Subset-Specific Regulation of the Lymphatic Exit of Recirculating Lymphocytes In Vivo

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Subset-Specific Regulation of the Lymphatic Exit of Recirculating Lymphocytes In Vivo

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The blood-to-lymph recirculation of lymphocytes is required for the maintenance of immune surveillance and the dissemination of memory. Although the ability of lymph-borne cells to recirculate has been well documented, relatively less is known about the migration capacity of PBLs. We have found a clear preference for PBLs to recirculate through s.c. rather than intestinal lymph nodes. This preference could be directly attributed to the migratory characteristics of γδ-T cells. γδ-T cells were found to express significantly higher levels of L-selectin than other subsets, suggesting that at least some of this preferential migration could be attributed to their interaction with ligands on vascular endothelium. More detailed experiments showed that γδ-T cells migrated through lymph nodes with greater efficiency than αβ T cells or B cells, which clearly indicated an enhanced ability of γδ-T cells to exit lymph nodes in the effenter lymph independent of entry from the blood. This hypothesis was supported by histological examination, where γδ-T cells were found almost exclusively in the interfollicular traffic areas within lymph nodes. These data indicate that γδ-T cells are the most active recirculating lymphocyte subset in ruminants and suggest new mechanisms to regulate the traffic of lymphocyte subsets through normal lymph nodes.

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There are two distinct populations of T cells found in mammals, differentiated by their use of TCR gene products (1). Although many functions of αβ-T cells are reasonably well understood, it has been more difficult to define a distinct function for γδ-T cells. Important differences have been found between the two subsets. Unlike αβ-T cells, γδ-T cells appear to recognize native rather than processed Ag, and most γδ-T cells do not express either the CD4 or CD8 coreceptors. In addition, the number and distribution of γδ-T cells differ considerably among species. In mice and humans, γδ-T cells form only a minor proportion of the PBL pool, whereas in ruminants they may constitute up to 50% of PBLs in young animals (2). Experiments in mice have suggested that γδ-T cells may demonstrate a particular tropism to localize in epithelial tissues (3). Furthermore, the use of murine γ and δ receptor genes is restricted, apparently due to an ordered release from the thymus and similarly ordered colonization of the tissues during development of the immune system (3). Large numbers of γδ-T cells can also be found in the skin of ruminants, but there is relatively more variability in receptor gene products (4).

Lymphocytes are unique in their capacity to not only migrate selectively into tissues, but to continually recirculate between the blood and the tissues via the lymph. Large animal models offer advantages of scale for the study of lymphocyte recirculation because it is possible to directly collect the lymph draining a variety of tissues in unanesthetized animals over extended periods of time. As a result, more is known regarding the recirculation of lymphocytes in sheep than in any other species (5–7). Using in vivo systems, at least three distinct pools of lymphocytes can be identified that recirculate preferentially through s.c. lymph nodes (SCLNs), intestinal lymph nodes, and skin, respectively (8–10). The relative contribution of each pool to the overall immune complement in animals is as yet unknown. In the original experiments, which defined these preferential recirculation pathways, recirculating lymphocytes harvested from lymph were the test population. Although the blood is an obligate midpoint in the recirculation of lymphocytes, relatively few studies have examined the migratory capacity of PBLs; therefore, the relative contribution of each homing pool to the overall recirculating pool remains unknown.

Phenotypic analysis of blood, lymph, and lymphoid tissues have revealed important differences in the relative concentrations of lymphocyte subsets in vivo. Although γδ-T cells are a significant component of ruminant peripheral blood, afferent lymph (draining nonlymphoid tissues), and efferent lymph (draining lymph nodes), they are only a minor population within lymph nodes (11). This, as well as the observation that they form a major lymphoid population within epithelial tissues, has led investigators to suggest that γδ-T cells recirculate preferentially through nonlymphoid tissues like skin and gut (2). The precise mechanism for this preferential migration is unclear. Most migration studies have examined the ability of lymphocyte subsets to bind and migrate across vascular endothelial cells lining the postcapillary venules of lymph nodes (12–14). In rodents, this traffic endothelium has been termed “high endothelial venules,” and although ruminant postcapillary venules do not exhibit the morphological characteristics of high endothelial venules, their function is clearly conserved between species (15). It is important to remember that the physiological recirculation of lymphocytes involves not only transeendothelial migration, but also the successful transit of lymphocytes through solid tissue and their exit in the lymph. Relatively little is known regarding the importance of cytokines, extracellular matrix, and lymphatic endothelial cells in regulating these processes (5, 16).

The experiments reported here were designed to examine the migratory properties of PBLs. Surprisingly, γδ-T cells were found...
to be the most active recirculating lymphocyte in the peripheral blood. The data clearly indicate that γδ-T-cells recirculate through lymph nodes in higher numbers than other lymphocytes, which may be linked to their elevated expression of the homing molecule L-selectin. In addition, we found that γδ-T-cells also migrated through lymph nodes with greater efficiency than other lymphocyte subsets and were largely confined to traffic areas within the node. This localization of γδ-T-cells within lymph nodes clearly correlated with expression of the Meca-79 Ags by endothelial cells within the node. These data clearly indicate the importance of extravascular components in the regulation of lymphocyte traffic through lymph nodes and in the regulation of peripheral immune surveillance.

Materials and Methods

Experimental animals

Randomly bred sheep aged from 6 to 12 mo were obtained from Versuchs- betrieb Sennweid (Olsberg, Switzerland). Handling and treatment of the animals was according to protocols approved by the regional government authority, the Kantones Veterinarant.

Surgical procedures

Cannulation of afferent popliteal lymphatics, efferent prescapular, intestinal, and popliteal lymphatics was as previously described (17). During general anesthesia, a shortened roentgenography catheter (Becton Dickinson, Franklin Lakes, NJ) attached to a three-way stopcock was inserted into the jugular vein.

Cell collection and purification

Lymph. Lymph was collected in sterile containers containing a small amount of heparin/penicillin solution as previously described (17). Effluent lymphocyte lymphocytes (ELLs) were centrifuged at 450 × g for 7 min and used for cell labeling or analysis as described below.

Blood. Blood was sampled in 7.5% EDTA via the indwelling jugular cannula. PBLs were isolated by centrifugation over Percoll gradients and labeled as described (18). For samples intended for FACS analysis, erythrocytes were lysed using 0.16 M Tris/17 mM NH4Cl (17). Cells were washed twice in PBS and counted on a Coulter ZM cell counter (Coulter Pharmaceutical, Palo Alto, CA).

Cell labeling

Labeling of lymphocytes with PKH-26, PKH-2 (Sigma, St. Louis, MO), and 5-and 6-carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Eugene, OR) was as previously described (17, 19). Labeling of lymphocytes with CellTracker Green CMFDA (5-chloromethylfluorescein diacetate; Molecular Probes) and CellTracker Orange CMTMR (5-(and-6)-((4-chloromethyl)benzoyl)amino)-tetramethylrhodamine; Molecular Probes) was performed using 5 μM dye in PBS, under identical conditions to those used for CFSE. These labels have all been used extensively for cell tracking experiments and show no subset-specific toxicity (our unpublished observations).

Short-term cell tracking experiments

Blood (15 ml/kg; 350–500 ml) was withdrawn, and PBLs were isolated as previously described (19). Yields ranged from 3.0 × 10⁸ to 1.4 × 10⁹ cells. Cells were then labeled with either PKH-26 (three animals) or PKH-2 (three animals) and resuspended in 20 ml saline for injection. A similar number of ELLs collected from either mesenteric lymph nodes (MLNs; three animals) or prescapular lymph nodes (three animals) were labeled with the complementary label and injected at the same time. Lymph and blood samples were collected for the next 40 h. Lymphocytes were isolated from each sample as described, fixed in 1% paraformaldehyde in PBS, and stored at 4°C until analysis on a FACSscan (Becton Dickinson). Cells were gated on the basis of forward and side scatter to include only mononuclear cells in fluorescence analysis.

Homing of lymphocyte subsets to SCLNs

ELLs were collected from a cannulated efferent lymphatic in four animals (5 × 10⁸, 4.3 × 10⁸, 3.9 × 10⁹, and 3.0 × 10⁹ cells, respectively). Cells were labeled with either CellTracker Green (three animals) or CellTracker Orange (one animal) and reinjected i.v. Three hours after injection, a normal prefemoral lymph node was harvested from each animal, and single-cell suspensions were prepared. Lymph node cells were phenotyped by flow cytometry for conventional T and B cell subsets, and the proportion of each subset within the labeled cell pool was calculated. At least 500 labeled cells were analyzed per sample, requiring analysis of 1 to 2 × 10⁹ total lymph node cells. The percentages of injected CD4⁺, CD8⁺, γδ-TCR⁺, and B cells recovered from the lymph node were then calculated. To determine the efficiency of homing of each subset between animals, this number was then normalized to obtain the percent recovery of injected cells per 10⁹ lymph node cells.

Lymphatic infusion of lymphocytes

One hundred milliliters of blood was purified over Percoll as described and labeled with PKH-26 or CFSE. In other experiments, a similar number of ELLs from efferent prescapular lymph were labeled with the alternate label. Both populations were resuspended to a final concentration of 10⁹ cells/ml, and a maximum of 2 × 10⁹ cells of each type were infused into two popliteal afferent lymphatics over a total time of 1 h. Before and following infusion, a solution of 5 U/ml heparin in sterile saline was infused at 2 ml/h into cannulated afferent lymphatics to maintain patency of the system. As a control for effects of heparin on lymphocyte traffic, labeled lymphocytes were simultaneously injected intralymphatically and i.v., and the recovery was compared with control animals without efferent lymphatic cannulation and heparin treatment. In all experiments, popliteal efferent lymph was collected and phenotyped for the next 48 h.

Immunophenotyping

Blood and lymph samples were collected at various time points following injection of labeled blood cells. Abs against CD4 (mAb 17D; Ref. 20), CD8 (mAb 7C2; Ref. 19), γδ-TCR (mAb 86D; Ref. 21), CD72 (mAb 2-104; Ref. 22), CD21 (mAb 2-87; Ref. 19), CD22 (mAb 135A; Ref. 20), β2 integrin (mAb 101.1; Ref. 23), α4 integrin (mAb 218.1; Ref. 23), T19 (mAb F10-197; Ref. 21), and L-selectin (mAb D1u-29; Ref. 23) were used to stain labeled PBLs and labeled ELLs recovered from peripheral blood or s.c. efferent lymph, respectively. Immunophenotyping was as previously described (19). FITC- and PE-conjugated secondary Abs were used in conjunction with PKH-26- and PKH-2 (or CFSE-) labeled cells, respectively. Cells were fixed in 1% paraformaldehyde in PBS and stored at 4°C until analysis on a FACScan (Becton Dickinson). At least 500 labeled cells and as many as 4000 were analyzed to determine cell phenotypes.

Immunohistology

Animals were sacrificed with T-61 euthanasia solution, and normal (prescapular, popliteal, or prefemoral) SCLNs were excised and frozen on dry ice in OCT mounting medium. Following cryosectioning, sections were reacted with Abs against CD4 (mAb 17D; Ref. 20), CD8 (mAb 7C2; Ref. 19), γδ-TCR (mAb 86D; Ref. 21), CD72 (mAb 2-104; Ref. 19), CD21 (mAb 2-87; Ref. 19), and the peripheral node addressin, PNAd (Meca 79; American Type Culture Collection, Manassas, VA).

Statistics

Multivariate ANOVA using a nonlinear regression analysis is according to Glantz and was performed using the Minitab 10Xtra statistical package for Power Macintosh (24). Normalization for each set of data is described with each figure. In general, it was useful to calculate the percentage of injected cells recovered per unit time, divided by the total number of cells collected, and then normalize per 10⁹ total cells in the sample. This gave a numerical calculation of the percentage of injected cells recovered per 10⁹ cells collected over a given time period, a standard measurement that has been previously described (17).

Results

PBLs recirculate preferentially through SCLNs

By definition, all recirculating lymphocytes must at some time be found either in the blood or the lymph. Although some PBLs do not actively recirculate between the blood and the lymph, the recirculating lymphocytes within the blood should be an unbiased sample of the recirculating lymphocyte pool (19). Therefore, it is possible to assess the relative sizes of the blood-borne homing pools by directly labeling and tracking PBLs. In other words, if the
Previous experiments have indicated that memory CD4<sup>+</sup>gd<sup>-</sup>T cells are the major recirculating population. Previous experiments have indicated that memory CD4<sup>+</sup> T cells are largely responsible for tissue-specific recirculating patterns observed using recirculating ELLs in vivo (23, 26, 27). To test for the possibility that a single lymphocyte subset was responsible for the observed tissue-specific homing patterns of PBLs, labeled lymphocytes were collected from efferent intestinal or s.c. lymph at 12, 24, and 36 h after i.v. injection and phenotyped for CD4, CD8, γδ-TCR, and CD72 (Fig. 2). Of all labeled PBL subsets, only γδ-T cells were recovered in significantly higher numbers from efferent s.c. lymph than efferent intestinal lymph. To quantify this phenomenon, the total recovery of each cell subset over 36 h was calculated and corrected for the total number of cells collected from each lymphatic bed (Table I). Of all lymphocyte subsets analyzed, only γδ-T cells were significantly more likely to migrate through SCLNs than intestinal lymph nodes.

CD4<sup>+</sup> and γδ-TCR<sup>+</sup> T cells home to SCLNs in similar numbers

Two distinct mechanisms could account for the results obtained above. The first mechanism would suggest that γδ-T cells were more likely to cross the tissue endothelium within SCLNs than other lymphocytes, resulting in a higher recovery of this subset. This hypothesis was tested by measuring the ability of lymphocyte subsets to home to SCLNs. By flow cytometry, 3 h after injection of labeled cells, 0.05 ± 0.01% of the injected cells were recovered per 10<sup>9</sup> lymph node cells, but no labeled cells could be detected in the efferent lymph. Previous experiments have clearly demonstrated that the recirculation kinetics of labeled lymphocytes into efferent lymph parallels that seen in efferent lymph, and that virtually no labeled cells can be detected entering the lymph node via afferent lymph at this early time point (28, 29). However, using flow cytometry, sufficient numbers of labeled cells could be clearly differentiated within the lymph node and phenotyped for conventional T and B cell subsets (Fig. 3). There was no significant difference in the percentage of injected CD4<sup>+</sup> T cells recovered per 10<sup>9</sup> lymph node cells (0.05 ± 0.01%, n = 4), the percentage of injected CD8<sup>+</sup> T cells (0.04 ± 0.01%, n = 4), the percentage of γδ-T cells (0.06 ± 0.01%, n = 4), or the percentage of B cells (0.04 ± 0.01%, n = 4) at this early time point. This demonstrated

<table>
<thead>
<tr>
<th></th>
<th>Efferent Lymph</th>
<th>Overall</th>
<th>CD4</th>
<th>CD8</th>
<th>γδ-T Cells</th>
<th>B Cells</th>
</tr>
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<tbody>
<tr>
<td>s.c.</td>
<td>0.13 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.14 ± 0.04</td>
<td>0.08 ± 0.02</td>
<td>0.31 ± 0.09&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.04 ± 0.01</td>
<td></td>
</tr>
<tr>
<td>Intestinal</td>
<td>0.06 ± 0.02</td>
<td>0.09 ± 0.01</td>
<td>0.07 ± 0.02</td>
<td>0.13 ± 0.03</td>
<td>0.02 ± 0.01</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> Percentage of injected cells recovered over 36 h, normalized to the total number of cells collected (percentage of injected per 10<sup>9</sup> total cells collected).

<sup>b</sup> *p < 0.01; significantly different than all other subsets (ANOVA, Student-Newman-Keuls t test).
FIGURE 3. All lymphocyte subsets home to SCLNs in similar numbers in short-term homing assays. Identification of CFSE-labeled ELLs in prefemoral lymph node cells reacted with Abs against CD4, CD8, γδ-TCR, or CD21 (B cells). Nodes were harvested from four animals 3 h after injection of labeled cells, and sufficient total cells were analyzed to obtain a minimum of 500 labeled cells for phenotypic analysis. No labeled cells were detectable in efferent lymph at this time. Numbers not in parentheses represent the percentage of labeled cells in each quadrant as a proportion of total lymphocytes, whereas numbers in parentheses denote the percentage of labeled cells reactive with each Ab. Representative results from one of four animals are shown.

γδ-T cells transit lymph nodes better than other lymphocytes

that both γδ-T cells and CD4⁺ T cells were at least equally competent at entering the parenchyma of lymph nodes via the blood.

A second explanation for the enhanced recovery of peripheral blood γδ-T cells in lymph following i.v. injection would be that all lymphocytes entered the tissue equally well, but γδ-T cells were more likely to enter the lymph than other lymphocytes. To explore this possibility, it was necessary to devise a means to introduce recirculating lymphocytes directly within the parenchyma of a lymph node independent of transendothelial migration. To accomplish this, we cannulated several lymphatic vessels afferent to the popliteal lymph node in the direction of flow. When recirculating lymphocytes harvested from efferent lymph were labeled and infused via the afferent lymphatic, any differences in their proportions relative to the infused population would be due to differences in the rate that they migrated through the lymph node. In several animals, ELLs were separated into two pools and labeled with two differentiable tracking dyes. One population was then injected i.v., and the other was infused intralymphatically. The recovery of labeled cells was then monitored in the efferent lymph and lymph node independent of transendothelial migration. To accomplish this, we cannulated several lymphatic vessels afferent to the popliteal lymph node in the direction of flow. When recirculating lymphocytes were labeled with heparin, all subsets were collected at 6 and 24 h after injection (Fig. 3). As it was necessary to use a small amount of heparin in the lymphatic perfusate, we compared the recovery of i.v.-injected labeled ELLs from normal (n = 4) and heparin-perfused (n = 3) lymph nodes. Although heparin did cause a small increase in the recovery of i.v.-injected lymphocytes (−0.07% per 10⁹ cells), all subsets were similarly affected; therefore, heparin was not responsible for the subset-specific effects described below. When labeled cells were injected intralymphatically, labeled ELLs were always recovered in higher numbers than i.v.-injected cells. Peak recovery of all subsets occurred 24–36 h after either injection or infusion. Although statistical analysis clearly demonstrated that the recovery of either i.v.-injected or intralymphatically perfused lymphocyte subsets differed (p < 0.05, ANOVA), intra-animal variation in the kinetics of recovery made it difficult to isolate a time point when the recovery of one subset differed from the rest. Therefore, we calculated the total recovery of each subset over 36 h and normalized to the total number of cells collected (Table II). Regardless of the method of injection, the recovery of γδ-T cells was always significantly higher than other subsets. Because γδ-T cells were more likely to pass unhindered through a lymph node than other lymphocyte subsets, their histological distribution within lymph nodes would be expected to be significantly different from αβ-T cells or B cells.

γδ-T cells localize to areas of cell traffic within lymph nodes

Lymph nodes were harvested from a number of animals, and the distribution of lymphocyte subsets were compared (Fig. 5A). Few differences were evident in the medulla of the lymph node. However, marked differences were apparent in the cortex. B cells and

Table II. Normalized total recovery of labeled ELLs in lymph 36 h following i.v. injection or intralymphatic infusion

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Overall</th>
<th>CD4</th>
<th>CD8</th>
<th>γδ-T Cells</th>
<th>B Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>i.v. injection (n = 7)</td>
<td>0.21 ± 0.21</td>
<td>0.21 ± 0.21</td>
<td>0.18 ± 0.18</td>
<td>0.37 ± 0.06*</td>
<td>0.15 ± 0.03</td>
</tr>
<tr>
<td>Intralymphatic infusion (n = 6)</td>
<td>1.67 ± 0.53</td>
<td>1.97 ± 0.57</td>
<td>1.79 ± 0.64</td>
<td>2.75 ± 0.81†</td>
<td>1.89 ± 0.88</td>
</tr>
</tbody>
</table>

* Percentage of injected cells recovered over 36 h, normalized to the total number of cells collected (percentage of injected per 10⁹ total cells collected).
†, p < 0.01; *, p < 0.05; significantly different than other subsets similarly injected (ANOVA, Student-Newman-Keuls t test).
expression of L-selectin was significantly higher on γδ-T cells than on any other lymphocyte subset (Fig. 7).

**Discussion**

Tissue-specific lymphocyte recirculation pathways were originally demonstrated in ruminants over 20 years ago. Since that time, a great deal of effort has been directed at uncovering the molecular basis of the process. A major factor in the regulation of tissue-specific migration is the presence of adhesion molecules on the surface of recirculating lymphocytes and complementary vascular addressins on the surface of endothelial cells in different tissues. The presence of these molecules correlates with the differentiation status of lymphocytes, and it has been proposed that memory lymphocytes are primarily responsible for the described tissue-specific recirculation pathways. The significance of these patterns at the physiological level and the relative size of the individual pools remain unclear. When peripheral blood was labeled, any observed homing preference would presumably be due to differences in the relative numbers of lymphocytes homing to either s.c. or intestinal lymph. When this hypothesis was tested, we consistently saw a higher recovery of labeled cells in s.c. efferent lymph than intestinal lymph. Our results indicated either that the extraction of lymphocytes by SCLNs was more efficient than MLNs or that a specific subset of lymphocytes found in peripheral blood was more efficient at recirculating through SCLNs than intestinal lymph nodes. Although there was a slight, if statistically insignificant, increase in the recovery of all lymphocyte subsets in efferent s.c. rather than efferent intestinal lymph, only γδ-T cells were significantly more likely to be recovered in efferent s.c. than in efferent intestinal lymph. The fact that similar numbers of CD4+ T cells migrated through both intestinal and s.c. efferent lymph indicates that the relative sizes of these two homing pools must be of equivalent size in the peripheral blood. Nonetheless, peripheral blood γδ-T cells demonstrated a marked preference to recirculate through SCLNs.

Two observations were particularly interesting. First, the peak recovery of all subsets in either lymph compartment occurred at similar times, indicating similar kinetics for recirculation through...
both MLNs and SCLNs. Second, γδ-T cells were significantly more likely to be recovered in the efferent lymph of SCLNs than MLNs, indicating a marked tissue-specific migration pattern. It has previously been suggested that γδ-T cells demonstrate a marked tissue tropism for epithelial tissues and, therefore, are found in elevated numbers in afferent lymph (11). This hypothesis is based on static measurements of lymphocyte proportions in various tissue compartments, rather than a detailed analysis of the kinetic migration of lymphocyte subsets in vivo as is described here. It is important to remember that afferent lymph input accounts for only 10% of the cells found in efferent lymph and would therefore have to consist almost exclusively of γδ-T cells to account for the number leaving lymph nodes in the efferent lymph each hour (5). Although γδ-T cells are clearly found in elevated numbers in the afferent lymph and likely recirculate more effectively through epithelial tissues than other subsets, our data clearly demonstrate that they also recirculate effectively through ovine SCLNs. Given the clear importance of lymphocyte recirculation to immune surveillance, this implies an important role for the γδ-T cell in the immune response.

Although a great deal of work has been directed toward clarifying the mechanism whereby lymphocytes migrate across vascular endothelium and enter the tissues, it is important to remember that the process of lymphocyte migration involves the blood-to-lymph migration of cells. Relatively little is known regarding the mechanisms involved in regulating the movement of cells through tissues, and even less regarding their entry into lymphatics. Certain studies have investigated the importance of extracellular matrix and soluble molecules in regulating this process, and others have also implied important roles for soluble mediators in regulating the positioning of lymphocytes during inflammation (16, 32, 33, 34). It is assumed that similar processes are involved in positioning lymphocytes within lymph nodes. If little is known regarding the migration of cells through tissues, comparatively less is known regarding their entry into lymphatics. It is unclear as to whether the entry of lymphocytes into lymphatics is an active or a passive process, but it has been demonstrated that specific exit signals govern the migration of lymphocytes out of lymph nodes (35–37). Although recent studies have indicated that lymphatic endothelial cells may express some chemokines related to lymphocyte recruitment, their role in regulating the entry of cells into lymph remain unclear (33). It has been demonstrated that cytokine-activated lymphatic endothelial cells will bind lymphocytes in vitro, and it seems likely that they are capable of influencing the entry of cells into the lymph (38). Future experiments should concentrate on the role of these important cells in lymphocyte recirculation.

When we examined differences in the transit of a similar population of lymphocytes through lymph nodes, several observations were particularly significant. First, the peak recovery of all lymphocyte subsets occurred 24–36 h following infusion. Because this paralleled so closely what was observed following i.v. injection, it seems clear that it takes the average lymphocyte 1 day to migrate through a lymph node. This differs considerably for small molecules, which appear in efferent lymph within minutes of injection into the drainage area of a lymph node (39). Furthermore, we found that γδ-T cells were less likely to be retained within lymph nodes than other subsets. Based exclusively on the increased recovery of γδ-T cells following i.v. injection, it could be argued that the increased contribution of γδ-T cells from the afferent lymph relative to other subsets could explain the data. It is difficult to reconcile this hypothesis with the observation that they also transit lymph nodes more effectively than other subsets when introduced via the afferent lymphatic. It seems clear that γδ-T cells are particularly adept at leaving lymph nodes via the efferent lymph relative to other subsets. This conclusion was further supported by histological data that indicated that γδ-T cells were restricted to areas of recirculation. Previous data has indicated that bovine γδ-T cells may be inefficient at entering lymph nodes, despite expressing high levels of L-selectin (40). Although it is possible that differences exist between cow and sheep γδ-T cells, this seems unlikely. Although this observation may seem to contrast with data presented here, it should be noted that the previous study examined the ability of bovine γδ-T cells to accumulate within murine lymph nodes relative to other subsets, whereas our study measured their recirculation back to the efferent lymph. If γδ-T cells were, in fact, more likely to exit lymph nodes than other subsets, it would be expected that they would accumulate less efficiently within lymph nodes than other T and B cells, as was previously reported. In addition, previous data has shown that the concentration of γδ-T cells was much lower in lymph nodes than in either the afferent or efferent lymph, which suggests some important clues to the function of these cells in vivo (11). Although this cell does not appear to play a major role within normal lymph nodes, it has been found in sites of acute inflammation (41). It seems likely that the γδ-T cell plays a role in immune surveillance in vivo, potentially as an early monitor of stressed cells or tissue damage. Recent data has pointed to γδ-T cells as early responders to such pluripotent mediators as TNF-α (42). The presence of high levels of L-selectin may contribute to its migratory characteristics, making the γδ-T cell an early responder in vivo. Unlike αβ-T cells, ruminant γδ-T cells were, in fact, found to be less efficient at down-regulating L-selectin following activation, which may play a role in their increased ability to transit lymph nodes (40). One consequence of transendothelial migration of lymphocytes is the specific shedding of L-selectin during transit (43). Although no role has been demonstrated for L-selectin in the interaction with lymphatic endothelial cells, it should be stressed that there is virtually no data regarding the regulation of the entry of cells into lymph. It may be that increased levels of adhesion molecules allow the γδ-T cell to more effectively enter the lymphatics than other cell types, contributing to the results described in this paper. In addition to significantly higher levels of L-selectin than all other cells, it was intriguing that γδ-T cells also expressed uniformly high levels of aα and β1 integrin, similar to naive T cells. These high levels of integrins may contribute to the selective interaction of naive T cells and γδ-T cells with extracellular matrix proteins in the lymph node, further aiding their migration in vivo.
These data have clearly indicated the importance of nonvascular components in the regulation of lymphocyte traffic in vivo. Although it was possible that the enhanced ability of γδ-T cells to traffic lymph nodes could have been due to differences in their interaction with extracellular components within the node, it seems more likely that lymphatic endothelium was playing a major role. Future experiments will examine the migration of γδ-T cells during an immune response and investigate the relative importance of specific adhesion receptors on γδ-T cells in regulating their traffic through lymph nodes in vivo. Finally, it will be important to examine the interaction of all lymphocytes with lymphatic endothelial cells. It seems likely that lymphatics may play as important a role in regulating lymphocyte traffic as vascular endothelial cells.

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