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CD8 T Cells Activated in Distinct Lymphoid Organs Differentially Express Adhesion Proteins and Coexpress Multiple Chemokine Receptors

Andrew R. Ferguson and Victor H. Engelhard

Previous work from this laboratory showed that generation of memory CD8 T cells by different immunization routes correlates with control of tumors growing in distinct sites. We hypothesized that effector CD8 T cell expression of adhesion proteins and chemokine receptors would be influenced by activation in different secondary lymphoid organs. In this report, CD8 T cells were activated by immunization with bone marrow-derived dendritic cells via i.p., i.v., or s.c. routes. Three distinct populations of activated CD8 T cells arise in mesenteric, axillary/brachial, and mediastinal lymph nodes and spleen based on differential expression of α4β7 integrin, E-selectin ligand, and α4β1 integrin, respectively. In contrast, three subsets of CD8 T cells defined by differential expression of P-selectin ligand and chemokine receptors were induced irrespective of activation site. The majority of activated CD8 T cells expressed CXCR3, with one subset additionally expressing P-selectin ligand, and another subset additionally expressing CCR3, CCR4, CCR5, CCR6, and CCR9. In the mesenteric lymph node, a fourth subset expressed CCR9 and CXCR3 in the absence of CCR5. Similar homing receptor profiles were induced in the same sites after localized vaccinia immunization. Homing receptor expression on CD8 T cells activated in vivo was distinct, revealing influences of both dendritic cells and the lymphoid microenvironment. Collectively, these results identify previously undescribed populations of activated CD8 T cells based on adhesion protein expression and coexpression of chemokine receptors that arise after activation in distinct secondary lymphoid organs. The Journal of Immunology, 2010, 184: 4079–4086.

The process of T cell activation in secondary lymphoid organs (SLOs) is accompanied by the upregulation of several different adhesion proteins and chemokine receptors associated with recruitment to sites of inflammation in peripheral tissues (1). T cell migration to gut associated tissue and peripheral skin tissue occurs via distinct homing receptors. The α4β7 integrin and CCR9 are associated with specific homing of CD4 (2, 3) and CD8 (4, 5) T cells to the gut, and the ligands for P-selectin (PSL) and E-selectin (ESL) have been associated with CD4 (6, 7) and CD8 (8, 9) T cell homing to the skin. CD4 T cell homing to the skin is partially dependent on CCR4 (10, 11), but the role of this receptor in mediating CD8 T cell homing to the skin is unknown. In contrast, the α4β1 integrin and the chemokine receptors CXCR3, CCR5, CCR3, and CCR6 have been associated with homing of CD4 (12, 13) and CD8 (14–20) T cells to multiple inflamed sites. Although expression of several chemokine receptors has been associated with entry into peripheral tissues, the extent to which they are expressed coordinately or independently of one another on the same CD4 T cells has not been comprehensively evaluated (21–23), and has not been previously analyzed on CD8 T cells activated in vivo.

Several studies have sought to understand how the site of T cell priming influences their expression of homing receptors. Initial studies analyzing CD4 T cells demonstrated that T cells activated in mucosal and skin sites differentially express α4β7 and PSL (24). Some of these have analyzed the induction of homing receptors on CD8 T cells activated in vitro by resting dendritic cells (DCs) isolated from different SLO. DCs from skin draining lymph node (LN) were found to induce ESL and PSL, but not α4β7 or CCR9 (11, 25, 26). Conversely, Peyer’s patch or mesenteric (mes) LN DC induced α4β7 and CCR9 with minimal induction of ESL and PSL. Based on chemotaxis assays, expression of CCR2, CCR4, and CCR5 by these two populations of activated CD8 T cells appeared similar (11, 25). A possible limitation is that these studies used resting DCs, whose ability to induce homing receptors might differ from that of mature DCs. These in vitro data were complemented by in vivo studies. CD4 T cells activated in mes LN and cutaneous peripheral LN expressed α4β7 and PSL in a mutually exclusive fashion (24). In addition, CD4 T cells activated in both compartments migrated to a CXCR3 ligand, whereas only cells activated in mes LN migrated to a CCR9 ligand. Similarly, CD8 T cell effectors generated by s.c. immunization with bone marrow-derived DCs (BMDCs) expressed ESL and CCR4 but not α4β7, whereas those generated by i.v. BMDC immunization expressed α4β7 with minimal ESL and lower levels of CCR4 (27, 28). Blood-borne CD8 T cells generated by i.v. BMDC immunization expressed CCR4 on a subset of cells, and failed to express either ESL or α4β7. Similarly, CD8 T cells activated in mes LN by i.p. injection of vaccinia virus acquired α4β7, but not ESL or PSL, whereas those activated in inguinal LN by s.c. viral injection expressed ESL and PSL, but not α4β7 (16). These studies collectively support the concept that CD8 T cells activated in gut and skin draining SLO differentially express α4β7 compared with ESL and PSL. However, the adhesion proteins induced in other SLO remain incompletely described. In contrast, the induction of most chemokine receptors on activated CD8 T cells remains poorly described.
both in vitro and in vivo. Finally, the influence of activating CD8 T cells in the same SLO using different immunogens has not been directly compared.

A previous report from this laboratory demonstrated that BMDC immunization generated tumor-specific CD8 T cell memory that provided protection against the outgrowth of subsequently injected melanoma cells (29). Interestingly, the route of DC immunization dictated the ability to control tumors in different locations: s.c., but not i.v., immunization protected against s.c. tumor challenge, whereas i.v. immunization conferred significantly greater protection against lung metastases. We subsequently determined that i.v. immunization resulted in BMDC accumulation and activation of CD8 T cells only in the mediastinal (med) LN and spleen (29–32), whereas s.c. immunization in the scapular fold lead to BMDC accumulation and activation of CD8 T cells only in the axillary/brachial (ax/brach) LN (29, 30, 32). Conversely, i.p. immunization only activated CD8 T cells in the mes and med LN (31, 32). Expression of high levels of α4β7 was confined to those cells activated in mes LN, whereas α4β1 was expressed on T cells activated in both mes and med LN and spleen (31). However, neither the expression of chemokine receptors and selectin ligands on CD8 T cells activated in the mes LN, med LN, and spleen or the expression of homing receptors induced in the ax/brach LN after s.c. immunization were examined.

In the current work, we have conducted a more comprehensive evaluation of the homing receptor profiles induced on CD8 T cells activated in different compartments, and compared both BMDC and vaccinia virus immunogens. Our results extend earlier observations of differential adhesion molecule expression to define a new population of vaccinia virus immunogens. Our results extend earlier observations of differences in the homing receptor profiles induced on CD8 T cells activated in the mes LN, med LN, and spleen (31).

Materials and Methods

Mice, viruses, and viral infection

C57BL/6, OT-I RAG1−/−, and C57BL/6 Thy-1.1 mice were obtained from Charles River Laboratories (Wilmington, MA), Taconic Farms (Germantown, NY), and The Jackson Laboratory (Bar Harbor, ME), respectively. OT-I Thy1.1 mice were first generation crosses of OT-I RAG1−/− and C57BL/6 Thy-1.1 mice. All animals were maintained in pathogen-free facilities. Recombinant vaccinia virus expressing vaccinia-ova (OVA) was a kind gift from Dr. J. Yewdell (National Institute of Allergy and Infectious Diseases). All protocols were approved by the Institutional Animal Care and Use Committee.

Adaptive transfer of OT-I cells

Single-cell suspensions from spleen and pooled inguinal, ax/brach, cervical, mes, and med LN of OT-I Thy1.1 mice were labeled with 5 μM CFSE (Molecular Probes, Eugene, OR) in PBS/0.1% BSA for 15 min at 37°C prior to injection. One to two days before immunization, the indicated number of OT-I cells were injected i.v. into the dorsal tail vein of sex-matched recipients.

Immunogens and immunizations

CD40L-activated BMDC were generated as previously described (33). Prior to injection, BMDC were pulsed with 10 μM OVA257 (corresponding to residues 257–264 of chicken OVA) for 1 h at 37°C. Mice were immunized with 105 BMDC or 105 PFU OVA in 100–200 μl into the dorsal tail vein (i.v.), the peritoneal cavity (i.p.), or the scapular fold (s.c.).

FTY720 treatment

Mice were injected i.p. with 1 mg/kg FTY720 (a generous gift of Dr. V. Brinkmann [Novartis Pharma AG]) in 200 μl 48 h after adoptive transfer of OT-I cells (24 h after BMDC immunization) and then every 24 h until harvest.

In vitro activation of CD8 T cells by BMDC

OT-I cells were labeled with 1 μM CFSE in PBS/0.1% BSA for 15 min at 37°C. Cells (2.5 × 106) were plated in 96-well round bottom plates together with 2.5 × 104 CD40L-activated BMDC and cultured for 5 d before staining for FACS analysis.

Flow cytometric analysis of surface markers

Single-cell suspensions were incubated with anti-CD16/32 (eBioscience, San Diego, CA) or 5% normal rat serum to block Fc receptors. PerCP anti-CD90.1, APC-Alexa-750 anti-CD8α, Alexa-647 anti-α4, PE anti-α4 integrin, PE anti-α4β7, biotin anti-α4β7, PE-Cy7 anti-CD62L, biotin anti-CCR5, and biotin anti-CCR9 were from eBiosciences. PE-Cy7 anti-CD43 (clone 1B11), and alexa-647 anti-CCR3 was from Biologend (San Diego, CA). PE anti-CCR5 was from BD Pharmingen (San Jose, CA). PE anti-CXCR3, PE anti-CCR6, E-selectin fusion protein, and P-selectin fusion protein were from R&D Systems (Minneapolis, MN). Fusion proteins were detected using a PE anti-human IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Anti-mouse CCR4 (Caprolactics, Hardwick, MA) was detected using PE anti-goat IgG (Jackson ImmunoResearch Laboratories). Viability assay for in vitro activated cells was performed using LIVE/DEAD stain (Invitrogen, San Diego, CA). Samples were analyzed on FACSCalibur and FACScanto instruments (Becton Dickinson, San Jose, CA) using FlowJo software (Treestar, Ashland, OR).

Results

Expression of adhesion proteins is restricted to distinct SLO

To examine the differentiation of CD8 T cells in different SLO, we adaptively transferred 105 OT-I cells and immunized the recipients with BMDC that had been pulsed with the OVA257 peptide. FTY720 was administered to these recipients in order to deplete leukocytes.

FIGURE 1. Adhesion protein expression on CD8 T cells is restricted to distinct sites of activation. The 105 Thy 1.1+ OT-I cells were adoptively transferred into Thy 1.2+ C57BL/6 mice 1 d prior to immunization with 105 BMDC via the indicated route. One day after immunization, mice were treated with FTY720 daily until SLO injection. One to two days before immunization, the indicated number of OT-I cells were injected i.v. into the dorsal tail vein of sex-matched recipients.
was administered daily beginning 1 d after BMDC immunization as this drug causes retention of effector cells that would otherwise have left the LN (32), providing a complete assessment of cells activated in the priming LN. The induction of adhesion proteins was evaluated 4 d after BMDC immunization on CD8 T cells that were activated in different SLO and had undergone at least three cell divisions. These cells were CD62L(Low) (Fig. 1) suggesting that these cells were fully differentiated (34). Consistent with previously published data (31), a substantial fraction (45.5 ± 2.6% [n = 6]) of divided CD8 T cells activated in the mes LN by i.p. immunization with BMDCs expressed the α4β7 integrin (Fig. 1). Because the expression of α4β7 is detected by differential staining with anti-α4 and anti-αβ7, it was not possible to ascertain whether α4β7* cells activated in the mes LN were also α4β7*. However, a smaller fraction of activated CD8 T cells in the mes LN are α4*, α4β7*αε, and thus are α4β7* (Fig. 1). Few cells bound the E-selectin fusion protein (12.2 ± 10.6% [n = 8]) or expressed the altered glycoform of CD43 that is recognized by the Ab IB11 (13.2 ± 9.1% [n = 5]) and can act as an ELSL (35) (Fig. 1). However, a substantial fraction of activated CD8 T cells expressed PSL (Fig. 1, Table I). Although about half of the PSL* activated CD8 T cells coexpressed α4β7, only a third of the α4β7* cells coexpressed PSL (Fig. 2). This suggests that differentiation to express PSL or α4β7 were not mutually exclusive outcomes of T cell activation in mes LN, and reexpressed α4β7 expression as the dominant phenotype. CD8 T cells activated in inguinal LN after s.c. immunization in the flank showed similar patterns of expression of all of these adhesion proteins (data not shown). Therefore, CD8 T cells activated in skin draining LN results in a dominant population that expresses ESL and smaller populations coexpressing α4β1 and/or PSL.

Overall, the same major phenotypes were evident in the presence or absence of FTY720 and over a range of adoptively transferred numbers (10^3–10^6, data not shown). Collectively, these data establish that CD8 T cell activation in mes LN, med LN/spleen, and skin-draining LN leads to three dominant populations that differentially express α4β7, α4β1, and ESL/1B11, respectively.

Similar chemokine receptor profiles are induced on CD8 T cells irrespective of priming site

Based on the differential expression of adhesion proteins on CD8 T cells activated in different SLO, we wanted to determine whether there was a similar selectivity in the induction of chemokine

Table I. Homing receptor induction on CD8 T cells activated in distinct lymphoid organs

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<td>32 ± 2%</td>
<td>31 ± 9%</td>
<td>ND</td>
</tr>
<tr>
<td>CCR4 (n = 6)</td>
<td>30 ± 14%</td>
<td>36 ± 14%</td>
<td>30 ± 13%</td>
<td>42 ± 26%</td>
<td>8 ± 10%</td>
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<tr>
<td>CCR5 (n = 21)</td>
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<td>32 ± 14%</td>
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<tr>
<td>CCR6 (n = 15)</td>
<td>33 ± 10%</td>
<td>41 ± 15%</td>
<td>35 ± 13%</td>
<td>38 ± 17%</td>
<td>4 ± 3%</td>
</tr>
<tr>
<td>CCR9 (n = 17)</td>
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Mice were treated as described in Fig. 3. Values in parentheses indicate the number of mice evaluated for each receptor.

*AT only is the average of mes, med, and ax/brach LN, and spleen for all homing receptors, except CCR9.

#p < 0.05 compared with other lymphoid organs.

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#p < 0.05 compared with other lymphoid organs.

Average of med LN, ax/brach LN, and spleen.

Average of mes LN.
receptors. However, we observed that most chemokine receptors were expressed similarly regardless of activation sight. The majority of activated cells in all SLO expressed CXCR3 (Fig. 3, Table I). In contrast, CCR3, CCR4, CCR5, and CCR6 were all induced on much smaller subsets, although comparably in each SLO (Figs. 3, 4, Table I). We failed to detect any induction of either CXCR4 or CCR6 on activated CD8 T cells in all SLO (data not shown). Interestingly, we also observed CCR9 induction in all SLO, although the percentage of cells expressing CCR9 was clearly elevated in mes LN (Table I). These expression patterns were consistent over a range of adoptively transferred OT-I cells (data not shown). These results indicate that, in contrast to the adhesion proteins, induction of these chemokine receptors was not restricted to particular SLO.

Definition of CD8 T cell subsets based on coexpression of chemokine receptors and PSL

The expression of CCR3, CCR4, CCR5, and CCR6 on activated CD8 T cells was consistent with the possibility that all of these receptors were coexpressed. Therefore, we evaluated the extent to which cells expressing CCR5 also expressed CCR3, CCR4, and CCR6. Regardless of the LN examined, all activated CD8 T cells that expressed CCR5 also expressed these other chemokine receptors (Fig. 4). The coexpression of these chemokine receptors suggested that these cells might represent more differentiated effector cells. Consistent with this possibility, activated CD8 T cells expressing CCR5 also expressed PSL (Fig. 4). However, a proportion of PSL+ cells did not express CCR5. These results establish the existence of three subpopulations of activated cells in each SLO based on chemokine receptor expression: one expressing only CXCR3, one additionally coexpressing PSL, and one additionally coexpressing CCR3, CCR4, CCR5, and CCR6.

Based on the reported involvement of CCR9 in the selective migration of activated CD8 T cells to the small intestine (36), we were interested in understanding whether it was coexpressed with other chemokine receptors. In all LN examined, we found that the chemokine coexpressing population defined using CCR5 also coexpressed CCR9 (Fig. 4). However, in mes LN, we found an additional population that was CCR9+, but CCR5−. Furthermore, these cells expressed CXCR3. The presence of this additional population accounts for the elevated level of CCR9+ cells in mes LN compared with other SLO (Table I).

Induction of homing receptor expression by BMDCs in vitro

To examine whether the induction of multiple chemokine receptors in all SLO might be a consequence of the intrinsic programming capability of BMDCs, we analyzed their expression on CD8 T cells activated with BMDCs in vitro. A small fraction of T cells cultured in the absence of BMDC expressed low levels of CXCR3, CCR4, CCR5, CCR6, and CCR9 (Fig. 5, data not shown). The percentages of cells expressing CXCR3 and CCR9 were modestly increased in the presence of BMDC. In contrast, CCR4, CCR5, nor

FIGURE 3. Activated CD8 T cells express the same profile of chemokine receptors irrespective of the site of activation. Mice received 10⁶ OT-I cells and immunized as described in Fig. 1. Lymphocytes were gated for CD8⁺Thy1.1⁺ cells and analyzed for CFSE and the indicated chemokine receptor.

FIGURE 4. Coexpression of chemokine receptors and adhesion proteins on CD8 T cells activated in distinct SLO. Mice were treated as described in Fig. 3. CD8⁺Thy1.1⁺ lymphocytes that had undergone three or more divisions (as determined by CFSE dilution) were gated and analyzed for the indicated proteins.
CCR6 was induced on CD8 T cells activated in vitro by BMDCs. These results suggest that the in vivo expression of all these chemokine receptors on CD8 T cells is not a result of the intrinsic programming capability of BMDCs, but depends on additional factors in the lymphoid microenvironment.

We were also interested in how the expression patterns of adhesion proteins in different SLO in vivo were related to the intrinsic programming capability of BMDCs. Consistent with previous results (26, 31), α4 was induced on CD8 T cells activated by BMDCs in vitro, whereas α4β7 was not (Fig. 5). We expanded these results to include 1B11, which was expressed at a high level on most divided cells. Despite this, none of the activated cells expressed ESL (Fig. 5). PSL was induced on T cells cultured in vitro in the absence of DC stimulation making an examination of the induction of PSL unfeasible (data not shown). The lack of ESL and α4β7 induction in vitro contrasts with their selective induction in vivo, suggesting that induction is due to microenvironmental factors in specific LN that directly or indirectly augment the programming capabilities of BMDCs. Conversely, the absence of α4 expression on most CD8 T cells activated in the ax/brach LN, and the lack of 1B11 expression on cells activated in the mes LN, med LN, and spleen, demonstrates the existence of microenvironmental factors that suppress the intrinsic ability of BMDCs to induce these proteins on activated CD8 T cells.

**Homing receptor profiles induced by vaccinia immunization**

The in vitro results previously described demonstrate that the expression of homing receptors in vivo after BMDC immunization reflected a combination of the programming capabilities of BMDCs together with microenvironmental influences in the SLO. To compare the programming capabilities of BMDCs with those of endogenous DCs, we immunized OT-I adoptively transferred mice via i.v., i.p. or s.c. routes with Vac-OVA. As with BMDC immunization, mice were also treated with FTY720 and analyzed 4 d later. As was seen in mice immunized with BMDCs, substantial fractions of cells activated in the mes LN, med LN, and ax/brach LN by OVA expressed α4β7, α4β1, and ESL, respectively (Fig. 6). Similarly, in each SLO a subset of activated CD8 T cells expressed PSL (Fig. 6). CCR4, CCR5, CCR6, and CCR9 were expressed on subsets of cells activated in all SLO that were comparable in size to those elicited by BMDCs, with the subset of cells expressing CCR5 entirely included within the PSL + subset (Fig. 6, data not shown). However, in contrast to CD8 T cells activated by BMDCs, a higher percentage of those activated by OVA in ax/brach LN expressed α4β1 (Fig. 6). In addition, a population of cells activated in mes LN and med LN expressed 1B11, although few of these also expressed ESL compared with cells activated in the ax/brach LN. These results demonstrate that the patterns of homing receptors induced by BMDCs and by endogenous DCs targeted by a virus are very similar.
T cells activated in vitro by DCs from Peyer’s patches (25) or mes immunization with BMDCs (27, 28) and of CD8 T cells activated in vitro by DC from skin draining LN (25–27). However, we also identified a distinct differentiation pathway of CD8 T cells activated in the mes LN and spleen after i.v. BMDC or vaccinia immunization, which results in uniform expression of α4β7, but limited expression of α4β7 or ESL. Previous work demonstrated cells arising from i.v. BMDC immunization failed to accumulate in the gut (31), were unable to mediate skin contact hypersensitivity (27), and could not control s.c. tumor outgrowth (29). However, i.v. BMDC immunization elicited CD8 T cells that were able to protect mice against outgrowth of tumors in their lungs (29). Our results suggest that these functional properties are based on a homing receptor phenotype that is programmed in mes LN and spleen, which is distinct from that programmed in either skin draining LN or mes LN.

ESL and α4β7 are not induced on CD8 T cells activated in vitro. Thus, their induction in only certain LN in vivo exemplifies the positive influences the lymphoid microenvironment can exert on homing receptor programming. In contrast, an apparent suppressive influence of the lymphoid microenvironment on the homing receptor profiles induced by BMDCs was evident in the reduced expression of α4β1 on CD8 T cells activated in skin-draining LN, and the reduced expression of 1B11 on cells activated in mes and med LN and spleen. In both cases, this in vivo constraint was relieved when vaccinia was used as an immunogen instead of BMDCs. In skin-draining LN, the extent of cell division and the expression of chemokine receptors and ESL on

Discussion

The migration of activated CD8 T cells requires the expression of adhesion proteins and chemokine receptors to enable entry into peripheral tissues and tumors. In the current study, we examined the expression of homing receptors on CD8 T cells after in vivo activation in different lymphoid compartments by two different immunogens. Our experiments establish that activation of CD8 T cells by BMDCs in mes LN, med LN/spleen, and skin draining LN results in three dominant populations based on differential expression of α4β7, α4β1, and ESL. In contrast, regardless of SLO, most activated CD8 T cells expressed CCR3, with a subset additionally expressing PSL, and a subset additionally expressing CCR3, CCR4, CCR5, CCR6, and CCR9. The expression of adhesion proteins and chemokine receptors on activated CD8 T cells after vaccinia immunization was similar to that observed after BMDC immunization, indicating similar site-dependent and site-independent homing receptor profiles are induced by both endogenous and exogenous DCs. Collectively, these results identify previously undescribed populations of activated CD8 T cells based on adhesion protein expression and coexpression of chemokine receptors that arise after activation in distinct SLO.

Our observation that high levels of α4β7 are selectively expressed on CD8 T cells activated in mes LN is consistent with previous in vivo data analyzing CD8 effectors generated by i.p. immunization with BMDCs (27, 28) as well as the phenotype of T cells activated in vitro by DCs from Peyer’s patches (25) or mes LN (4). Similarly, our observation that ESL is selectively expressed on CD8 T cells activated in skin draining LN is consistent with the phenotype of CD8 T cells generated by s.c. immunization with BMDC (27, 28) and of CD8 T cells activated in vitro by DC from skin draining LN (25–27).

FIGURE 6. Vaccinia infection induces similar homing receptor profiles based on site of CD8 T cell activation. 10^6 Thy 1.1^ CFSE labeled OT-I cells were adoptively transferred into Thy 1.2^ C57BL/6 mice 1 d prior to immunization with OVA via the indicated route. Lymphocytes from the indicated SLO were gated for CD8^Thy1.1^ cells and analyzed for CFSE and the indicated proteins or analyzed cells that had undergone 3 or more divisions for the indicated proteins.

Our demonstration that PSL is expressed on significant fractions of CD8 T cells activated in every SLO by both BMDCs and vaccinia virus is at odds with previous work suggesting it is not induced on T cells activated in vitro by DCs from Peyer’s patches (25) or in mes LN by i.p. with vaccinia virus (16) or intralymphatic injection of BMDCs (28). This discrepancy may reflect the fact that these studies analyzed T cells at an earlier time point. In addition, we found the percentage of cells expressing PSL in mes LN was generally less than that in ax/brach LN, although this difference was barely below the level of statistical significance. However, our observation is
consistent with the idea that PSL is less tissue selective in T cell homing than ESL. In support of this, P-selectin itself is expressed by a much broader array of inflamed peripheral tissues than E-selectin (41). Collectively, our results support the importance of different lymphoid microenvironments in dictating the expression of α4β7 and ESL on activated CD8 T cells, whereas expression of PSL is not limited to distinct SLO.

An unexpected finding from these studies was the induction of CCR9 on activated CD8 T cells in all SLO in response to multiple immunogens. This contrasts with previous reports demonstrating that CCR9 is expressed by CD8 T cells activated in vivo in mes LN and not peripheral LN (28, 36) and after activation in vitro by DCs from gut draining SLO, but not skin draining LN (4, 25, 26). However, our results are in keeping with the demonstration that CD8 T cells activated in vitro by BMDCs express CCR9 (37) and that s.c. BMDC immunization elicits CCR9+ memory CD8 T cells (26). These discrepancies may reflect differences in the activation state of the DCs. In keeping with this possibility, the use of LPS dramatically increases CCR9 expression compared with OVA alone indicating the activation state of the DC can have an impact (42). How the activation state of DC compares between BMDCs activated by CD40L, vaccinia infection, and LPS remains unclear as the quality of activation in the peripheral LN in these experiments was not addressed. Nevertheless, our data demonstrate the expression of CCR9 by CD8 T cells activated in multiple sites.

Another surprising finding from this study was the extent to which multiple chemokine receptors were coexpressed on a subset of CD8 T cells activated in vivo. Previous work has associated the independent expression of CXCx3CR, CCR4, and CXCxR3 with functionally distinct CD4 T cell subsets (22), but also documented the coordinate expression of CCR4, CCR6, and CCR10 (21). Although previous studies established that CD8 T cells express CCR4, CCR6, CCR9, and CXCxR3 after BMDC immunization, the coexpression of these receptors on the same CD8 T cell was not evaluated (11, 27, 43). Our demonstration of the coexpression of six different chemokine receptors on a subset of activated CD8 T cells, with minimal evidence of cells expressing single chemokine receptors other than CXCxR3 and CCR9, suggests that the control of CD8 T migration will be largely dependent on the selective expression of chemokines by target tissues, rather than selective imprinting. Collectively our results suggest a model in which the site of CD8 T cell activation influences migratory potential through the selective induction of adhesion proteins, whereas chemokine receptor expression is less selective and potentially dependent on DC activation state.

The impact of adhesion proteins and chemokine receptors on migration of the CD8 T cells to peripheral nonlymphoid tissue remains to be explored. Of particular interest is the necessity of these proteins for the infiltration of CD8 T cells into tumors. An important component of future studies is the role of these homing receptors in CD8 T cell infiltration of tumors in different sites of the body. Understanding the homing receptor profile required for efficient CD8 T cell infiltration will indicate the best route of DC immunization to target particular SLO in relation to the tumor site. Applying this knowledge to the development of vaccines is an important consideration to ensure efficient targeting of the immune response to the desired peripheral tissue compartment.

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Disclosures
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