-homing receptor MAb on line KLH-1 homing. Data are expressed as the mean number of labeled cells detected in six mice ± SD.

the molecules that mediate this event in vivo (8, 24, 26, 34). In particular, the controversy involves the participation of α4β7–MAdCAM-1 interactions. We previously reported that approximately 30% of GT CD4 cells express increased levels of β7 integrin during MoPn infection compared CD4 cells from uninfected mice or those in the spleen (24). Additionally, MAdCAM-1 was found on infected but not uninfected GT tissues (24), and in vitro lymphocyte adherence was blocked with antibodies against MAdCAM-1 (26), suggesting that α4β7 interactions could mediate the recruitment of a subset of CD4 cells to the GT during infection. Perry et al. (34) reported that 15 to 30% of infiltrating T cells expressed low levels of β7 and did not detect MAdCAM-1 in the local genital mucosa; they concluded that lymphocyte trafficking to the GT was mediated by other homing receptor-adhesion molecule pairs. However, subsequent studies from that group showed that MAdCAM-1 was present in the oviducts of infected mice (8). Expression of integrin homing receptors and adhesion molecules does not necessarily imply function since conformational changes are required to mediate adhesion (35). Therefore, in vivo functional studies are necessary. To this end, we evaluated the GT homing potential of two MoPn-specific, $CD4^+$ Th1 clones and a KLH-specific, $CD4^+$ Th1 cell line. As previously reported (24), we found that the noninflamed genital mucosa did not support lymphocyte trafficking. However, following infection, the two *Chlamydia*-specific clones as well as the KLH-specific line readily migrated to the GT. We also found that MoPn-specific clones derived from mice following infection favored recirculation through mucosa-associated lymph nodes and expressed high levels of the mucosal homing receptor α4β7. Furthermore, blocking α4β7 interaction with its ligands significantly reduced recruitment of clone P-MoPn but not the KLH-1 line to the GT in vivo. Taken together, these data suggest that although multiple homing receptor-adhesion molecule pairs may mediate homing to the *Chlamydia*-infected genital mucosa, natural infection generates a subset of protective T cells that utilize α4β7.

Prior studies have indicated that $CD4^+$ Th1 cells are essential for both the resolution of and immunity to chlamydial genital infection (30, 42). Although the homing of clone P-MoPn may not entirely reflect the trafficking of the heterogeneous T-cell population that develops in response to genital infection, it is likely to be representative of a crucial subset of T cells, i.e., protective and antigen specific. In support of this, we found that anti-α4β7, anti-α4, and anti-CD44 could reduce the GT migration of $CD4^+$ cells isolated from the ILN of MoPn-infected mice (K. A. Kelly et al., unpublished data). Finally, although a cell line may represent a number of clones all with different migration patterns, the two MoPn-specific clones derived from the same line exhibited similar migration patterns. Therefore, it is highly likely that the results found using clone P-MoPn will accurately reflect the overall migration potential of *Chlamydia*-specific T cells during infection.

Recently, Marelli-Berg et al. (28) found that antigen can also influence the rate of migration across endothelial monolayers in vitro. Although we cannot discount the influence of antigen on the rate of migration in vivo, our data suggest that clone P-MoPn migrates across the endothelium at a higher rate than the KLH-1 line. To rule out the possibility that decreased GT homing of KLH-1 compared to P-MoPn, observed at 18 h, was not not due simply to antigen-specific retention within the infected GT, we examined additional time points following adoptive transfer. We found that the amount of time allowed for recirculation following adoptive transfer affected the number of T cells found within GT. The ratios of P-MoPn to KLH-1 within the GT were similar at 6 h and at 18 h. Only at later time points (36 h) did this ratio increase, indicating that retention or proliferation of MoPn-specific clones within the GT occurred at time points later than 18 h. Nonetheless, we cannot rule out the possibility that the increased rate of migration seen for clone P-MoPn relative to KLH-1 was solely dependent on antigen specificity and not increased expression of α4β7. Taken together, these data support the supposition that antigen-specific clones are retained in tissues following antigenic activation. In addition to antigen-specific retention, clone P-MoPn appears to migrate across the GT endothelium at a higher rate compared to the KLH-1 line.

Likewise, antigen also appeared to influence the recirculation of clone P-MoPn to the local draining lymph nodes since labeled clone P-MoPn was observed in both the ILN and MLN after infection. Both the ILN and MLN were shown to contain viable MoPn 7 days following MoPn infection (11), which may in part account for the increased trafficking of P-MoPn to these tissues compared with the KLH-1 line. Also, although only weak expression of MAdCAM-1 was observed on the venular endothelium within the ILN during MoPn infection (K. A. Kelly, unpublished observation), a greater number of cells were found at this site than in the MLN. Thus, the presence of specific antigen and possibly other factors associated with inflammation also contribute to the trafficking of memory T cells to secondary lymphoid tissues as well as extra-lymphoid tissue sites.

Although the primary ligand of α4β7 is MAdCAM-1, this

heterodimer, when expressed at high levels on lymphocytes, has the capacity to bind to both MAdCAM-1 and VCAM-1 (5) in vitro. However, the consequences of this in vivo are not known (8). This study is the first to propose that $\alpha4\beta7^{hi}$ cells may transmigrate via VCAM-1 in vivo. The administration of anti-LPAM-1 to recipient mice in vivo blocked the recruitment of statistically greater numbers of clone P-MoPn than treatment of mice with anti-α4. This is remarkable since there are 5 to 10 times fewer venules expressing MAdCAM-1 in the inflamed GT compared with VCAM-1-expressing venules (26). Since antichlamydial lymphocytes can arise from MLN (10), α4β7 could be preactivated and compete effectively with α4β1-expressing cells. Alternatively, α4β7 integrin may be preferentially activated over α4β1 within the GT via chemokine production. Although intriguing, further studies are needed to support this proposition.

Other homing receptors in addition to α4β7 are likely to participate in recruiting lymphocytes to the GT. Chlamydiae have been shown to elicit an array of inflammatory cytokines such as tumor necrosis factor alpha (3, 14), IFN-γ (9), IL-1, IL-6, and various chemokines (37, 48). Accordingly, we found that P-MoPn, N-MoPn, and KLH-1 all expressed CD44, which has been shown to be one of the principal homing receptors mediating the trafficking of activated T cells to sites of inflammation (15) and possibly intestinal sites (8). Although we have not examined the effects of anti-CD44 antibody treatment on the recruitment of P-MoPn or KLH-1 in vivo, we would anticipate that this molecule would also be involved in GT homing. Indeed, we found that anti-CD44 inhibited GT homing of $CD4^+$ cells isolated from the ILN of genitally infected mice (Kelly et al., unpublished). Interestingly, clone P-MoPn had a GT homing potential in mice infected with a different serovar of *C. trachomatis*, serovar E, that was similar to that displayed by line KLH-1 in MoPn-infected mice (data not shown). However, clone P-MoPn did not proliferate in vitro to serovar E (data not shown). Serovar E infection in mice has been shown to induce an inflammatory response of much less intensity compared to infection with MoPn (13). Thus, the magnitude of lymphocyte recruitment must also be influenced by inflammatory changes that occur within the GT during infection.

In our model of murine chlamydial genital infection, the pattern of lymphocyte recruitment to the GT appears to be shaped by the influence of local inflammation or host-pathogen interaction, as well as the unique tissue site. For instance, MAdCAM-1 has not been observed on the endothelium of normal uterus or ovarian tissue in humans (6) or mice (24). However, this molecule is expressed during murine MoPn infection (8, 24, 26). Likewise, VCAM-1 is also temporarily induced in the GT following chlamydial infection (24, 34). In addition to expressing α4β7, clone P-MoPn expressed the homing receptor α4β1 (VLA-4), which has been shown to facilitate recruitment to nonmucosal sites of inflammation through interaction with VCAM-1 (27). Blocking studies in vivo revealed that both α4β7 and to a lesser degree α4β1 participate in recruitment to the GT. In contrast, α4β1–VCAM-1 interactions have not been shown to be involved in lymphocyte recruitment to the intestinal wall even during inflammation (38). Taken together, these data suggest that recruitment to the GT, specifically during chlamydial infection, is unique from that of intestinal tissues and nonmucosal inflammatory sites but contains aspects from both situations. It remains to be determined how competition among these ligands for recruitment to the GT in vivo during a natural *Chlamydia* infection would select for a distinct population of memory cells.

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REFERENCES

1. **Andrew, D. P., C. Berlin, S. Honda, T. Yoshino, A. Hamann, B. Holzmann, P. J. Kilshaw, and E. C. Butcher.** 1994. Distinct but overlapping epitopes are involved in alpha 4 beta 7-mediated adhesion to vascular cell adhesion molecule-1, mucosal addressin-1, fibronectin, and lymphocyte aggregation. J. Immunol. **153:**3847–3861.
2. **Anonymous.** 1997. *Chlamydia trachomatis* genital infections—United States, 1995. Morbid. Mortal. Wkly. Rep. **46:**193–198.
3. **Ault, K. A., O. W. Tawfik, M. M. Smith-King, J. Gunter, and P. T. Terranova.** 1996. Tumor necrosis factor-α response to infection with *Chlamydia trachomatis* in human fallopian tube organ culture. Am. J. Obstet. Gynecol. **175:**1242–1245.
4. **Barron, A. L., R. G. Rank, and E. B. Moses.** 1984. Immune response in mice infected in the genital tract with mouse pneumonitis agent (*Chlamydia trachomatis* biovar). Infect. Immun. **44:**82–85.
5. **Berlin, C., E. L. Berg, M. J. Briskin, D. P. Andrew, P. J. Kilshaw, B. Holzmann, I. L. Weissman, A. Hamann, and E. C. Butcher.** 1993. α4β7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell **74:**185–195.
6. **Briskin, M., D. Winsor-Hines, A. Shyjan, N. Cochran, S. Bloom, J. Wilson, L. M. McEvoy, E. C. Butcher, N. Kassam, C. R. Mackay, W. Newman, and D. J. Ringler.** 1997. Human mucosal addressin cell adhesion molecule-1 is preferentially expressed in intestinal tract and associated lymphoid tissue. Am. J. Pathol. **151:**97–110.
7. **Brunham, R. C., J. Kimani, J. Bwayo, G. Maitha, I. Maclean, C. L. Yang, C. X. Shen, S. Roman, N. J. D. Nagelkerke, M. Cheang, and F. A. Plummer.** 1996. The epidemiology of *Chlamydia trachomatis* within a sexually transmitted diseases core group. J. Infect. Dis. **173:**950–956.
8. **Butcher, E. C., M. Williams, K. Youngman, L. Rott, and M. Briskin.** 1999. Lymphocyte trafficking and regional immunity. Adv. Immunol. **72:**209–253.
9. **Byrne, G. I., B. Grubbs, T. J. Marshall, J. Schachter, and D. M. Williams.** 1988. Gamma interferon-mediated cytotoxicity related to murine *Chlamydia trachomatis* infection. Infect. Immun. **56:**2023–2027.
10. **Cain, T. K., and R. G. Rank.** 1995. Local Th1-like responses are induced by intravaginal infection of mice with the mouse pneumonitis biovar of *Chlamydia trachomatis*. Infect. Immun. **63:**1784–1789.
11. **Cotter, T. W., K. H. Ramsey, G. S. Miranpuri, C. E. Poulsen, and G. I. Byrne.** 1997. Dissemination of *Chlamydia trachomatis* chronic genital tract infection in gamma interferon gene knockout mice. Infect. Immun. **65:**2145–2152.
12. **Csencsits, K. L., M. A. Jutila, and D. W. Pascual.** 1999. Nasal-associated lymphoid tissue: phenotypic and functional evidence for the primary role of peripheral node addressin in naive lymphocyte adhesion to high endothelial venules in a mucosal site. J. Immunol. **163:**1382–1389.
13. **Darville, T., C. W. Andrews, K. K. Lafoon, W. Shymasani, L. R. Kishen, R. G. Rank, C. W. Andrews, Jr., and K. K. Laffoon.** 1997. Mouse strain-dependent variation in the course and outcome of chlamydial genital tract infection is associated with differences in host response. Infect. Immun. **65:**3065–3073.
14. **Darville, T., K. K. Lafoon, L. R. Kishen, and R. G. Rank.** 1995. Tumor necrosis factor-alpha activity in genital tract secretions of guinea pigs infected with chlamydiae. Infect. Immun. **63:**4675–4681.
15. **DeGrendele, H. C., P. Estess, and M. H. Siegelman.** 1997. Requirement for CD44 in activated T cell extravasation into an inflammatory site. Science **278:**672–675.
16. **Ferguson, T. A., and T. S. Kupper.** 1993. Antigen-independent processes in antigen-specific immunity. A role for alpha 4 integrin. J. Immunol. **150:**1172–1182.
17. **Hamann, A., D. P. Andrew, D. Jablonski-Westrich, B. Holzmann, and E. C. Butcher.** 1994. Role of α4-integrins in lymphocyte homing to mucosal tissues in vivo. J. Immunol. **152:**3282–3293.
18. **Hanninen, A., C. Taylor, P. R. Streeter, L. S. Stark, J. M. Sarte, J. A. Shizuru, O. Simell, and S. A. Michie.** 1993. Vascular addressins are induced on islet vessels during insulitis in nonobese diabetic mice and are involved in lymphoid cell binding to islet endothelium. J. Clin. Investig. **92:**2509–2515.
19. **Igietseme, J. U., K. H. Ramsey, D. M. Magee, D. M. Williams, T. J. Kincy, and R. G. Rank.** 1993. Resolution of murine chlamydial genital infection by the adoptive transfer of a biovar-specific TH1 lymphocyte clone. Regul. Immunol. **5:**317–324.
20. **Igietseme, J. U., and R. G. Rank.** 1991. Susceptibility to reinfection after a primary chlamydial genital infection is associated with a decrease of antigen-specific T cells in the genital tract. Infect. Immun. **59:**1346–1351.
21. **Igietseme, J. U., I. M. Uriri, S. N. Kumar, G. A. Ananaba, O. O. Ojior, I. A. Momodu, D. H. Candal, and C. M. Black.** 1998. Route of infection that induces a high intensity of gamma interferon-secreting T cells in the genital tract produces optimal protection against *Chlamydia trachomatis* infection in

mice. Infect. Immun. **66:**4030–4035.
22. **Katz, B. P., B. E. Batteiger, and R. B. Jones.** 1987. Effect of prior sexually transmitted disease on the isolation of *Chlamydia trachomatis*. Sex. Transm. Dis. **14:**160–164.
23. **Kawai, T., H. Shimauchi, J. W. Eastcott, D. J. Smith, and M. A. Taubman.** 1998. Antigen direction of specific T-cell clones into gingival tissues. Immunology **93:**11–19.
24. **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.
25. **Kelly, K. A., E. A. Robinson, and R. G. Rank.** 1996. Initial route of antigen administration alters the T-cell cytokine profile produced in response to the mouse pneumonitis biovar of *Chlamydia trachomatis* following genital infection. Infect. Immun. **64:**4976–4983.
26. **Kelly, K. A., J. C. Walker, S. H. Jameel, H. L. Gray, and R. G. Rank.** 2000. Differential regulation of CD4 lymphocyte recruitment between the upper and lower regions of the genital tract during *Chlamydia* infection. Infect. Immun. **68:**1519–1528.
27. **Lobb, R. B., and M. E. Hemler.** 1994. The pathophysiologic role of α4 integrins in vivo. J. Clin. Investig. **94:**1722.
28. **Marelli-Berg, F. M., L. Frasca, L. Weng, G. Lombardi, and R. I. Lechler.** 1999. Antigen recognition influences transendothelial migration of CD4+ T cells. J. Immunol. **162:**696–703.
29. **McDermott, M. R., and J. Bienenstock.** 1979. Evidence for a common mucosal immunologic system. I. Migration of B immunoblasts into intestinal, respiratory, and genital tissues. J. Immunol. **122:**1892–1898.
30. **Morrison, R. P., K. Feilzer, and D. B. Tumas.** 1995. Gene knockout mice establish a primary protective role for major histocompatibility complex class II-restricted responses in *Chlamydia trachomatis* genital tract infection. Infect. Immun. **63:**4661–4668.
31. **Pal, S., T. Fielder, E. M. Peterson, and L. de la Maza.** 1993. Analysis of the immune response in mice following intrauterine infection with the *Chlamydia trachomatis* mouse pneumonitis biovar. Infect. Immun. **61:**772–776.
32. **Parr, M. B., L. Kepple, M. R. McDermott, M. D. Drew, J. J. Bozzola, and E. L. Parr.** 1994. A mouse model for studies of mucosal immunity to vaginal infection by herpes simplex virus type 2. Lab. Investig. **70:**369–380.
33. **Parr, M. B., and E. L. Parr.** 1990. Antigen recognition in the female reproductive tract. I. Uptake of intraluminal protein tracers in the mouse vagina. J. Reprod. Immunol. **17:**101–114.
34. **Perry, L. L., K. Feilzer, J. L. Portis, and H. D. Caldwell.** 1998. Distinct homing pathways direct T lymphocytes to the genital and intestinal mucosae in *Chlamydia*-infected mice. J. Immunol. **160:**2905–2914.
35. **Picker, L. J., and E. C. Butcher.** 1992. Physiological and molecular mechanisms of lymphocyte homing. Annu. Rev. Immunol. **10:**561–591.
36. **Ramsey, K. H., L. S. F. Soderberg, and R. G. Rank.** 1988. Resolution of chlamydial genital infection in B-cell-deficient mice and immunity to reinfection. Infect. Immun. **56:**1320–1325.
37. **Rasmussen, S., L. Eckmann, A. J. Quayle, L. Shen, Y. Zhang, D. J. Anderson, J. Fierer, R. S. Stephens, and M. F. Kagnoff.** 1997. Secretion of proinflammatory cytokines by epithelial cells in response to *Chlamydia* infection suggests a central role for epithelial cells in chlamydial pathogenesis. J. Clin. Investig. **99:**77–87.
38. **Salmi, M., and S. Jalkanen.** 1997. How do lymphocytes know where to go: current concepts and enigmas of lymphocyte homing. Adv. Immunol. **64:**139–218.
39. **Samlowski, W. E., B. A. Robertson, B. K. Draper, E. Prystas, and J. R. McGregor.** 1991. Effects of supravital fluorochromes used to analyze the in vivo homing of murine lymphocytes on cellular function. J. Immunol. Methods **144:**101–115.
40. **Stamm, W. E.** 1999. *Chlamydia trachomatis* infections: progress and problems. J. Infect. Dis. **179**(Suppl. 2)**:**S380–S383.
41. **Streeter, P. R., E. L. Berg, B. T. N. Rouse, R. F. Bargatze, and E. C. Butcher.** 1988. A tissue-specific endothelial cell molecule involved in lymphocyte homing. Nature **331:**41–46.
42. **Su, H., and H. D. Caldwell.** 1995. CD4+ T cells play a significant role in adoptive immunity to *Chlamydia trachomatis* infection of the mouse genital tract. Infect. Immun. **63:**3302–3308.
43. **Tidswell, M., R. Pachynski, S. W. Wu, S. Q. Qiu, E. Dunham, N. Cochran, M. J. Briskin, P. J. Kilshaw, A. I. Lazarovits, D. P. Andrew, E. C. Butcher, T. A. Yednock, and D. J. Erle.** 1997. Structure-function analysis of the integrin β7 subunit. Identification of domains involved in adhesion to MAdCAM-1. J. Immunol. **159:**1497–1505.
44. **Tilney, N. L.** 1971. Patterns of lymphatic drainage in the adult laboratory rat. J. Anat. **109:**369–383.
45. **Westrom, L., R. Joesoef, G. Reynolds, A. Hagdu, and S. E. Thompson.** 1992. Pelvic inflammatory disease and fertility. A cohort study of 1,844 women with laparoscopically verified disease and 657 control women with normal laparoscopic results. Sex. Transm. Dis. **19:**185–192.
46. **Westrom, L. V.** 1994. Sexually transmitted diseases and infertility. Sex. Transm. Dis. **21:**S32–S37.
47. **Williams, M. B., and E. C. Butcher.** 1997. Homing of naive and memory T lymphocyte subsets to Peyer's patches, lymph nodes, and spleen. J. Immunol. **159:**1746–1752.
48. **Wyrick, P. B., S. T. Knight, T. R. Paul, R. G. Rank, and C. S. Barbier.** 1999. Persistent chlamydial envelope antigens in antibiotic-exposed infected cells trigger neutrophil chemotaxis. J. Infect. Dis. **179:**954–966.

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