Blockade of both L-selectin and \( \alpha_4 \) integrins abrogates naive CD4 cell trafficking and responses in gut-associated lymphoid organs

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Abstract

The recirculation of naive lymphocytes from blood to lymph that is initiated in high endothelial venules (HEV) of secondary lymphoid organs such as lymph nodes and Peyer’s patches (PP) is regulated by multiple interactions of adhesion receptor/counter-receptor pairs involving both selectins and integrins. We showed previously that blocking of only L-selectin is sufficient to ablate trafficking of naive CD4 cells and the development of their responses in peripheral lymph nodes but not in PP where \( \alpha_4\beta_7 \) integrins are thought to primarily regulate entry. However, although antibody to \( \alpha_4 \) integrins partially inhibited homing of naive CD4 cells to PP and not to lymph nodes, there was no effect on the development primary responses in these tissues or spleens. Since previous studies indicate that both \( \alpha_4\beta_7 \) integrins and L-selectin regulate adhesion of naive cells to PP HEV, we examined the effect a blockade of both adhesion pathways on the recirculation of naive CD4 cells. There was no detectable homing of naive CD4 cells to PP or lymph nodes when interactions with both receptors were inhibited, resulting in a profound depletion of naive CD4 cells and loss of antigen responses in these sites. In contrast, increased numbers of naive CD4 cells and responses of higher magnitude were found in the spleen. The results demonstrate recirculation of naive CD4 cells through tissues where entry is controlled through HEV is essential for the local generation of primary responses.

Introduction

The continuous recirculation of lymphocytes is thought to be crucial for the development of primary immune responses to foreign antigen. Activation of naive T and B cells occurs only within the context of organized lymphoid tissue (1,2), suggesting that the blood to tissue migration of lymphocytes facilitates localization of rare cells of appropriate specificity, thereby enabling initiation of responses to antigen. Although entry of lymphocytes from the blood into the spleen is apparently not restricted, extravasation into peripheral lymph nodes (PLN), mesenteric lymph nodes (MLN) and Peyer’s patches (PP) occurs through specialized high endothelial venules (HEV), and is regulated by the interactions of adhesion molecules expressed on lymphocytes with counter-receptors expressed on HEV (reviewed in 3–5). L-selection, which is highly expressed on naive cells (6), is the principle homing receptor regulating lymphocyte entry into PP or lymph nodes when interactions with both receptors were inhibited, resulting in a profound depletion of naive CD4 cells and loss of antigen responses in these sites. In contrast, increased numbers of naive CD4 cells and responses of higher magnitude were found in the spleen. The results demonstrate recirculation of naive CD4 cells through tissues where entry is controlled through HEV is essential for the local generation of primary responses.

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a rolling behavior, and possibly participating in the up-
regulation of the avidity of integrins (17) that include \( \alpha_4 \beta_7 \) and \( \alpha_4 \beta_2 \) (LFA-1). Activated integrins mediate firm attachment to
the vessel wall and transmigration (reviewed in 5,18). Studies
of lymphocyte interactions with HEV in GALT suggest that
sequential interactions of multiple adhesion receptor/counter-
receptor pairs regulate lymphocyte recirculation.

Although it is well established that naive cells recirculate,
at any given time, only a limited fraction of cells are engaged
in interactions with HEV and, of those cells, only some
extravasate from the blood into tissue (19). Although it is not
clear why some cells and not other, apparently identical cells
interact with HEV, it is thought that this aspect of lymphocyte
recirculation may be a random process (4). It is possible
that additional, as yet undiscovered chemotaxants or
chemokines affect the transendothelial migratory capacity of
individual cells. Our previous studies of naive CD4 cells
indicate that a blockade of L-selectin alone is sufficient to
abolish trafficking to PLN and cause depletion of naive cells,
resulting in impaired primary responses only in that tissue
(6). Trafficking to PP is partially blocked, with a limited and
transient loss of naive CD4 cells and no effect on immune
responses (15). The implication of these results is that a
requirement for L-selectin usage in PP can be bypassed,
either by \( \alpha_4 \beta_7 \) alone or by \( \alpha_4 \beta_7 \) in combination with other
receptors. Studies by other investigators indicate lymphocyte
homing to PP is also only partially inhibited when \( \alpha_4 \beta_7 \) integrins
are blocked, demonstrating a role for additional receptors.
Moreover, when interactions with both L-selectin and \( \alpha_4 \beta_7 \)
are prevented, lymphocytes no longer adhere to PP HEV (12).
However, since multiple adhesive interactions are required for
lymphocyte extravasation through HEV and several alternative
adhesion receptor/counter-receptor pairs could be potentially
utilized if a particular pathway were unavailable (reviewed in
3), we investigated whether a blockade of only L-selectin and
\( \alpha_4 \) integrins, the two major homing receptors, would be
sufficient to abolish not only homing into sites where entry is
controlled by HEV, but also localized development of immune
responses. We show that highly purified, naive CD4 cells are
indeed unable to enter GALT or PLN when interactions with
both homing receptors are prevented by blocking antibodies.
Under these conditions, the generation of primary CD4
responses to antigen is also abolished in these sites due to
profound depletion of naive CD4 cells.

Methods

Mice

C57BL/6 and B10.BR mice were obtained from Jackson
Laboratories (Bar Harbor, ME). V\( _{j}^{3} \)N\( \alpha_{t}^{11} \) TCR transgenic mice
(20) on the B10.BR background were bred in our animal
facility at The Scripps Research Institute. Adult mice of the
same age and sex were used in individual experiments.

Antibodies

The cell lines secreting rat mAb to L-selectin (CD62L, MEL-
14, IgG2a), \( \alpha_4 \) integrin (PS/2, IgG2b), CD8 (3.155, IgM),
B220 (RA3-6B2, IgG2a) and anti-\( \alpha_4 \)\( \beta_7 \) (CA4.12, IgM) were
obtained from the ATCC (Rockville, MD). Purified anti-CD44
(IRAWB14.4, rat IgG2a) was the generous gift of J. Lesley
(Salk Institute, La Jolla, CA). Rat anti-CD45 (M1/9.3.4.HL.2,
IgG2a and M189.18.7.HK, IgG2b) from the ATCC were used
as isotype controls. mAbs were produced in ascites from cells
grown in nude mice. IgG2a and IgG2b from the sera of
normal rats (Caltag, Burlingame, CA) were used as additional
isotype controls. Antibodies were affinity purified using Protein
Affinity purified DAKT32 (anti-\( \alpha_4 \)\( \beta_7 \), rat IgG2a) was purchased
from PharMingen (La Jolla, CA). For flow cytometry, FITC-
labeled anti-L-selectin, -CD44, -CD45RB, -V\( _{y}^{\gamma} \)11, rat IgG2a
and IgG2b isotype controls, and the second step reagent,
FITC–mouse anti-rat IgG2a were obtained from PharMingen.
Phycoerythrin (PE)–anti-CD4 (GK1.5) was obtained from
Becton Dickinson (Mountain View, CA). PE–anti-B200 and
FITC–anti-CD8 were obtained from Caltag.

 Trafficking of naive CD4 cells

CD4 cells were isolated from spleens and pooled lymph
nodes of TCR transgenic mice by magnetic (MACS) sorting
(Miltenyi Biotech, Auburn, CA). CD4+ cells, B cells and
macrophages/dendritic cells were depleted using anti-CD8,
anti-class II and anti-B220 mAb followed by incubation with magnetic beads conjugated to anti-rat \( \kappa \) chain and passage of
the cells over MACS columns according to the manufacturer’s
instructions. To remove potentially activated cells, high den-
sity, resting cells were then obtained by Percoll density
gradient centrifugation (four layers, 45, 53, 62 and 80%), and
collected from the interface of the 80 and 62% layers. These
cells were 95–98% CD4+. The cells were labeled with 10 \( \mu \)Ci
\( ^{51} \)C-labeled sodium chromate (NEN Dupont, Boston, MA)/107
cells at 37°C for 20 min. Between 3 \( \times \) 10^5 and 5 \( \times \) 10^6 labeled
cells were treated for 20 min at 4°C with 100 \mu g each of rat
IgG2a/IgG2b (anti-CD45), 100 \mu g anti-CD44 alone, 100 \mu g
anti-L-selectin alone, 100 \mu g anti-\( \alpha_4 \) alone or the latter two
mAb in combination. The cells were then injected i.v. in 0.2 ml
to into groups of four B10.BR recipients that were 8 weeks of
age. After 2 h, distribution of cells in the tissues of recipient
mice was measured with a \( \gamma \)-counter. Blood values are
computed for a 2.0 ml volume. Data for each organ are shown
as percent of total c.p.m. injected. Recovery of radioactivity
ranged from 60 to 75% of that injected.

Responses of CD4 cells

Primary effector CD4 cells were generated by i.p. injection
of 4-month-old C57BL/6 mice with 100 \mu g keyhole limpet
hemocyanin (KLH; Calbiochem, La Jolla, CA) precipitated
with 1.8 mg alum and of 10^9 Bordatella pertussis vaccine
organisms 1 day after i.v. injection of 200 \mu g each of normal
rat IgG2a/IgG2b, 200 \mu g anti-L-selectin, 200 \mu g anti-\( \alpha_4 \) or
both. On day 4 after priming with KLH, CD4 cells were
enriched to >85–90% from PLN, MLN, PP and spleens
by magnetic sorting, as indicated above. CD4 cells were cultured at
1 \times 10^5/well in triplicate in 200 \mu l RPMI 1640 (Irvine Scientific,
Santa Ana, CA) supplemented with 7% FCS (Hyclone, Logan,
UT), 200 \mu g/ml penicillin, 200 \mu g/ml streptomycin, 4 mM
l-glutamine, 10 mM HEPES and 5 \times 10^{-5} M 2-mercaptoethanol
in 96-well flat-bottomed plates (Costar, Cambridge, MA).
For re-stimulation of KLH-specific CD4 cells, T cell-depleted
spleens from unimmunized mice pulsed with KLH or
ovalbumin were used at $2 \times 10^5$/well as antigen-presenting cells (APC), as previously described (6). To assess KLH-specific responses, culture supernatants were harvested at 36 h and tested for secretion of IL-2 by measuring proliferation of the NK cell line as described (6). Serial dilutions of test supernatants were assayed in triplicate and referenced to recombinant murine IL-2 (PharMingen). The data are half-maximal units (U) where 1 U of IL-2 equals 14 pg protein.

**Immunofluorescent staining and flow cytometry**

CD4 cells were analyzed for expression of surface markers by two-color analysis as described (6,15). Between $2 \times 10^5$ and $5 \times 10^5$ cells were stained for 15 min with 0.05–1.0 µg mAb in 100 µl PBS containing 1% BSA and 0.05% NaN₃. Cells were stained initially with mAb that were directly labeled with FITC as indicated in the text and then with PE-anti-CD4 mAb. Shown are histograms generated from 5000 gated events using a FACScan flow cytometer (Becton Dickinson).

**Results**

Blockade of α₄ integrins and L-selectin ablates trafficking of naive CD4 lymphocytes to PP

To provide a homogeneous population of naive CD4 cells, we used TCR transgenic mice specific for pigeon cytochrome c that have >90% T cells that carry the V₅α11, V₅β3 transgenic receptor (20,21). Resting CD4 cells were purified as indicated in Methods. As shown in Fig. 1, the resulting population was 98% CD4⁺ (top left panel) that were IL-2R⁻ (not shown) and exclusively transgenic as indicated by expression of V₅α11 (top right panel). This population also exhibited characteristics typically associated with naive CD4 cells: uniform expression of CD45RB and CD44 with an absence of low or high expressing cells respectively (middle panels). In terms of homing receptors (bottom panels), the purified CD4 cells expressed high levels of L-selectin and low levels of α₄β₇.

In initial experiments to evaluate the roles of the homing receptors, α₄β₇ and L-selectin, in trafficking of naive cells, the purified CD4 cells were labeled with 51Cr and incubated with 100 µg of mAb to L-selectin (MEL-14) or α₄ integrin (PS/2). For comparison we also used mAb to CD44, a molecule which may play a role in initial, rolling interactions of lymphocytes with endothelium (22) using an mAb (IRAWB14.4) which blocks lymphocyte migration into inflamed skin during a contact sensitivity response (29). As an isotype control, cells were incubated with 100 µg each of rat IgG2a and IgG2b anti-CD45 mAb. The cells were injected i.v. into separate groups of four syngeneic, B10.BR recipients and 2 h later lymphocyte homing was evaluated by the distribution of c.p.m. in various organs of recipient mice. As shown in Fig. 2, MEL-14 blocked naive CD4 cell homing to PLN and MLN by >90% compared to the control, whereas mAb to α₄ integrin (PS/2) or CD44 were without effect. Both MEL-14 and PS/2 partially blocked homing to PP to a similar extent (68 and 54% respectively) while no inhibitory effect was seen with mAb to CD44.

To determine if blocking both α₄β₇ and L-selectin together would further affect homing of naive cells to PP, 51Cr-labeled CD4 cells were treated with 100 µg of mAb to either L-selectin or α₄ integrin, or both mAb together. As shown in Fig. 3 (middle panel), while homing to PP was reduced by only 28% with MEL-14 alone and by 66% with PS/2 alone; most dramatically, the combination completely blocked trafficking of naive CD4 cells to this site from a high of 0.7% of injected cells in the control to 0.1%, the limit of detection for the assay. Similar numbers of control and mAb-treated naive CD4 cells were found in spleen, blood and non-lymphoid tissues. No differences were seen in the trafficking of isotype control treated and untreated CD4 cells (not shown). Comparable results were obtained in three experiments. Similar results were also obtained when CD4 cells isolated from lymph nodes of young, normal B10.BR mice were as a source of naive cells (not shown). The results show that migration of naive CD4 cells into PP was not detectable when interactions with both L-selectin and α₄ integrins were prevented, directly implicating both receptors in trafficking into these intestinal lymphoid tissues.
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Effect of an in vivo blockade of L-selectin and α4 integrins on naive CD4 cells

To further delineate the roles of α4β7 and L-selectin in naive CD4 trafficking to GALT, we quantitated CD45RBhi, CD44lo CD4 cells in PLN, MLN, PP as well as spleen on day 5 after injection of the blocking mAb to L-selectin or α4 integrin, or the isotype controls into groups of six mice. The injected mAb have a half-life in excess of 2 weeks and are readily detectable in the sera of recipient mice (not shown). Since expression of high versus low levels of CD44 is generally considered to be the most reliable indication of prior antigen exposure in mice on the C57BL/6 or C57BL/10 background, we used this marker to indicate changes in the proportion of naive CD4 cells as a consequence of adhesion molecule blockade. In agreement with our previous studies (6,15), administration of MEL-14 reduced the naive CD4 cell numbers in PLN >90%, to virtually undetectable levels (Fig. 4). MEL 14 partially reduced naive CD4 cell population in MLN (67%) but had no effect on their numbers in PP. In contrast, PS/2 treatment reduced naive CD4 cell numbers found in PP by 70% but had no effect on this population in either PLN or MLN. However, treatment with both MEL-14 and PS/2 caused a dramatic depletion of the naive CD4 population in PP and MLN to undetectable levels. Comparable data were obtained in terms of total lymphocyte recovery from the individual organs of mAb treated animals (not shown) which correlated with the size of the organs observed at the time of excision. In these experiments as well as the homing studies described above, we found that the anti-α4 mAb, PS/2, was considerably more effective that the lower affinity anti-α4β7 mAb, DAKT32 (14), in terms of blocking of homing and depletion of naive CD4 cells from PP (not shown). Although the α4 chain also associates with the β7 integrin chain, this heterodimer preferentially recognizes VCAM-1 on activated endothelium and is not therefore involved in normal lymphocyte recirculation (11). These results demonstrate that when both L-selectin and α4 were blocked, naive cells were profoundly depleted from PP and MLN, whereas inhibition of interactions with L-selectin...
To determine the effects of blocking L-selectin and α4 on naive CD4 cell function, we evaluated primary KLH-specific responses of CD4 cells in PLN, MLN, PP, and spleen from mice pretreated with either MEL-14 or PS/2, alone, or a combination of both in comparison to isotype control treated mice. Groups of six mice were injected with mAb 1 day prior to immunization with KLH. On day 4 after priming (day 5 after mAb injection), IL-2 production by CD4 cells re-stimulated with KLH-pulsed APC was measured (Fig. 6). As shown previously, MEL-14 treatment prevented development of KLH-specific CD4 cells in PLN but not PP and spleen (6,15), while responses in MLN were reduced by 73%. PS/2 treatment alone had no effect on responses of CD4 cells from PLN or spleen and only caused a minor reduction responses from MLN (20%) and PP (33%). Pretreatment of animals with both MEL-14 and PS/2 completely abolished primary CD4 responses in both MLN and PP. Responses in spleen were unaffected by mAb treatment, and in the case of animals receiving both MEL-14 and PS/2, the splenic responses were significantly higher (170%) than those found in the isotype control treated animals. These data, representative of three experiments, conclusively demonstrate that treatment with the mAb to both L-selectin and α4 integrin interferes with the entry of naive CD4 cells into MLN and PP but not with their function.

Discussion

Newly generated naive T cells exiting the thymus join the recirculating lymphocyte pool and are thought to engage in a continuous process of migration from blood to lymph for the duration of their lifespan unless exposure to specific antigen occurs as they traverse through secondary lymphoid organs. In this study, by completely blocking all trafficking through tissues where entry is regulated through HEV, we demonstrate that recirculation of naive CD4 cells is essential for the development of primary immune responses. Studies of homing with bulk lymphocyte populations indicate that several adhesion molecules which include L-selectin as well as the integrins α4β7 and α4β2 (LFA-1) participate in the regulation of lymphocyte migration through HEV (7,12,14,16,23,24). Our studies demonstrate that interactions with L-selectin are critical for entry of naive CD4 cells into PLN but not PP (Figs 2 and 3) (6,15). However, although L-selectin is not essential for migration into PP, studies of lymphocyte homing and attachment to Peyer’s patch vessels by intravital microscopy have shown that this receptor can provide the initial interactions with PP HEV that promote initial lymphocyte rolling, and that α4β7 mediates subsequent rolling and firm adhesion through interactions with their respective ligands on the mucin-like and Ig domains of the mucosal addressin cell adhesion molecule 1 (MAdCAM-1) (12,25). In this report, we show that when both molecules are blocked, entry of naive CD4 cells into PP is completely abolished.

By using a highly defined, homogeneous population of resting, CD4 cells (Fig. 1) to analyze the capacity of naive cells to home to various tissues, we eliminate the possible contributions of subsets of T and B cells that are heterogeneous with respect to adhesion molecule expression (26). Thus, our results which demonstrate that blocking of either homing receptor partially inhibits localization of naive CD4 cells in PP indicate that both homing receptors can directly contribute to interactions with PP HEV (Figs 2 and 3). However, α4β7 integrin is clearly the major receptor regulating entry into PP since in vivo treatment with anti-α4 mAb resulted in substantial depletion of this population from PP, while a blockade of L-selectin over several days had no effect on this tissue (Fig. 4, see also ref. 5). Naive CD4 cells can bypass a requirement for L-selection during recirculation...
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Fig. 5. Combined treatment in vivo with MEL-14 and PS/2 mAb depletes naive CD4 cells from GALT. Groups of 6 C57BL/6 mice were injected i.v. with 200 µg MEL-14 alone, PS/2 alone, both MEL-14 and PS/2 or rat IgG2a/2b as a control as in Fig. 4. On day 4 post-injection, expression of CD44 by CD4 cells in spleen, PLN, MLN and PP was determined by flow cytometry for CD4 cells from mice treated with the mAb indicated on the left (filled histograms) overlaid onto the controls (open histograms). Values shown are log MFI for cells from the mAb treated mice.

Fig. 6. Combined treatment in vivo with MEL-14 and PS/2 mAb inhibits development of primary CD4 responses in GALT. Groups of six C57BL/6 mice were injected i.v. with 200 µg MEL-14 alone (■), PS/2 alone (▲), both MEL-14 and PS/2 (●) or normal rat IgG2a/2b (■) as a control as in Fig. 4. On day 1 after mAb injection, mice were immunized with 100 µg alum precipitated KLH and 10⁹ pertussis vaccine organisms. On day 4 after priming, CD4 cells were isolated and cultured in triplicate at 5x10⁵/ml with 1x10⁶/ml KLH-pulsed APC. IL-2 production into culture supernatants was measured after 36 h. No responses were obtained to APC pulsed with an irrelevant antigen, ovalbumin (not shown).

Through GALT and may either use α4β7 alone for initial tethering and rolling interactions (27,28) or may substitute other adhesion molecules that have been implicated in lymphocyte–endothelial interactions. We have recently confirmed a direct role of LFA-1 (CD11a/CD18) in the migration of naive CD4 cells into PP (24), and find that a combination of mAb to this receptor and to either L-selectin or α4 can also completely block homing (L. M. Bradley, unpublished observations), demonstrating that alternative combinations of receptors can be utilized by the naive population during the process of extravasation from blood into lymphoid tissues. Although recent in vitro studies suggest a potential role for CD44 in lymphocyte trafficking (22), our data (Fig. 2) support previous in vivo studies that this molecule is not involved in lymphocyte extravasation into PLN but rather in their migration to sites of inflammation (29).

When interactions with both L-selectin and α4 integrin were prevented for an extended period by in vivo administration of blocking mAb, naive CD4 cells were profoundly depleted from PP, as well as from MLN and PLN (Figs 4 and 5). The results indicate that naive CD4 cells residing in these tissues at the time of mAb administration exit but cannot then re-enter. Naive CD4 cells are not, however, depleted from the animal under these conditions, since they are clearly evident in the spleen. Thus, in the presence of an antibody blockade to both homing receptors which eliminates lymphocyte recirculation through lymphoid tissues via HEV, naive CD4 cells remain circulating in the blood and are routed through the spleen. These data confirm the lack of a requirement for homing receptor usage for lymphocyte entry into the spleen.
In our mAb blocking studies to date, the only adhesion molecule we have identified as important for lymphocyte migration and responses in the spleen is LFA-1 (CD11a, CD18) (L. M. Bradley and S. R. Watson, unpublished observations), which appears to have a general role in cell adhesion as well as transmigration (30) and can function in co-stimulation (31).

Due to the depletion of naive CD4 cells that occurred when mAb to L-selectin and α4 were administered in combination, primary responses to antigen were absent in all of the sites where entry is controlled through HEV, but were present in the spleen. These results provide a conclusive demonstration that although migration of naive lymphocytes into tissues via HEV appears to be random (4) and <20% of cells in the blood are estimated to be engaged in the process of extravasation through HEV per day (19), recirculation of the naive CD4 population is essential for the generation of immune responses in these locations. The results provide proof that not only do the homing receptors L-selectin and α4β7 play direct roles in lymphocyte adhesion to HEV, but also that a blockade of only these two receptors is sufficient to ablate blood to lymph recirculation of naive CD4 cells. The finding that a small population of memory CD4 cells (5% or less of the total CD4 population), measured here by expression of high levels of CD44, was demonstrable in PLN, MLN and PP after depletion of naive cells by the combined mAb treatment (Fig. 5) is consistent with the notion that memory CD4 cells, which we have shown to be contained exclusively within the L-selectin subset in the mouse (32), either do not recirculate via HEV or have the capacity to use other receptors for this process (33–35). However, memory cells are clearly a minority population in lymph nodes and PP as compared to the spleen (6,32), and it is also possible that some memory cells are to a large extent retained in sites where they were initially exposed to antigen.

Our data reveal that migration of naive CD4 cells into MLN is regulated similarly to PP where blocking of both L-selectin and α4 is required to completely ablate both the trafficking and responses of naive CD4 cells. The homing receptor requirements observed in our functional studies are likely to reflect availability of counter-receptors expressed on HEV. Although both L-selectin and α4β7 are expressed on naive lymphocytes (Fig. 1), their endothelial ligands are expressed in a tissue-restricted manner (reviewed in 36). Molecules bearing ligands for L-selectin (PLN addressins or PNAd) are found in high levels in PLN but in much lower levels in PP, whereas MadCAM-1, the major ligand for α4β7 (28), is expressed predominantly in PP, but is absent from PLN in adult mice (25). Both addressins are, however, represented on HEV of MLN and PP, and a subset of MadCAM-1 molecules in GALT bears PNAd and may therefore perform the function of counter-receptor for both homing receptors (37). However, while the homing receptor usage for extravasation of naive CD4 cells is explicable in terms of distribution of ligands for the respective molecules on HEV, from the stand point of the need for widespread accessibility of naive cells to secondary lymphoid organs, the greater adhesion receptor usage in GALT as opposed to PLN is unclear. It is possible that observed flexibility evolved to ensure that naive lymphocytes gain ready access to lymphoid sites in vicinities of high antigen exposure such as the gut. In terms of the general significance of these observations, it is noteworthy that activated endothelium present in sites of chronic inflammation where trafficking of naive cells can be observed (38) is similar to mucosal sites in that both PNAd and MadCAM are expressed (39). Together, our data underscore the significance of recirculation by naive lymphocytes in the immune system and demonstrate a hierarchical dependence of naive CD4 cells upon either L-selectin or α4 for extravasation into secondary lymphoid tissue where entry is through HEV which mirrors the known distribution of the ligands for these homing receptors in peripheral versus gut mucosal lymphoid tissues.

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Abbreviations

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<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>APC</td>
<td>antigen-presenting cell</td>
</tr>
<tr>
<td>GALC</td>
<td>guard-associated lymphoid tissues</td>
</tr>
<tr>
<td>HEV</td>
<td>high endothelial venules</td>
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<tr>
<td>KLH</td>
<td>keyhole limpet hemocyanin</td>
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<tr>
<td>MadCAM-1</td>
<td>mucosal addressin cell adhesion molecule 1</td>
</tr>
<tr>
<td>MFI</td>
<td>mean fluorescence intensity</td>
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<tr>
<td>MLN</td>
<td>mesenteric lymph node</td>
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<tr>
<td>PE</td>
<td>phycoerythrin</td>
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<tr>
<td>PNAd</td>
<td>peripheral lymph node addressin</td>
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<td>PP</td>
<td>Peyer’s patches</td>
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