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Bovine γδ T Cell Subsets Express Distinct Patterns of Chemokine Responsiveness and Adhesion Molecules: A Mechanism for Tissue-Specific γδ T Cell Subset Accumulation

Eric Wilson,‡ Jodi F. Hedges,† Eugene C. Butcher,* Michael Briskin,‡ and Mark A. Jutila†

Subsets of γδ T cells localize to distinct tissue sites in the absence of exogenous Ag stimulation or development of effector/memory cells. Selective lymphocyte homing from the blood into tissues is controlled by a multistep process involving vascular and lymphocyte adhesion molecules, and G protein-linked chemokine receptors. The role of these mechanisms in the tissue tropism of γδ T cells is still poorly understood. In this study, we demonstrate that a subset of γδ T cells, most of which express an antigenically distinct TCR and are characterized by coexpression of CD8, selectively accumulated in tissues that expressed high levels of the mucosal vascular addressin, mucosal addressin cell adhesion molecule 1. These cells expressed higher levels of α4β7 integrins than other γδ T cell subsets and selectively migrated to the CCR7 ligand secondary lymphoid-tissue chemokine (CCL21). Integrin activation by CCL21 selectively increased CD8⁺γδ T cell binding to recombinant mucosal addressin cell adhesion molecule 1. These results suggest that the tropism of circulating CD8⁺γδ T cells for mucosal tissues is due, at least in part, to selective developmental expression of adhesion molecules and chemokine receptors. The Journal of Immunology, 2002, 169: 4970–4975.

Considerable effort has been devoted to the study of γδ T cells; however, many aspects of this population are still poorly understood. One intriguing characteristic demonstrated in studies with mice, cattle, nonhuman primates, and humans is that discrete subsets of γδ T cells, defined by their TCR usage, specifically accumulate in certain tissues and organs in the absence of exogenous Ag stimulation (reviewed in Ref. 1). For example, mouse γδ T cells associated with the epidermis (2, 3) and the mucosal epithelia of the vagina, uterus, and tongue (4, 5) express distinct combinations of γ- and δ-chains. Other distinct TCR-defined subsets of γδ T cells are found in the blood, spleen, intestine, lung, liver, and mammary glands (reviewed in Ref. 1). The homing and accumulation of γδ T cells is of particular interest, as the presence or absence of TCR-defined subsets of γδ T cells dramatically influences the outcome of some infectious diseases (6, 7). Thus, defining the molecular events that control the selective tissue distribution of these cells is warranted.

Cattle provide a useful model for the study of γδ T cells because, unlike in adult humans, these cells constitute a predominant population of lymphocytes in young animals. One tissue-specific γδ T cell subset in cattle expresses CD8 and CD2, lacks the GD3.5 Ag, and most cells within the population express an antigenically distinct TCR (8, 9, 10) (referred to in this study as CD8⁺γδ T cells). Bovine CD8⁺γδ T cells are found in increased numbers in the spleen, gut lamina propria, and mesenteric lymph nodes (MLN).4 Conversely, other TCR-defined subsets of γδ T cells (which do not express CD8) are found predominantly in blood, peripheral lymph nodes (PLN), and skin (9, 10). The ability to collect large numbers of peripheral blood γδ T cells, as is possible in calves, provides a unique opportunity to study the molecular mechanisms controlling the tissue-specific accumulation of these cells.

Leukocytes are generally recruited from the circulation into tissues through selective interactions with the vascular endothelium. A multistep process mediated through a series of adhesion and signaling molecules present on both lymphocyte and endothelial surfaces is responsible for the slowing, arrest, and transendothelial migration of circulating leukocytes (11). Adhesion molecules (selectins, integrins, and their corresponding ligands) participate in tethering, rolling, and adhesion of lymphocytes. Chemokines can function as critical regulators of lymphocyte homing by triggering integrin-dependent arrest of rolling lymphocytes, as well as subsequent transendothelial migration into tissues (12, 13). Analysis of the role of chemokines in γδ T cell subset homing and accumulation has been limited, due in part, to the small numbers of γδ T cells in the mouse, making functional assays difficult.

In our initial study of primary bovine γδ T cells, we found that most CD8⁺γδ T cells, unlike CD8⁻γδ T cells, do not express L-selectin and E-selectin ligands and do not efficiently home to sites of s.c. inflammation (10). The molecular mechanisms that contribute to the selective homing and accumulation of CD8⁺γδ T cells into mucosal tissues have not been determined. In the current study, we show that the majority of peripheral blood CD8⁺γδ T

4 Abbreviations used in this paper: MLN, mesenteric lymph node; PLN, peripheral lymph node; CCL, CCR ligand; MAdCAM-1, mucosal addressin cell adhesion molecule 1; CHO, Chinese hamster ovary; PBS-HS, CXCL, CXC ligand; RT, reverse transcription.
cells express functionally higher levels of the αβ7 integrin than CD8γδ T cells. Additionally, CD8γδ T cells selectively migrate to the CCR7 ligands secondary lymphoid-tissue chemokine (CCR ligand (CCL21) and macrophage inflammatory protein-3β (CCL19) in vitro chemotaxis assays. CCL21 stimulation also results in significantly increased efficiency of CD8γδ T cell binding to mucosal address cell adhesion molecule 1 (MAdCAM-1), compared with CD8γδ T cells. These results demonstrate that functional differences in adhesion molecule expression and chemokine sensitivity in γδ T cell subsets likely contribute to their unique accumulation patterns in vivo.

Materials and Methods

Animals

Holstein calves were purchased from local producers and housed at the Montana State University Large Animal Facilities at the Veterinary Molecular Biology Laboratory (Bozeman, MT). Cattle used in this study were born calves from 1 to 6 mo of age. Tissue samples were collected from animals upon necropsy and were cut and frozen in OCT freezing medium (TissueTek, Elkhart, IN). For immunohistology, frozen sections were air-dried, fixed in acetone, and stained as previously described (14).

Cells and cell lines

Peripheral blood was collected into sodium heparin anticoagulant tubes by venipuncture and PBMCs were purified by Ficoll-Hypaque (Sigma-Aldrich Co., St. Louis, MO) gradient centrifugation. Lymphocytes were isolated from the thymus, lymph nodes, and spleen by first finely mincing tissues with a razor blade. Samples were then dounced in a tissue homogenizer to release lymphocytes from the stromal elements. Cell suspensions isolated from various tissues were then passed through a layer of Nyrex fabric (Fairmount Fabrics, Hercules, CA) to remove cell aggregates, and the resulting cell preparation was stained for FACS analysis or used in functional assays. Intraepithelial lymphocytes were isolated by exteriorizing the lumen of the small intestine, washing extensively with PBS, and gently scraping the luminal surface with a razor blade. Cell suspensions were then dounced in a tissue homogenizer, and isolated as described above.

Mouse MAdCAM-1 transfected Chinese hamster ovary (CHO) cells and nontransfected CHO cells have been previously described (15).

mAbs used in this study

The following mouse mAbs were used: GD3.8, which recognizes a pan epitope on the bovine γδ TCR (8); GD3.5, which recognizes a lineage-specific Ag on γδ T cells (16); CC58, which recognizes bovine CD8, kindly provided by C. Howard (Institute for Animal Health, Compton, U.K.) (17); MMH23, which recognizes human and bovine CD18 (DAKO, Carpinteria, CA); PV4-101, which recognizes bovine CD29 (VMRD, Pullman, WA); HP2/1, which recognizes CD49d (Serotec, Raleigh, NC); and 7G11, which recognizes human MAdCAM-1 and cross-reacts with ovine and bovine MAdCAM-1 (18). The following mAbs were used: FIB30, a rat anti-β7 integrin mAb that has previously been shown to specifically bind human and mouse β7 integrin (19) and cross-reacts with bovine β7; and MEC367, a function-blocking mAb which recognizes mouse MAdCAM-1 (20). SK208, a rat mAb which recognizes mouse neutrophils (M. A. Jutila, unpublished observations), and EL81 (21), a mouse mAb that recognizes human E-selectin, were used as isotype-matched negative controls (Ref. 10 and 16) in combination with GD3.8 were compared. The GD3.5 mAb distinguishes the inflammatory CD8γδ T cells from the CD8γδ T cell. Specifically, GD3.5 stains CD2 γδ T cells in neonates (10). Greater than 90–95% of the GD3.5 cells are CD8γδ and virtually 100% of these cells express CD2 (10, 16). Stained γδ T cell subsets populations were sorted at 10,000 cells/s on a FACSVantage cell sorter (BD Immunocytometry Systems). Purity of the sorts was confirmed by analysis of the sorted populations on a FACS Calibur.

Chemotaxis assays

Bovine PBMC were analyzed in chemotaxis assays using transwell 24-well tissue culture inserts (5-μm pore size; Corning, Corning, NY) as previously described (16). Briefly, 1 × 10^6 cells were added to each insert in 5 ml of medium. Chemokine preparations, or medium alone, were added to the lower well. All migration assays were done in RPMI 1640 with 10% bovine serum at 37°C plus 10% CO2 for 2 h. Optimal concentrations of chemokines (all from PeproTech, Rocky Hill, NJ) were determined and the following concentrations were used in each experiment: 10–15 nM recombinant human stromal-cell-derived factor-1α (CXC ligand (CCLX1);1); 100 nM recombinant murine CCL21; and 150 nM recombinant murine CCL19. Phenotypic analysis and quantification of the migration of the γδ T cell subsets was determined by a flow cytometric approach, as previously described (24). Briefly, following migration, 50,000 15-μm polystyrene beads were added to each sample as an internal counting standard. The ratio of beads to each lymphocyte subset was determined for the total input cells, and the cells that migrated into the lower chambers, and was used to calculate the percentage of migration of each cell subset. γδ T cell subsets were determined by multicolor FACS analysis, as described above.

MAdCAM-1 binding assay

Bovine peripheral blood was collected and PBMC-purified, as described in Cells and cell lines. Mononuclear cells were then incubated for 24 h at 37°C in T-175 flasks (Nunc, Naperville, IL) containing RPMI medium supplemented with 10% FBS. The nonadherent lymphocyte population was washed in HBSS (Life Technologies, Grand Island, NY) and resuspended at 5 × 10^6 cells/ml in DMEM/BSA before use in functional assays. Adhesion assays were performed in T-25 flasks containing confluent monolayers of MAdCAM-1-transfected or nontransfected CHO cells. Briefly, MAdCAM-1-binding cells were separated from whole lymphocyte preparations by incubating 1 × 10^7 PBMC in 2 ml of medium under constant rotation on a horizontal rotator (30 rotations/min) for 10 min. Unbound cells were decanted and the flasks were washed twice in HBSS. Lymphocytes bound to CHO cells were then eluted by treatment with 2 mM EDTA/PBS. Following elution of adherent cells, polystyrene beads were added to each sample to facilitate counting. The phenotype and number of cells collected from adhesion assays was then determined by multicolor flow cytometric analysis, as described above. In experiments to test the effects of chemokines on lymphocyte adhesion, 100 nM soluble CCL21 or 15 nM CXCL12 (final concentration) was added for the final 2 min of the 10-min incubation. Specificity controls for MAdCAM-1 mediated adhesion of lymphocytes included the MAdCAM-1 blocking mAb MEC367 (50 μg/ml) and the isotype-matched negative control mAb SK208 (50 μg/ml).

mRNA analysis

Bovine PBL were collected by histopaque gradient centrifugation at 1300 × g, and sorted into CD8γδ and CD8γδ γδ T cell populations. Preliminary analysis suggested that mRNA degradation occurred immediately following the sorting procedure (data not shown). Therefore, sorted cells were rested overnight in complete RPMI medium, then treated for 4 h with 5 μg/ml Con A and 1 ng/ml recombinant human IL-2 to stimulate transcription of message down-regulated during the 8-h staining and sorting process. RNA was extracted from 1 × 10^6 cells (pooled from multiple animals) of each population with TRIzol (Life Technologies) according to the manufacturer’s instructions. The RNA was treated with DNase for 1 h.
at 37°C and extracted again with phenol-chloroform (1:1), and precipitated with a 0.1 volume of 5 M NaCl and a 2.5 volume of ethanol. Total bovine PBLs were similarly stimulated and the RNA was extracted for use as template to construct standard curves.

Relative CCR7-specific RNA message in CD8+ γδ and CD8- γδ T cells was quantified by measuring SYBR green incorporation during real-time quantitative RT-PCR using the relative standard curve method (without determining the exact quantity of target RNA). Bovine expressed sequence tags homologous to human and mouse CCR7 sequences were selected. A contig of bovine CCR7 was then constructed from the sequences with accession numbers AW347844, AW447807, AW632094, and BF073678 and the Vector NTI software (Applied Biosystems, Foster City, CA). Bovine CCR7-specific primers were designed using the Primer Express software, and the pair with the lowest penalty was selected (5'-AGCACGTTGAGGCCTTGAT-3', 3'-5'GGCGGATGTGACGAGTTA-3'). Primers specific for bovine 18S RNA (endogenous control) and β2 integrin were similarly designed (5'-5'CCT GCTGGTGTTCACGTCAG-3', 3'-5'GCTGCAGCTGTA GAGGCGAT-3', 18S 5' primer; 5'-TCGAGGGCTGTAATTGGA-3', 18S 3' primer; 5'-CCCAAGATCCACTAGGCTT-3'). The standard curve was constructed using total bovine PBL RNA starting at 4 μl per 20 μl reverse transcription (RT) reaction, diluted serially to 0.25 μl per RT reaction. The RT was performed with Superscript RT and random primers (Life Technologies) according to the manufacturer's protocol. One micro-liter of each RT reaction was subsequently used in the PCR. The PCRs used to generate the standard curve were performed in duplicate, and the γδ T cell subset samples were performed in triplicate. The PCR was cycled and data was collected on the Applied Biosystems GeneAmp 5700 sequence detection system (Foster City, CA) and all calculations were performed as described in user bulletin no. 2 for the ABI PRISM 7700 sequence detection system.

CD8+ γδ T cells accumulate in tissues expressing MadCAM-1

In an effort to determine whether subsets of γδ T cells accumulated in sites expressing MadCAM-1, γδ T cell subset analysis of various mucosal and nonmucosal organs was performed. The percentage of γδ T cells as a function of total lymphocytes varied greatly from tissue to tissue as previously reported (26–29). However, the percentage of CD8+ γδ T cells as a function of total γδ T cells correlated directly with the amount of MadCAM-1 expressed in the tissue (Table I). For example, at sites expressing high levels of MadCAM-1, such as the intestinal lamina propria and the MLN, large numbers of γδ T cells coexpressed CD8. In contrast, in PLN, which contained minimal levels of MadCAM-1, the percentage of CD8+ γδ T cells was similar to that found in peripheral blood (Table I).

Peripheral blood CD8+ γδ T cells express high levels of α4 and β2 integrins

To determine whether CD8+ γδ T cells in the circulation express the MadCAM-1 counter receptor α4β2, FACs analysis of γδ T cell subsets was performed. Multicolor FACs analysis of bovine peripheral blood γδ T cells showed that the fluorescence of the anti-β2 integrin stain on CD8+ γδ T cells (mean 554) was ~1.5 fold higher (p < 0.01) than that on CD8- γδ T cells (mean 322) (Fig. 2). The expression of the α4 integrin on CD8+ γδ T cells was also higher than on CD8- γδ T cells, but expression of the β1 (CD29) and β2 (CD18) integrins did not differ statistically between the two populations (data not shown).

### Table I. MadCAM-1 expression correlates with the CD8+ γδ T cell-γδ T cell ratio in a tissue

<table>
<thead>
<tr>
<th>Tissue</th>
<th>MadCAM-1</th>
<th>γδ T cells (% of total lymphocytes)</th>
<th>CD8+ γδ T Cells (% of total γδ T cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>–</td>
<td>17.8 ± 3.6</td>
<td>20.9 ± 6.4</td>
</tr>
<tr>
<td>Thymus</td>
<td>–</td>
<td>9.6 ± 0.4</td>
<td>28.2 ± 4.3</td>
</tr>
<tr>
<td>MLN</td>
<td>++</td>
<td>3.7 ± 1.7</td>
<td>30.8 ± 5.9</td>
</tr>
<tr>
<td>MLN</td>
<td>+++</td>
<td>2.4 ± 0.7</td>
<td>64.8 ± 9.8</td>
</tr>
<tr>
<td>Intestinal lamina propria</td>
<td>++++</td>
<td>20.2 ± 7.8</td>
<td>63.2 ± 5.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>++</td>
<td>20.0 ± 6.2</td>
<td>50.6 ± 10.0</td>
</tr>
</tbody>
</table>

* MadCAM-1 expression was determined by immunoperoxidase staining and lymphocyte data was generated through multicolor FACs analysis of tissue homogenates.

* MadCAM-1 expression was judged on a scale of no detectable MadCAM-1 (−), occasional positive vessel (±), to the majority of vessels being positive for MadCAM-1 (+++, +++)

* Statistically significant difference compared to peripheral blood (p < 0.01). Data was from five animals.

### Statistical analysis

Results were analyzed using the paired Student t test. Significant p values are indicated.

### Results

#### Distribution of bovine MadCAM-1

The distribution of bovine MadCAM-1 closely resembled the MadCAM-1 distribution described in the mouse (20, 25), with high expression at mucosal sites and minimal expression in peripheral tissues. MadCAM-1 was expressed at high levels on venules in the MLN as well as throughout the ileal mucosa and the lamina propria (Fig. 1). Most PLN examined had undetectable levels of MadCAM-1; however, in some PLNs, minimal expression of MadCAM-1 was occasionally seen in the paracortical area. This observation is similar to the PLN expression of MadCAM-1 described in sheep (18). A considerable amount of MadCAM-1 was expressed in the splenic marginal zone, extending into the red pulp area (Fig. 1). MadCAM-1 expression was not seen in the skin or the thymus (data not shown).
Peripheral blood CD8γδ T cells express higher β7 integrin levels than CD8γδ T cells as determined by three-color FACS analysis. A, Anti-β7 staining on CD8γδ (dotted line) and CD8γδ T cells (solid line). Mean fluorescence of isotype-matched negative control Ab staining was <10 U. B, Pooled data from 12 experiments showing mean fluorescence ± SEM of anti-β7 staining on CD8γδ and CD8γδ T cells. *, Statistically significant difference at p < 0.01.

Peripheral blood CD8γδ T cells preferentially bind recombinant mouse MadCAM-1

To determine whether the different levels of integrin expression on CD8γδ and CD8γδ T cells correlated with functional differences in adhesion, we examined the ability of these subsets to bind MadCAM-1. As expected, a variety of cells in the PBL preparations bound MadCAM-1, including αβ T cells (data not shown), B cells (data not shown), and γδ T cells (Fig. 3). CD8γδ T cells were recovered at a higher frequency (2.5-fold) than CD8γδ T cells (p < 0.01) (Fig. 3), indicating that the differences observed in αβ and β7 expression levels by flow cytometry, resulted in measurable functional differences in the ability of these cells to bind MadCAM-1. However, the overall level of binding of the untreated cells was low, suggesting that only a fraction of the total cells in either population expressed activated αβ7 integrins.

Peripheral blood CD8γδ, but not CD8γδ, T cells migrate to soluble CCR7 ligands

Transwell migration chambers were used to analyze the chemotactic response of bovine γδ T cell subsets to various mouse and human chemokines. Recombinant mouse CCL21 and CCL19 and human CXCL12 were shown to cross-react in the bovine system and mediate the migration of bovine lymphocytes (data not shown and Fig. 4). CXCL12 elicited an equally robust response by both γδ T cell subsets (Fig. 4). In contrast, CCL21 (Fig. 4) and CCL19 (data not shown), which bind the CCR7 chemokine receptor, preferentially stimulated the directional migration of CD8γδ T cells.

CCL21 specifically enhances the adhesion of CD8γδ T cells to MadCAM-1-transfected CHO cells

Chemokine-mediated activation of αβ7 has been shown to result in the firm adhesion of rolling lymphocytes to MadCAM-1 (30–32). Therefore, we next tested whether the minimal αβ7/MadCAM-1 binding shown in Fig. 3 could be augmented by chemokine activation in a γδ T cell subset-specific fashion. The same MadCAM-1 adhesion assay, with the addition of chemokine for the final 2 min of the 10-min adhesion assay, was used. The addition of soluble CXCL12 resulted in an increase of γδ T cell binding to MadCAM-1, with both γδ T cell subsets increasing ~4-fold over the levels recovered from MadCAM-1 binding assays without CXCL12. In contrast, binding assays in which CCL21 was added resulted in a selective ~4-fold increase in the binding of CD8γδ T cells with no increase in the binding of CD8γδ T cells (Fig. 5).

Comparison of mRNA levels for CCR7 and β7 integrin in sorted CD8δ- and CD8γδ T cells

Sorting approaches were established to compare CCR7 and β7 transcript levels in CD8γδ and CD8γδ T cells by real-time quantitative RT-PCR. Initial sorts using anti-CD8 mAb were ineffective (purity of the CD8γδ cells preparation averaged 60%, data not shown). As such, GD3.5 mAb, which recognizes inflammatory CD8γδ T cells (with ~90–95% of the CD3.5− γδ T cells being CD8γδ; Refs. 10 and 16), was used. Use of GD3.5 mAb plus GD3.8 provided sorts that were ~98% pure (data not shown), which were used in real-time RT-PCR analysis of CCR7 and β7 transcripts. The results of the CCR7 and β7 standard curves can be summarized by the slope, y-intercept, and R² values of the resulting lines (y = −5.2486x + 26.373, R² = 0.938 and y = −5.4098x + 13.857, R² = 0.9502, respectively). These values were used to calculate the volume of target RNA in the two γδ T cell subsets. The values were then normalized to 18S by division with the corresponding 18S value and the SD determined. The results indicated that the CD3.5− γδ T cells contained significantly greater amounts of CCR7 transcripts than CD3.5− γδ T cells (~3-fold), even though both populations were sorted and cultured under identical conditions. As another comparison, levels of β7 transcripts were also analyzed in both subsets. CD3.5− γδ T cells had significantly greater amounts of β7 transcripts than CD3.5− γδ T cells (Fig. 6). Because the predominant CD3.5− γδ T cell population is CD8γδ and virtually all CD3.5− γδ T cells are CD8δ− in the animals used.

**FIGURE 2.** Peripheral blood CD8γδ T cells express higher β7 integrin levels than CD8γδ T cells as determined by three-color FACS analysis. A. Anti-β7 staining on CD8γδ (dotted line) and CD8γδ T cells (solid line). Mean fluorescence of isotype-matched negative control Ab staining was <10 U. B. Pooled data from 12 experiments showing mean fluorescence ± SEM of anti-β7 staining on CD8γδ and CD8γδ T cells. *, Statistically significant difference at p < 0.01.

**FIGURE 3.** CD8δ− γδ T cells preferentially bind MadCAM-1-transfected cells. The y-axis represents the percent of input cells bound to a confluent monolayer of CHO cells or MadCAM-1-transfected CHO cells. CD8δ− γδ T cells; CD8γδ T cells. Data represent mean γδ T cell adhesion ± SEM from five experiments. *, Statistically significant difference at p < 0.01.

**FIGURE 4.** CD8δ− γδ T cells preferentially migrate to CCL21 compared with CD8δ− γδ T cells. y-axis represents the percent migration of each cell type to either CXCL12 or CCL21. CD8δ− γδ T cells; the migration of CD8δ− γδ T cells. Mean ± SEM from seven experiments are shown. *, Statistically significant difference at p < 0.01.
in this study, we conclude that the differences in transcript levels were due to differences in the CD8⁺ and CD8⁻ subsets, which is consistent with the flow cytometric analysis reported above.

Discussion

Our results suggest that the differential expression of adhesion molecules and chemokine receptors contribute to subset-specific accumulation of γδ T cells. Although tethering and rolling of leukocytes on the endothelium is an essential first step in the migration process, ultimately, the migration of a cell requires chemokine-mediated integrin activation, leading to tight adhesion and finally migration along a chemotactic gradient. The chemokine CCL21 is of particular interest in that CCL21/CCR7 interactions have been shown to play a pivotal role in the migration of T cells into secondary lymphoid organs (33–35). Furthermore, CCR7 ligands have been shown to induce rapid firm adhesion of lymphoid cells rolling on MAdCAM-1 in vitro and in vivo (30, 31). Our data demonstrate that CCL21 selectively mediates chemotaxis and augments MAdCAM-1 binding of CD8⁺γδ T cells which provides a mechanism to explain the preference of this cell population for MAdCAM-1 expressing secondary lymphoid tissues. Indeed, increased frequency of CD8⁺γδ T cells correlates directly with high levels of MAdCAM-1 in Peyer’s patch and MLN. Although some CD8⁺γδ T cells express high levels of αβ2 and small numbers specifically bind to MAdCAM-1 transfectants, the addition of CCL21 to binding assays does not increase the number of CD8⁺γδ T cells binding MAdCAM-1. Thus, as a population in circulation and in the absence of active immunization/Ag challenge, CD8⁺γδ T cells express the necessary chemokine receptors and adhesion molecules that likely direct their homing to MAdCAM-1 expressing secondary lymphoid tissues. The finding that CD8⁺γδ T cells are found in increased numbers in the lamina propria, a site which has not been shown to express CCL21, suggests that other chemokines may play a role in the homing of these cells to some tissues. The chemokines thymus-expressed chemokine (CCL25) and mucosae-associated epithelial chemokine (CCL28) which have been shown to be expressed within the small and large intestines (36–38) or more globally expressed chemokines, such as CXCL12, may play a role in CD8⁺γδ T cell homing to nonsecondary lymphoid mucosal tissues.

Analysis of RNA levels shows that the differences in integrin and chemokine receptor expression in CD8⁺ and CD8⁻γδ T cells (as defined by the GD3.5 mAb) are likely controlled at the level of gene transcription. These results, in addition to our earlier studies (10), suggest that the differentiation process leading to the segregation of the CD8⁺ and CD8⁻γδ T cell populations, leads to dramatic gene regulation events that alter the function of both populations. An ongoing functional genomic analysis of each subset supports this view and suggests that over 300 genes are differentially regulated in these subsets. The genes expressed by CD8⁺γδ T cells are consistent with an inflammatory or activated phenotype, whereas, of the genes expressed by CD8⁻γδ T cells are anti-inflammatory (N. Meissner, J. Hedges, M. A. Jutila, unpublished observations). The selective chemokine receptor and adhesion molecule profiles of these subsets likely ensures that the subset with the appropriate functional activity is delivered to the “right” tissue. The factors that regulate the gene expression patterns leading to the trafficking phenotypes of the CD8⁺ and CD8⁻γδ T cells are currently under investigation.

Although no differential adhesion molecule or chemokine receptor expression has been previously described for human γδ T cell subsets that account for tissue-specific accumulation, Glatzel et al. (39) have recently shown that the chemokine receptors CCR5 and CXCR1 are differentially expressed on Vδ2 and Vδ1 cells. In their study, the authors showed that CCR5 is expressed predominantly on CD45RO Vδ2Vγ9 cells and suggested that these cells represent a population of phospho-Ag-activated effector/memory cells, rather than naive γδ T cells. Although CXCR1 is expressed predominantly on Vδ1 cells (39), a previous study, which analyzed total γδ T cell migration, did not show a response to the CXCR1 ligand IL-8 (40). However, Vδ1 cells typically constitute a small minority of γδ T cells in the peripheral circulation, thus additional work is needed to determine whether CXCR1 mediates specific migration of these cells. In preliminary experiments, we analyzed the migration of circulating adult human CD8⁺ and CD8⁻γδ T cells in response to CCL21 and did not detect differences in the migration of the two subsets. Perhaps just as importantly, differences in αβ expression were likewise not detected. Additionally, there are differences in the type of CD8 molecule found on γδ T cells from adult humans vs those in newborn ruminants, with respect to the type of CD8 molecule they express, which likely impacts these results. In humans, many CD8⁺γδ T cells express the CD8αα homodimer (41); whereas, most circulating CD8⁺γδ T cells in young cattle express the CD8αβ heterodimer (42). Thus, if a human correlate to the results reported in this study exists, it may reside in a subset of the overall CD8⁺ pool of γδ T cells (those that express the CD8αβ heterodimer, for example) or at different stages of development (i.e., in neonates). In support of this possibility, we have found that in human γδ T cells expanded in vitro by the addition of phospho-Ag preparations from Mycobacterium sp, plus IL-2/IL-15, the CD8⁺ percentage increases and these cells express higher levels of β, than CD8⁻γδ T cells (J. F. Hedges and M. A.
Jutila, unpublished observations). It may be that human CD8αγδ T cells, which selectively respond to certain infectious agents and/or are defined by specific Vγδ and/or Vδ6 usage, are phenotypically similar to CD8+γδ T cell in calves.

In conclusion, through the use of functional analyses of primary cells, we have demonstrated that γδ T cell subsets exhibit functional differences in respect to both the adhesion molecules expressed on their cell surface and their responsiveness to chemokines. The functional differences described in this study show clear differences in γδ subsets that may result in differential regulation of these cells at each step of the multistep model of lymphocyte homing.

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References